

# A single-pheromone model accounts for empirical patterns of ant colony foraging previously modeled using two pheromones

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## ARTICLE INFO

### Keywords:

Ant  
Colony  
Foraging  
Pheromone  
Decision-making  
Psychophysics

## ABSTRACT

In a 2009 paper, Dussutour et al. proposed that big headed ants (*Pheidole megacephala*) employ two attractant pheromones during foraging: one for exploration and another during food gathering. This claim was consistent with, and argued to be supported by, laboratory studies of ant exploration and food-gathering in a Y-maze apparatus. The authors measured foraging activity and colony foraging choice in terms of the number of ants choosing different branches over time, where experimental conditions modified the history of food availability at the end of each branch. They built a two-pheromone mathematical model to account for observed rates and proportions of ants traversing the left versus right branch. Here we show that the main reported experimental observations can be explained by a one-pheromone model. Our findings show that it is plausible, but unnecessary, to hypothesize that these ants employ two distinct pheromones in order to account for the two principal results of the Dussutour et al. study, and therefore, the study falls short of dispositive evidence for a two-pheromone model. More broadly, we highlight that patterns of animal behavior can be ambiguous with respect to sensory and cognitive mechanisms, hopefully motivating future modeling efforts that perform formal comparison across models with different structure.

## 1. Introduction

Understanding the sensory, cognitive, and behavioral strategies employed by animals is difficult because direct measurements of relevant mechanisms (e.g. chemical, neural) are often impossible to obtain. In such cases, investigators can engage in indirect inference via statistical modeling based on limited evidence. These indirect inferences about behavioral and cognitive processes are limited by the types of measurements available for any given experimental setup, as well as constrained by investigators' conceptions of the possible strategies available to organisms in their evolutionary context. In the case of eusocial insects such as ants, a great body of field observational data supports theoretical frameworks that relate ecological considerations to nestmate and colony-level behaviors, and the apparent strategic rationales behind them (Friedman et al., 2020; Gordon, 2014, 2019; Lanan, 2014).

Eusocial insects communicate directly, via physical interactions, as well as indirectly through chemical (i.e. pheromone) signaling. This type of mass communication via pheromone deposition is a stigmergic

process, in that it belongs to a class of collective behavioral algorithms involving entity-niche feedback processes (Heylighen, 2016a, 2016b; Theraulaz & Bonabeau, 1999). To investigate the cognitive bases of ant behavior, observational and manipulative experiments can be carried out in the field (Fleischmann et al., 2018; Friedman et al., 2018), as well as under controlled laboratory settings (Entler et al., 2016; Gordon & Mehdiabadi, 1999).

One well-used experimental paradigm in the behavioral sciences is the Y-maze (similar to the T-maze), which presents a binary path choice to individuals in the context of a foraging trip. The decisions of any forager at the branch point can be influenced by their own history (via learning and memory), as well as influenced by prior actions of nestmates depositing pheromones near the decision point (Czaczkes, 2018). For Y-maze setups and behavioral experiments, direct chemical and neurological measurements are often lacking, though the focal phenomena of the study is often related to the chemical or neural basis of behavior. In such cases, the qualities and effects of pheromones must be inferred from gross behavioral outcomes, and thus importantly hinge on degrees of freedom employed in the statistical modeling of empirical

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<https://doi.org/10.1016/j.cogsys.2023.02.005>

Received 27 June 2022; Received in revised form 6 December 2022; Accepted 13 February 2023

Available online 17 February 2023

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results.

It is well established that some ants deposit pheromone trails as behavioral cues to others in foraging for food (Czaczkes et al., 2015; Feinerman & Korman, 2017; Gissis et al., 2018; Lanan, 2014). Multi-component trail pheromones and other glandular secretions have been characterized in many insects including termites (Sillam-Dussès et al., 2010; Wen et al., 2014) and ants (Cerdá et al., 2014) (e.g. in pharaoh ants, (Jackson et al., 2006, 2007), in *Pheidole* (Ali et al., 2007)). In multiple ant species, multiple pheromones and blends of pheromones are used in different contexts, serving different behavioral functions (Czaczkes et al., 2015; Robinson et al., 2008). Confidence in these conclusions, specifically about the type and influence of pheromones that ants use, depends on an inferential chain of evidence and assumptions that are best continually re-examined for soundness.

Here we consider the stigmergic regulation of foraging activity in the ant species *Pheidole megacephala*. We review the two-pheromone model (2PM) of foraging activity proposed by Dussutour et al. 2009 (Dussutour et al., 2009), and provide an alternative one-pheromone model (1PM) involving plausible sensory-cognitive mechanisms. This work, in the spirit of methodological refinement, suggests specific followup experiments that could disambiguate the 1PM and 2PM, and highlights that alternative models of animal cognition (including stigmergic mechanisms of collective cognition) must be formally compared in order to draw well-founded conclusions.

## 2. The two-pheromone model (2PM)

This section reviews the two-pheromone model (2PM) presented in Dussutour et al. (2009), as well as the primary behavioral experiments involving the ant species *Pheidole megacephala* performed in that work. The 2PM model of ant colony foraging proposes that there are two chemically-distinct pheromones, both of which are attractants for foragers (e.g. biasing the nestmate to forage in the direction where attractant pheromones are at higher concentration). The first hypothesized pheromone is named the “exploration pheromone” (E), and is described as “a long-lived signal that acts as an ‘external long-term memory’ of the environment allowing the colony to rapidly establish a new trail.” Functionally, this E pheromone is hypothesized to allow “the colony to more quickly mobilize foragers when food is discovered.” The second hypothesized pheromone is named “foraging pheromone” (F) which “evaporate[s] quickly allowing the colony to abandon a depleted food source.”

The exponential decay times of the E and F pheromones are estimated. However, direct chemical measurements are not presented in this study (and from literature searching, we were unable to find followup experiments providing any chemical measurements of *P. megacephala* trails). Additionally in the 2PM, the authors make “two assumptions about the interaction between the pheromones.” The first assumption is that “the exploration pheromone [E] has a positive feedback on the deposition of foraging pheromone [F].” The second assumption made by the authors is that “the foraging pheromone [F] has a negative feedback on the deposition of exploration pheromone [E].”

The authors use this 2PM to fit empirical behavioral observations of *P. megacephala* made in the laboratory in the Y-maze paradigm. Below, we will describe and evaluate the relevant empirical findings, and contrast the 2PM with a 1PM. The 1PM model we propose accounts for the observed data in a way that is both parsimonious and biologically realistic. The important note to make here, which will be explored in greater detail in the Discussion, is that the assertion of a model’s *a priori* plausibility and consistency with empirical data does not constitute dispositive evidence that the model is superior to alternative models, absent direct consideration among specified plausible models.

## 3. Experimental patterns of colony foraging

Here we present the experiments conducted by Dussutour et al.

(2009), and describe how the 2PM is used to interpret the results. The authors conducted four experiments, numbered 1–4. The 2PM is introduced through the first two experiments, then shown to be consistent with the fourth. Their Experiment 3 is tightly coupled to assumptions of their model hypothesis and we do not address it here.

### 3.1. Experiment 1 and Experiment 2

Experiment 1 and Experiment 2 compare ant colony foraging behavior under different combinations of three treatments of the Y-maze branches (Exploration plus Foraging [E + F], Exploration [E], and Not explored [N]). In both experiment 1 and 2, the colony engages first in exploration of one of the maze branches (Branch A), followed by a one hour “foraging phase.” In each experiment, following the foraging phase, a second branch (Branch B) is added at the decision line, giving rise to a “test phase.” During the test phase, after choosing a branch, “ants walking towards each branch were gently removed with a paintbrush as they crossed the decision line to prevent reinforcement of either branch.”

The E + F treatment is consistent on Branch A between the two experiments, which differ in the history of the second branch, Branch B. The experimental paradigm is illustrated in their Fig. 1 (reproduced here in Fig. 1a\*).

In Experiment 1, Branch B was novel and unexplored (N treatment), and there was no opportunity for ants to mark the path in advance of the experimental trial. The authors measure and plot the proportion of ants choosing the E + F versus N branches during the test phase. Essentially this experiment measures the effect of pheromone deposited during food-gathering on decision-making at the decision juncture, comparing marked and unmarked branches.

In Experiment 2, Branch B at test time had been explored, and presumably marked by some stigmergic mechanism, by a sister subcolony during the Exploration-foraging phase (E treatment). The authors measure and plot the proportion of ants choosing the E + F versus E branches during the test phase, where the E branch had the opportunity to be marked under exploration but no food had been placed.

The results from Experiment 1 and 2 are shown in their Fig. 2 (Fig. 1b here). The salient observation is that in the E + F vs E condition, preference for the E + F branch drops off within 25 min, while in the E + F vs N condition, preference for the E + F branch persists for about 90 min. They proceed to build a mathematical model assuming two distinct pheromones, one for exploration (E) and another for exploitation (foraging, F). Under this 2PM model, they estimate decay rates for these respective hypothesized pheromones.

In their Discussion, with respect to the divergence between the paths in their Fig. 2 (Fig. 1b here) the authors write, “the observation that the initial frequency of ants choosing the E + F branch is the same in the E + F vs N and E + F vs E experiments but these frequencies diverge after 15 min ([Dussutour et al.] Fig. 2) is difficult to explain with a single pheromone.” That the (unpublished) difficulty of constructing a single pheromone model is taken as part of the “behavioral evidence that *P. megacephala* uses two different pheromones,” is our central contention and focus of the following modeling.

## 4. A One-Pheromone Model (1PM)

In contrast with the authors’ claims that these patterns are “difficult to explain with a single pheromone,” here we formulate a one-pheromone Model (1PM) that accounts for the observed experimental results using a single attractant pheromone.

The 1PM model incorporates four main assumptions, which are grounded in biologically plausible mechanisms of insect perception,

\* Dussutour et al. (2009) Figs. 1, 2, 10 reproduced with permission, Journal of Experimental Biology.

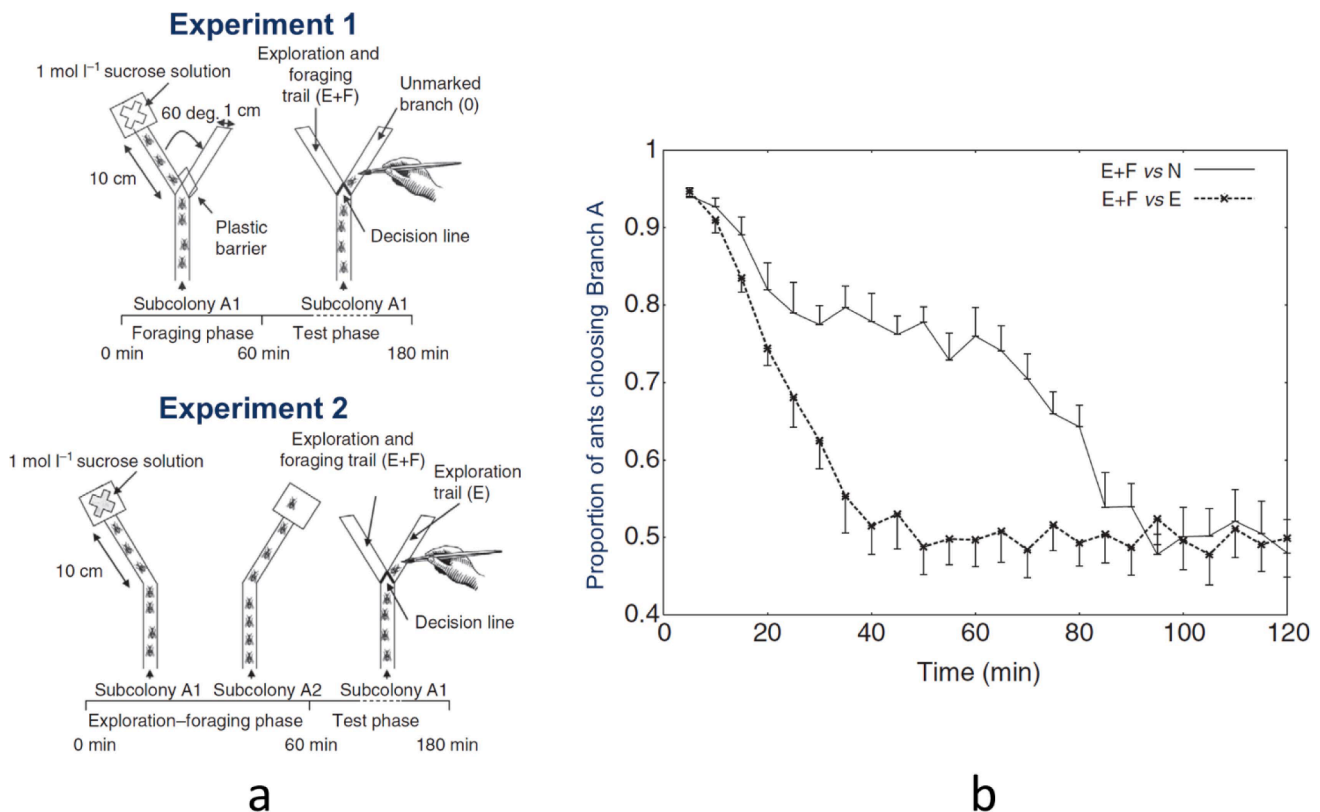


Fig. 1. a. Dussutour et al. Fig. 1, showing the setup of Experiment 1 and 2. b. Dussutour et al. Fig. 2 showing the results of Experiment 1 and 2.

cognition, and action.

1. First, we posit that ants deposit a single attractant pheromone whether exploring (E) or exploiting/gathering food (which Dussutour et al. call foraging, F). This is the crux of the difference between the 2PM and 1PM. We assume that ants deposit more of the pheromone when returning from a found resource (this could be on the return trip, verifying the presence of a seed) than if they do not find food, leaving stronger pheromone trails on paths leading to food.
2. Second, as with the 2PM, we assume that pheromone concentration decays exponentially with time.
3. Third, we model the perception of the downstream pheromone signal (e.g. behavioral impact) as a nonlinear amplification of pheromone concentrations. Amplification is stronger at lower pheromone levels, so the amplification curve will be concave downward (see Fig. 3). This type of signal amplification has been found commonly to govern sensory perception across species (Billock & Havig, 2018), and it is an established feature of the insect olfactory system (Gorur-Shandilya et al., 2017; Singh et al., 2019). Such methods have previously been applied to ant foraging (e.g. in the case of path selection in Argentine ants by (von Thienen et al., 2015)).
4. Fourth, we model branch preference (choice behavior) as related to the absolute difference in perceived pheromone concentration, rather than the ratio of pheromone perceived on each branch. This difference measurement is modulated by the robustness of the signal; under weaker levels of pheromone overall, deciding about the stronger branch is less certain and so the foragers behave as to hedge their bets by allocating traversals down both branches.

In the following paragraphs, we provide a qualitative overview of the 1PM. Following that, we present the mathematical details and computational implementation of the 1PM and perform parameter fitting based upon the experimental results of Dussutour et al., 2009.

#### 4.1. A qualitative overview of the 1PM

Qualitatively, the pattern observed in Experiment 1 and Experiment 2 of Dussutour et al. (their Fig. 2) is explained under a 1PM as follows. Assume that exploration (E) involves depositing pheromone, but exploitation (E + F) deposits substantially more so. At the outset, the pheromone signal from the E + F treatment is robustly stronger than either the E treatment or N (no prior ant traversal). The initial preference is therefore strong for the E + F branch under both experimental conditions.

Then, under exponential decay of pheromone (an assumption common to 1PM and 2PM), even initially high amounts of pheromone rapidly decay in strength. In experimental condition 2, the E + F and E signals both weaken, but under nonlinear amplification, the signal difference between the measurements becomes smaller rapidly, reducing to negligible within 25 min. By contrast, in Experiment 1 the difference between the weakened – yet amplified – E + F signal and background noise level (N treatment), persists until the pheromone evaporates almost completely at 90 min. The following sections elaborate on this qualitative explanation with a one-pheromone mathematical and simulation model, based upon the exact empirical results presented by Dussutour et al. (2009).

#### 4.2. Mathematical formulation and simulation of the One-Pheromone Model

Mathematically, the One-Pheromone Model (1PM) presented here consists of two stages, a measurement stage which includes non-linear signal amplification, and a preference stage which leads to action selection. We choose functional forms and parameters for each of these stages based on the data presented in Dussutour's Fig. 2.

In general, the overall composed preference function (including both amplification and preference stages) can be expressed as:

$$p_A = f(ph_A, ph_B) \tag{1}$$

where  $p_A$  is the probability of an ant choosing Branch A, estimated by the fraction of ants observed on Branch A in any interval (as per the Y-axis of Fig. 1B). The variables  $ph_A$  and  $ph_B$  are the amount of pheromone physically present on branches A and B, respectively.

The preference function can be visualized as a surface in two-dimensions (where  $ph_A$  and  $ph_B$  are the X and Y axes, and  $p_A$  is the elevation on that landscape). The Dussutour et al. experiments do not tell the entire shape of this surface, but they do provide data points for two paths across it. This data is sufficient to constrain a plausible analytical one-pheromone model entailing a handful of degrees of freedom whose parameters can be chosen to fit the data.

To motivate the functional form of a single pheromone model, consider a plot of the observed preference fraction on a two-dimensional plane, as shown in Fig. 2. The horizontal ( $ph_A$ ) axis is the amount of pheromone present on the E + F branch. The vertical ( $ph_B$ ) axis is the amount of pheromone on the other branch. Under the N treatment (Experiment 1), this is always at some noise level. Under the E treatment (Experiment 2)  $ph_B$  will decay from some non-negligible amount of pheromone.

Since we have no direct measurement of the amount of pheromone present, for the purposes of modeling we assume some initial amount of pheromone at the outset, with exponential decay through time down to the noise level. Pheromone decay or evaporation produces tracks of circles in Fig. 2. In Fig. 2, we choose an arbitrary scale for the initial pheromone amount, such that initially the E + F treatment has a value of 10. Under an assumption that exploration deposits less pheromone than exploitation, we choose the value 1 for the initial E treatment pheromone amount. These precise values do not matter for the structure of the argument; what matters is the assumption that the E branch has less pheromone than the E + F branch.

The exponential decay rate can be estimated from the time it takes for pheromone to evaporate from the initial value to the noise level. For example, if the initial F + E decays to a noise level 1/500 as strong over 90 min, then the decay rate is approximately  $-0.069$  per minute. Due to

exponential decay, most of the pheromone amounts are very small. Hence we display the values at three different scales, successively from left to right.

Biological sensors are in general designed to cover great dynamic range in the values of the property measured. For ecological utility, it is likely that ants have evolved to measure very scant amounts of pheromone. In other words, an ant’s pheromone sensor is likely, to some extent, to invert the exponential loss of signal strength over time seen in Fig. 2. We suggest that this inversion takes the form of a power law amplification as shown in Fig. 3.

Under this amplification, the paths of Experiment 1 and 2 shown in Fig. 2, spread out more evenly as shown in Fig. 4.

The transition from biased preference (large circles) to equal preference (small circles) is neither sharp nor a linear relationship. Instead, it presents more like a soft threshold whose height profile (in the range 0.5–1.0) in the  $ph'_A$ - $ph'_B$  plane can be modeled by a product of two sigmoid squashing functions as illustrated in Fig. 5, and as explained below.

$$f(ph'_A, ph'_B) = \frac{\sigma'_m(d_m(ph'_A, ph'_B)) \times \sigma'_r(d_r(ph'_A, ph'_B)) + 1}{2} \tag{2}$$

The two sigma functions  $\sigma'$  are the standard logistic function, with parameters for slope, offsets, and range.

$$\sigma'_m(x) = \frac{1}{1 + \exp(-s_m(x - d_{0_m}))} - \frac{1}{1 + \exp(s_m d_{0_m})} \tag{3}$$

$$\sigma'_r(x) = s_0 + (1 - s_0) \frac{1}{1 + \exp(-s_r(x - d_{0_r}))} \tag{4}$$

The distance functions,  $d_m(ph'_A, ph'_B)$  and  $d_r(ph'_A, ph'_B)$ , are distances on the  $ph'_A$ - $ph'_B$  plane to the sigmoid functions’ offset curves,  $d_{0_m}$ , and  $d_{0_r}$ , respectively.

The  $\sigma'_m$  term gauges distance from the midline  $ph'_A = ph'_B$ , where there is equal amount of pheromone on each branch, and hence, ants will prefer neither branch. The offset term,  $d_{0_m}$ , sets the width of a channel of proximity to the midline, within which the amounts of pheromone are considered similar enough to drive preference for

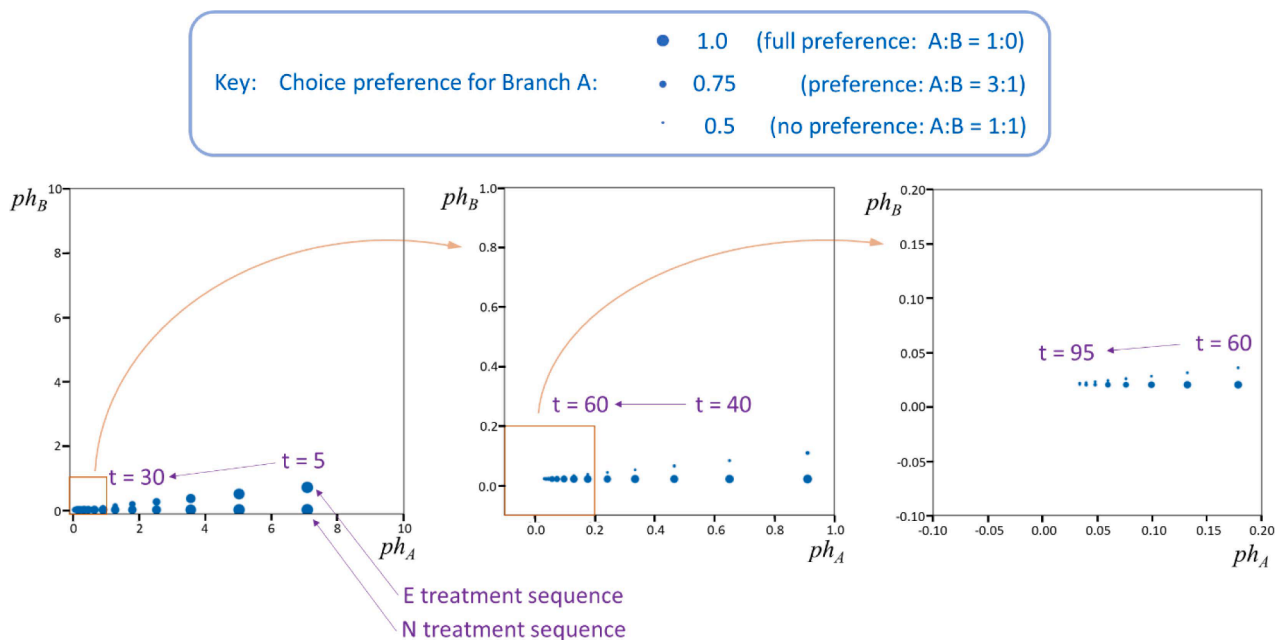
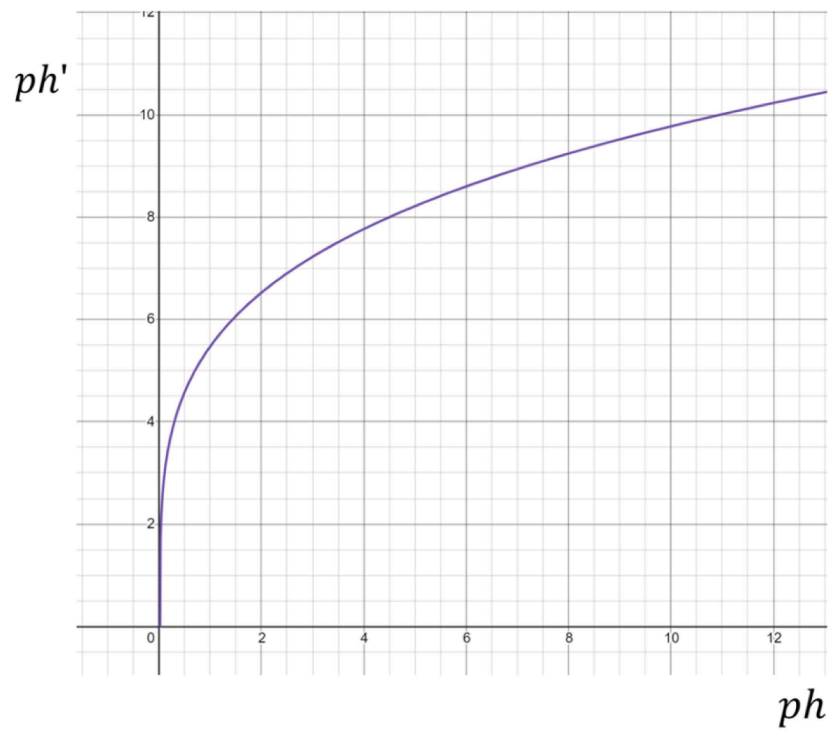


Fig. 2. Empirical observations from Dussutour experiments assuming a one-pheromone model. Horizontal axis is the concentration of pheromone on branch A ( $ph_A$ ), vertical axis is the concentration of pheromone on branch B ( $ph_B$ ) – Note the Y axis scale changes across the 3 graphs. Circles depict tracks of amount of hypothesized pheromone over time, for Experiments 1 and 2, under exponential evaporation. Lacking empirical data, we choose plausible relative initial values (farthest right circles in the leftmost, zoomed out plot) of 10 and 1, respectively, for  $ph_A$  and  $ph_B$  in the E + F versus E condition (Experiment 2). Circle diameter is choice preference, where large diameter is 1.0 (total preference) and small diameter is 0.5 (no preference). The values depicted as circle sizes were read off from Dussutour Fig. 2.



$$ph' = M(ph) = A(ph - B)^C$$

Fig. 3. Proposed pheromone measurement function M is a power-law amplification of the raw pheromone level  $ph$ , subtracting a small base level noise factor,  $B$ .

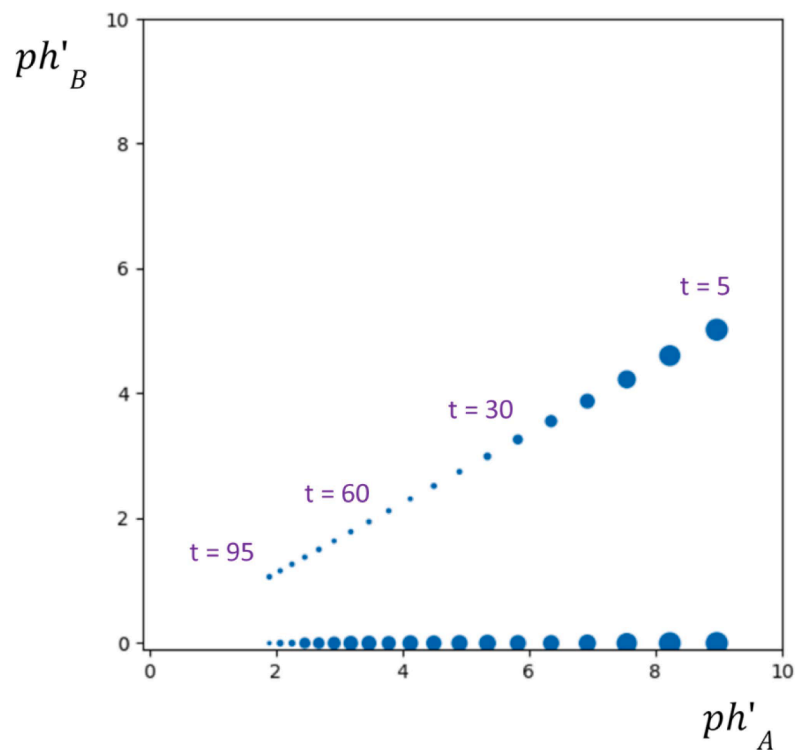


Fig. 4. The data of Fig. 2 (derived from Dussutour et al. Fig. 2) under power law sensory amplification using parameter values  $A = 5.5$ ,  $B = 0.02$ ,  $C = 0.25$ .

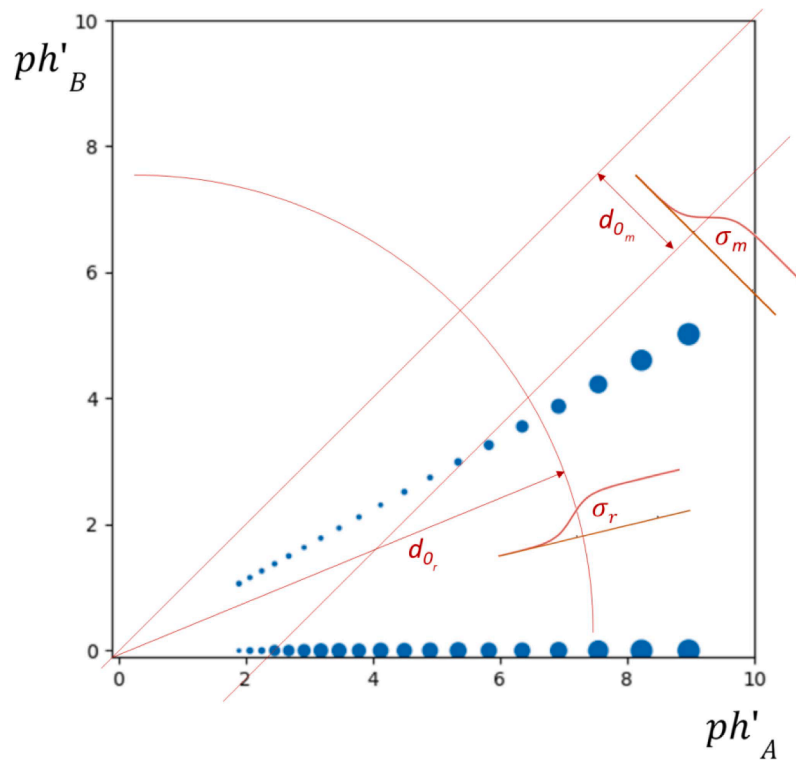


Fig. 5. Modeling the branch preference function as a product of sigmoid squashing functions.

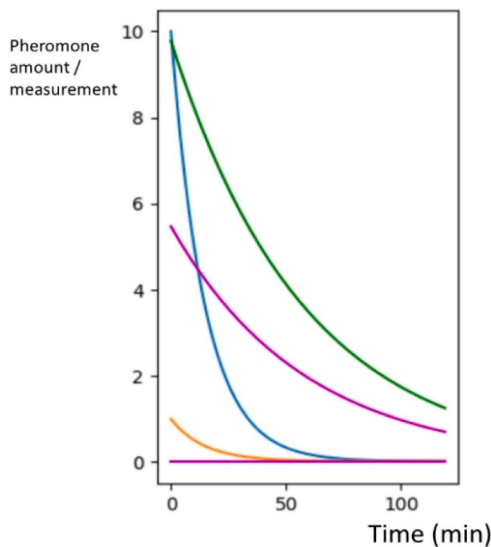
neither branch versus the other.

The  $\sigma_r$  term gauges robustness in the combined amount of pheromone present in terms of distance from the origin where  $ph'_A = ph'_B = 0$ . The offset term,  $d_{0_r}$ , sets the amount of pheromone above which the difference between the two branches becomes robustly distinguishable. Below pheromone amounts  $d_{0_r}$ , there is a limit imposed (by the  $s_0$  term) on the degree of preference for either branch. A typical value for this parameter sets this limit at about 0.8, or a ceiling of 80% preference

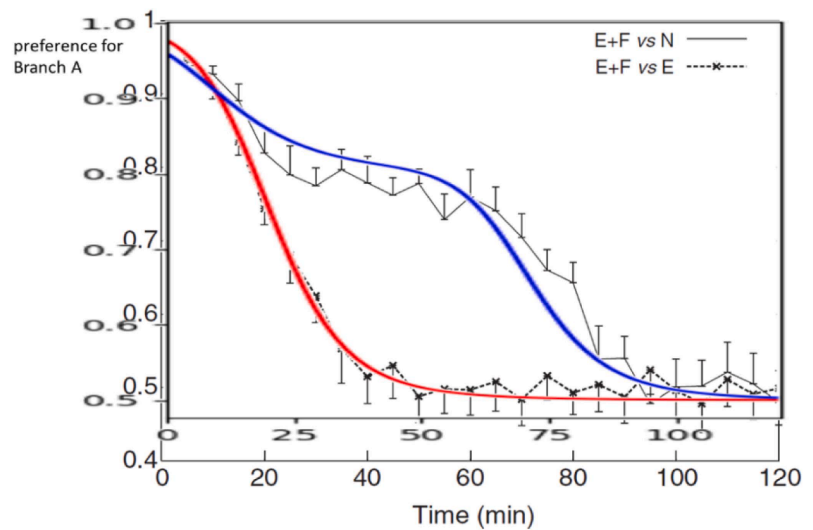
when the total pheromone level is weak.

The product of these terms in Eq. (2) models that strong branch preference occurs under a conjunction of two factors: ( $r$ ) sufficient amplified pheromone signal is transmitted to robustly measure any differences between the two branches, and ( $m$ ) the signal difference between the two branches is significant.

Consequently, the model expresses preference for the E + F branch (larger circles) when the amplified E + F signal is significantly greater



a



b

Fig. 6. a. Simulated trajectories of pheromone amounts and measurements under the one-pheromone model. Blue: Hypothetical physical pheromone amount for E + F branch. Orange: Hypothetical physical pheromone amount for E branch. Lower magenta: physical pheromone amount for N branch (noise level). Green: amplified pheromone measurement for E + F branch. Upper magenta: amplified pheromone measurement for E branch. b. Preference fractions for the E + F vs N (Blue, Experiment 1) and E + F vs E (Red, Experiment 2) conditions, plotted over Dussutour et al. Fig. 2. Parameter values were found by manual adjustment and are listed in the Table of Parameter Values, below. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

than the alternative signal, that is, for data points at greater distance from the  $ph'_A = ph'_B$  midline. And, the strongest preference for the E + F branch (largest circles) occurs when the total signal is greatest, as reflected in distance from the origin. This consideration is reflected in the “bump” in the decay of the E + F vs N plot in Dussutour’s Fig. 2. Values for the free parameters of the model can be found by manual adjustment or by numerical optimization.

With respect to their Fig. 2, Dussutour et al. write: “For example, the observation that the initial frequency of ants choosing the E + F branch is the same in the E + F vs N and E + F vs E experiments but these frequencies diverge after 15 min (Fig. 2) is difficult to explain with a single pheromone.” Under the single-pheromone measurement and preference model presented here, a simulation of ant behavior yields trajectories of pheromone amounts and amplified signals as shown in Fig. 6a. The resulting preference curves are shown in Fig. 6b, plotted on top of Dussutour Fig. 2 to demonstrate the close fit.

Clearly, a one-pheromone model can very handily account for the observation that the initial frequency of ants choosing the E + F branch is the same in the E + F vs N and E + F vs experiments but these frequencies diverge after 15 min.

#### 4.3. Dynamic environment experiment

Dussutour et al. performed another experiment, Experiment 4, called “dynamic environment,” in which they switch food between left and right branches and observe the time course of ant preference for each branch. This data is presented in their Fig. 10. They postulate that redirection to the food-containing branch is due to feedback modulation of the deposit of exploitation pheromone (F) in relation to exploration pheromone (E): “The model showed that the existence of negative feedback of foraging pheromone on the production of exploration pheromone allows an efficient switch back to the path where the source was located previously, after the intermediate period of unavailability of this source (2nd switch in our experiment).”

We find that a one-pheromone model is also able to account for the dynamic environment experimental result. We conducted a simulation of Dussutour et al. Experiment 4, with ant preference following the model above, and pheromone deposit occurring at a rate of 0.01 per ant on food branches (exploitation, F) and 0.001 per ant on no-food branches (exploration, E). Going by Dussutour’s report, we set total ant traffic in the range of 50/min. Under the model parameters used to fit Dussutour’s Fig. 2, we arrive at the preference curve of Fig. 7a.

Notably, ant preference does flip with change of food location. But

this curve shows some different features such as a significant branch indifference “shelf” when switching branches. It is possible however to fit model parameters using numerical optimization methods, still using the one-pheromone model formulation (see [Numerical Method, Code & Data Availability](#) section, for details). An example is shown in Fig. 7b. Here, a constrained BFGS method was used to optimize model parameters for dynamic environment experimental data read from Dussutour et al. Fig. 10. The parameters,  $d_{exploit}$  and  $d_{explore}$  are simulated pheromone deposit rates for ants on Food and No-food branches, respectively.

Obviously, any empirical curves can be fit under appropriate model structure given enough parameters. The model proposed here may be considered a member of a family of analytical forms whose parameters are relatively few and based in the principles of the domain. In general, good fits are obtained when parameter values are adjusted by 50% or more in trade-off with one another. In this way, the model is not dependent on precise adjustment of any parameter. Rather, well-fitting parameter values form a lower-dimensional manifold that is a projection down from the full parameter space. Through numerical optimization, a single set of values can be found that reasonably fits both Dussutour et al. Fig. 2 and Fig. 10. This is shown in Fig. 8.

#### 5. Discussion & conclusions

Here we have described the two-pheromone model (2PM) that was used by Dussutour et al. (2009) to model the stigmergic regulation of colony foraging behavior in big headed ants (*Pheidole megacephala*). We then propose an alternative one-pheromone model (1PM), which accounts for experimental evidence that was previously used to justify a conclusion that *P. megacephala* uses two pheromones to manage group exploration and exploitation of a food source (Dussutour et al., 2009). Our approach brings a two-stage computational model that decouples signal amplification from choice preference decision. Methodologically, we placed the computation in a general framework, then worked backward from experimental data to infer a plausible decision strategy that could easily be implemented in a simple nervous system.

In their abstract the authors write that they “establish that the ants’ behavior is consistent with the use of two different pheromone signals, both of which recruit nestmates.” We bring no contention against the claim that the empirical findings are “consistent” with a 2PM, and we also concur with the authors in that it is plausible that foraging trails consist of multiple chemical components with differential volatility and behavioral impacts (Czaczkes et al., 2015). Indeed, to the limited extent that glandular contents have been chemically analyzed in *P. megacephala*

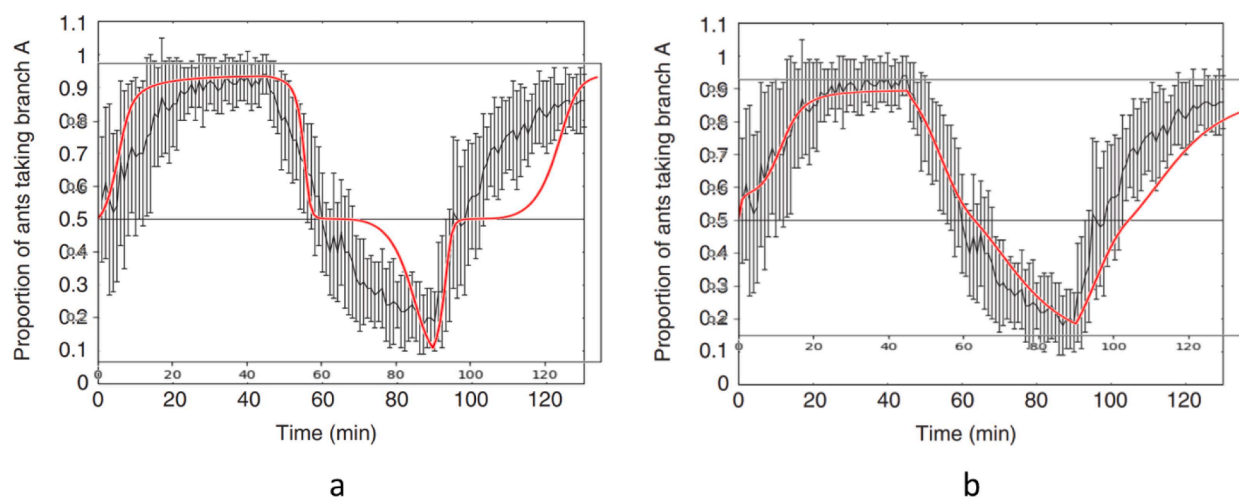


Fig. 7. a. Simulation of Dussutour et al. Experiment 4 under a one-pheromone model, using parameters estimated above to match empirical results from their Experiments 1 and 2. Simulated branch preference (Red) is plotted on top of Dussutour Fig. 10. b. Simulation of Dussutour et al. Experiment 4 using parameters found by numerical optimization to better fit the observed time course of branch preference switching. Optimized parameter values are listed in the table. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

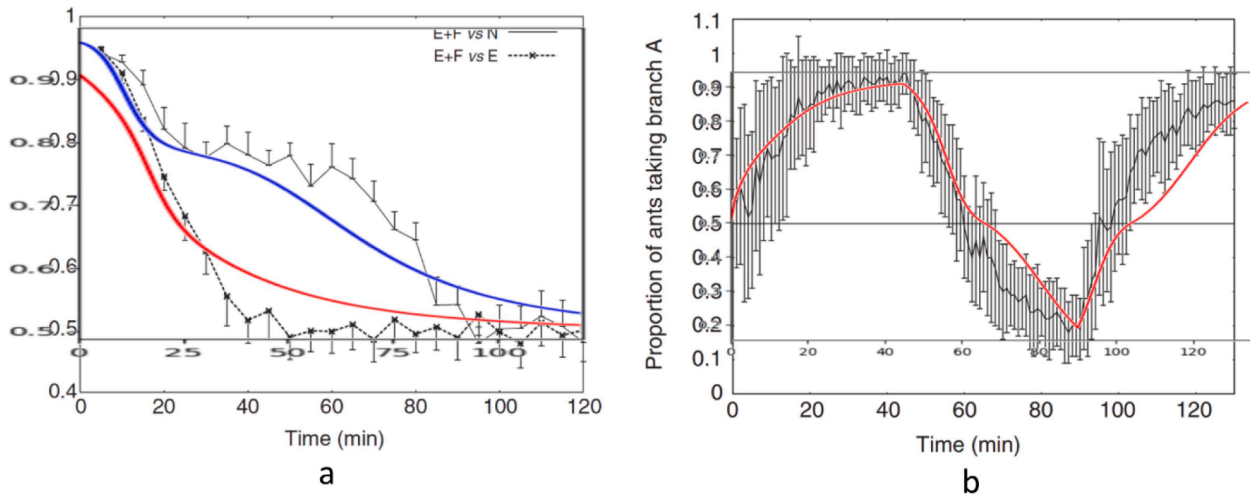


Fig. 8. One-phormone model simulations using a common set of parameter values optimized for Dussutour et al.’s Experiments 1, 2, and 4 as provided in their Figs. 2 and 10. Parameter values are listed in the table.

(e.g. Dufour gland in (Ali et al., 2007)), there are multiple components. Our concern arises from the Discussion section, where the claim is made that the modeled results provide “behavioral evidence that *P. megacephala* uses two different pheromones, a long-lasting pheromone during exploration and a short-lasting foraging pheromone during recruitment to a food source.” Our concern is both methodological and substantive. Without explicit comparison among alternative models with different number of pheromone components, the finding that the 2PM is consistent with the empirical results (per the claim in the Abstract) cannot be taken to constitute positive evidence for a two-pheromone foraging mechanism (as claimed in the Discussion). Regardless of the direct reading or interpretation of the original text, in subsequent literature, the experiments of Dussutour et al. have been widely cited specifically as positive evidence that ants use two pheromones in their foraging trails (e.g. (Czaczkes et al., 2015; Flanagan et al., 2013; Hills et al., 2015; Jeanson et al., 2012; Kolay et al., 2020; Lanan, 2014; Ma et al., 2013; Malíčková et al., 2015; Middleton & Latty, 2016; Vogel et al., 2015; Zabzina et al., 2014)).

Our model is certainly subject to overhaul, and the small number of free parameters would benefit from further adjustment with more data. In particular, the Dussutour et al. experiments cover only a small portion of the  $ph_A - ph_B$  plane of differential pheromone levels on the two branches of the Y-maze. It would be informative to gather branch preference data for other regions of this space.

In their paper, Dussutour et al. do not show predictions from their 2PM model charting the time course of ants’ collective Y-maze branch choice as measured and presented in their Figs. 2 and 10, so it is impossible to compare our 1PM model with theirs on this basis. Nonetheless, yet other experiments could be performed to distinguish our 1PM models from theirs or others. For example, Dussutour et al. report that, under some circumstances, their 2PM model predicts oscillations of pheromone concentrations when ants are presented with two branches both containing food. Our model makes a different prediction, namely that traffic and pheromone deposition equalizes without oscillation. Additionally, the two branch Y-maze experimental setup could be extended to three or more branches. Other experimental variations would involve lengthening or shortening branches, and varying environmental conditions such as temperature, lighting, surface properties, etc. In such cases, our current 1PM aggregate simulation would need to be enhanced toward an agent-based modeling framework, whereby individual simulated ants would traverse simulated paths and make stochastic decisions at branches.

The Y-maze paradigm itself merits methodological reflection, as well as consideration of the best practices for this experimental setup

(Czaczkes, 2018). It assumes that ant traffic is primarily governed by the physical presence of pheromone underfoot, and further, that this pheromone is unaffected by individuals during the experimental test phase. This assumption would not necessarily hold, however, if ants deposit some pheromone on their outgoing trip before being brushed off the trail. Ideally, the presence and composition of pheromone would be measured independently by a physical sensing apparatus throughout the experiment.

Another consideration is the microstructure of sensing and decision-making at the decision line. How does an ant know how much pheromone lies ahead on each alternative path? Do they interrogate each path for some distance, then backtrack to test the other? Methods such as high-speed, high-resolution video capture and analysis offer promise in deciphering insects’ sensory-decision machinery (Mueller et al., 2019; Walter & Couzin, 2021).

Limited gross behavioral data places only weak constraint on hypotheses about the physical, sensory, and cognitive mechanisms underlying complex observed behaviors. For this reason, it is incumbent to formally and rigorously consider alternative accounts. Similar to statistical power analysis, behavioral experiments need to search in experimental phase space in an informed way. Another path forward is to use integrated frameworks for ant colony cognitive modeling such as Active InferAnts (Friedman et al., 2021), that can be elaborated to encompass various sensory-cognitive phenomena as technology advances and new experiments are performed.

Table of Parameter Values.

Ref. Figure	Figs. 6, 7a	Fig. 7b	Fig. 8
parameter selection method	manual adjustment	num. optimization	num. optimization
A	5.5	2.27	4.92
B (noise level)	0.02	0.02	0.02
C	0.25	0.264	0.298
$ph_{A_0}$	10.0	10.0	10.0
$ph_{B_0}$	1.0	1.0	1.55
$s_m$	4.0	0.687	1.58
$d_{0m}$	2.0	2.1	1.61
$s_0$	0.6	0.261	0.617
$s_r$	0.8	1.93	1.81
$d_0$	8.0	9.4	7.71
$d_{exploit}$	0.011	1.0	0.0082
$d_{explore}$	0.0011	0.001	0.001

## Numerical Method, Code & Data Availability

Code and Data for this article can be found at <https://github.com/docxology/ant-pheromone>.

The program is written in Python. Numerical optimization is done using the `optimize.minimize` function of the SciPy scientific computing library. Optimization method used is L-BFGS-B (Limited-memory Broyden–Fletcher–Goldfarb–Shanno method with Box constraints). This method allows for hard bounding of certain parameter values. `noise_level` was clamped at 0.02. Value optimized is sum squared error between model prediction and empirical data as read from Dussutour et al. figures. Starting parameter values for optimization were the initial parameter values of Fig. 6 set by manual adjustment.

## Funding

DAF was supported by the USA National Science Foundation (grant award 2010290).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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