A new approach in understanding pheromone based collective behavior of ants

Wolfhard von Thienen

Dissertation zur Erlangung des Doktorgrades der
Naturwissenschaften
an der Fakultät für Biologie der Ludwig-Maximilians-Universität

München

Martinsried, 2016

Diese Dissertation wurde angefertigt unter der Leitung von
Prof. Dr. Dirk Metzler
am Institut für Evolutionsbiologie an der Ludwig-Maximilians-Universität München
und
Dr. Volker Witte
am Institut für Verhaltensökologie an der Ludwig-Maximilians-Universität München

Erstgutachter: Prof. Dr. Dirk Metzler
Zweitgutachter: Prof. Dr. Herwig Stibor

Tag der Abgabe: 25.02.2016
Mündliche Prüfung am: 24.06.2016

ERKLÄRUNG

Hiermit versichere ich an Eides statt, dass meine Dissertation selbständig und ohne unerlaubte Hilfsmittel angefertigt worden ist.

Die vorliegende Dissertation wurde weder ganz, noch teilweise bei einer anderen Prüfungskommission vorgelegt.

Ich habe noch zu keinem früheren Zeitpunkt versucht, eine Dissertation einzureichen noch an einer Doktorprüfung teilzunehmen.

Martinsried, den 25.02.2016 ___________________

Wolfhard von Thienen

DECLARATION OF COAUTHOR CONTRIBUTIONS

All of the studies were designed by myself with input from Dr. Volker Witte and Prof. Dirk Metzler.

I developed the models and software and interpreted the results. Dr. Witte provided introduction into the biological aspects of my research while Prof. Metzler introduced me into the statistical as well as modelling techniques. Both checked the manuscripts and suggested ways to improve them. For the first study, Prof. Choe from the University of California in USA provided me with data from his experiments with *Linepithema humile* to be able to compare his results with the model I developed. I wrote all manuscripts and appendices.

_____________________ ___________________

Wolfhard von Thienen Prof. Dirk Metzler

LIST OF PUBLICATIONS

von Thienen W, Metzler D, Choe D-H, Witte V (2014) Pheromone communication in ants: a detailed analysis of concentration dependent decisions in three species. Behav Ecol Sociobiol 68:1611-1627

von Thienen W, Metzler D, Witte V (2015) Modelling shortest path selection of the ant Linepithema humile using psychophysical theory and realistic parameter values. Journal of Theoretical Biology 372:168-178

von Thienen W, Metzler D, Witte V (2016) How memory and motivation modulate the responses to trail pheromones in three ant species. Behav Ecol Sociobiol 70:393-407

Table of contents

Summary and aim of this dissertation

Ants are remarkable in the way they form large, highly organized and structured colonies, consisting of up to several million individuals. This is achieved despite the small brains of individual ants and their limited information about the complex states of the colony and its environment, as well as the lack of a central guiding instance. Still, the ant colony is able to act as a whole and collectively finds solutions to the problems that it is challenged with, allowing the survival and propagation of the colony. This remarkable behavior of ant colonies is achieved by means of self-organization, which has become an important research field in biology with ants serving as model organisms. As result, researchers have successfully explained aspects of self-organization in ants by developing models of their collective behavior (Camazine et al. 2001b; Hölldobler and Wilson 2009).

In this context, the results of the research group led by Jean Louis Deneubourg from Belgium are most important. Based on the finding that ants respond to trail pheromones in a probabilistic manner, they developed models to explain some fundamental properties of the social behavior of ant colonies. For instance, they explaied how ants are able to collectively find the shorter of two different paths between nest and food (shortest path experiments) or how ant colonies are able to select the higher quality food source if food sources of different quality are available (Beckers et al. 1992; Beckers et al. 1993). We refer to this class of models as the *Deneubourg model* and to its underlying mathematical function as *the Deneubourg choice function (DCF)*. The DCF has been used by other models that explain different aspects of collective ant behavior, i.e. how they coordinate the division of labor between the members of the colony (Bonabeau et al. 1996), the influence of noise (Dussutour et al. 2009a), the path efficiency in artificial networks (Vittori et al. 2006), the symmetry breaking in foraging behavior (Lanan et al. 2012), the role of multiple pheromones (Dussutour et al. 2009b) and foraging in dynamic environments (Bandeira de Melo and Araújo 2011; Ramsch et al. 2012).

Although the Deneubourg model can explain important aspects of the social behavior of ant colonies, it bears some shortcomings:

- Computer simulations could not fully reproduce the experimental results of the shortest path experiments, despite the fact that the parameters of the DCF were freely adjusted to get an optimal fit to the experiments.
- Realistic parameter values for the DCF were never deduced experimentally.

- To our knowledge, the parameters of the DCF lack biological interpretation.
- The model does not include a theory of perception describing the relation between pheromone perception and ant behavior.

The aim of this dissertation was to overcome these shortcomings by measuring the exact dose-response relationship between pheromone concentration and ant response, deduce all relevant parameter values for the DCF, find a new model based on a theory of perception, test and compare both models and apply the new model to important biological aspects of ant colony behavior.

The results presented in this dissertation may be summarized as follows:

- The Deneubourg model in its original conception does not explain the experimental results if we use realistic parameter values that were deduced from experiment.
- As the missing theory of perception, *psychophysical theory* proved to be appropriate to describe the dose-response relationship between pheromone concentration and ant response. Most importantly it could be shown that *Weber's* law, a fundamental law in sensory physiology, is fulfilled. Important biological parameters like behavioral thresholds and error rates could be defined consistently and experimentally deduced.
- By incorporating *psychophysical theory* into the model, the modified Deneubourg model was able to qualitatively and quantitatively explain the shortest path experiments.
- Memory and motivation significantly altered the ants' behavior and their response to trail pheromones, resulting in the change of characteristic psychophysical parameters. For instance, the response threshold was shifted and the error rate in trail following changed in a way that we predicted.

We therefore suggest to refine one of the leading theories of collective ant behavior, the Deneubourg model, by integrating psychophysical theory into the model. Thus we may gain a more exact explanation of the experimental results, a clearer definition of important biological parameters and altogether a deeper insight into the collective behavior of ants.

General introduction

> *"With algorithms, the colony masters the problems natural selection has designed it to solve. The required information is distributed among the colony members. Thus, a distributed intelligence is greater than the intelligence of any one of the members, sustained by the increased pooling of information through communication."* (Hölldobler and Wilson 2009, "The Superorganism", p. 58)

A typical ant colony usually consists of many thousand individuals and in some species like wood ants or leaf cutter ants, this number may go up to several million. These individuals are constantly active in exploring for new food sources and bringing the food they found inside the nest, feeding and taking care of the brood, guarding and defending the colony, as well as finding new nest sites and moving the whole colony if the old nest is no longer suitable. Many ant species practice farming of aphids and live on the nectar they produce. Leaf cutter ants cultivate fungus which they feed with freshly cut plant material. The architecture of ant nests is often highly complex with different chambers, tunnels and a sophisticated system of air conditioning to control the climate within the nest. Around their nest, ants build complex networks of trails connecting the nest to food sites, often 50 or more meters away (Hölldobler and Wilson 1990; Seifert 2007; Hölldobler and Wilson 2009).

The most remarkable thing about this complex organization is that it works without a central guiding instance (Detrain and Deneubourg 2006). For this reason, ants have become an important model organism for research on self-organization (Camazine et al. 2001a). Self-organization allows patterns to emerge in a system on a global level, without the use of global information but instead by interactions between the components of the system using only local information (Camazine et al. 2001a, p. 8). It was shown that many aspects of the behavior of ant colonies can be explained by principles of self-organization (Camazine et al. 2001a, ch. 13 and 14; Detrain and Deneubourg 2006). Below is a list of some important properties of self-organization in ant colonies that have been described in the literature:

- A central control does not exist (Detrain and Deneubourg 2006).
- Individuals have only very limited knowledge about the state of the whole system and the environment (Detrain and Deneubourg 2006).
- Individual ants act on a small set of behavioral algorithms (Hölldobler and Wilson 2009, pp. 53-60).

- Ants use different kinds of signals for communication (tactile, acoustic, chemical), but predominantly using pheromones deposited in the environment (Hölldobler and Wilson 1990, p.227; Hölldobler 1995).
- Changes in the internal and/or external environment of the colony trigger positive and negative feedback loops, enabling structure and pattern to emerge (Camazine et al. 2001a, ch. 2).
- The colony as a whole reacts dynamically to the changes in the environment (Camazine et al. 2001a, ch. 3), shifting the colony into a state that represents a good solution to the problems that the environment has posed upon the colony (Hölldobler and Wilson 2009, p. 58).
- In many cases, colonies show a complete and abrupt transition from one stable state into another (*symmetry breaking* or *bifurcation*) (Detrain and Deneubourg 2006).

A key to the understanding of self-organization in ants is to understand the way they communicate, which is mainly based on pheromones (Wilson 1958). It was shown that ants do not respond to pheromones in binary manner but rather probabilistically: The higher the pheromone concentration an ant encounters, the higher the probability that it will respond to it (Hangartner 1969; Van Vorhis Key and Baker 1982; Choe et al. 2012; Perna et al. 2012). Based on these findings and principles of self-organization, models have been developed that explain some basics of collective ant behavior:

- How ant colonies are able to select the shorter of two paths of different lengths (Goss et al. 1989; Deneubourg et al. 1990).
- How ant colonies are able to select the higher quality food source if food sources of different quality are presented to the colony (Beckers et al. 1993).
- How ant colonies are able to coordinate the division of labor by caste specific response thresholds (Bonabeau et al. 1996).
- How ant colonies are able to find the shortest path between nest and food (Vela-Pérez et al. 2013).

Since these models are all very similar and go back to the work of the Belgium research group of J.L. Deneubourg, we refer to this class of models as the *Deneubourg model* and to its underlying mathematical function as the *Deneubourg choice function (DCF)*, see Box 1 below. The key elements of the model are, despite some variations, always the same: the amount of pheromone deposited in the environment contains information about the quality of the environment, ants respond probabilistically to different pheromone concentrations according to the DCF or slight modifications of it, and a positive feedback loop attracts the ants towards the most favorable environment.

Although the model was very successful in explaining important aspects of ant colony behavior, it has some shortcomings:

- In cases in which the model was tested by computer simulations that were compared to experimental results, the simulated results did not fit very well to the experimental results, see Fig. 1B (Goss et al. 1989; Deneubourg et al. 1990; Bonabeau et al. 1996). This was despite the fact that the parameter values of the DCF could be freely adjusted to fit the experiments. Thus, the models were able to explain the colony behavior only qualitatively and not quantitatively.
- The parameter values of the DCF were set freely to give an optimal fit to the colony behavior (Goss et al. 1989; Deneubourg et al. 1990; Bonabeau et al. 1996; Vela-Pérez et al. 2013) while real parameter values were never deduced by experiment (see Fig. 1).
- We know of no biological interpretations of the parameters of the DCF.
- The response of an ant to a pheromone is determined by the way it perceives the pheromone through its sensory organs, the further processing by its nervous systems and the final translation into behavior. Thus, a deeper understanding of the pheromone based behavior of an ant colony should be based on a theory of perception. This theory is missing in the Deneubourg model.
- The model did not distinguish between detection tasks and discrimination tasks. In detection tasks the animal has to detect a stimulus of varying intensity against a background with no stimulus (or just noise), while in discrimination tasks the animal has to distinguish a stimulus of varying intensity from a background stimulus of constant intensity. Both tasks lead to a different dose-response relationship. In the initial phase of self-organization, which mainly determines the development of the system, the ants have to perform detection tasks, while in the following phase, they have to perform discrimination tasks. These differences have not been considered by the model so far.

Box 1: The Deneubourg model,

(modified from von Thienen et al. 2014; von Thienen et al. 2015)

The Deneubourg model (Deneubourg et al. 1990; Beckers et al. 1992) is based on the finding that ants encode information about the environment by dropping varying amounts of pheromones on their trails. This information is then used by other individuals for directional choices (Wilson 1962). The model describes the decision ants take at a bifurcation of two pheromone trails: the trail with higher concentration is selected by the ants in a probabilistic and non-linear manner depending on the pheromone concentrations of the two trails given by the *Deneubourg choice function* with exponents ≈2:

$$p = \frac{\left(k + c_L\right)^b}{\left(k + c_L\right)^b + \left(k + c_R\right)^b} .$$

p – decision probability, c_L – pheromone concentration of left branch, c_R - pheromone concentration of right branch, b – exponent, k – constant.

The model could explain how ants are able to collectively select the shorter of two paths of different length between nest and food by depositing pheromone on their way towards the food and back (shortest path experiments) (Goss et al. 1989). The model explains the outcome of the experiments by initial differences between the pheromone concentrations of the two paths. When the paths are of different length, there is a time delay between the ants arriving at the food via the long compared to the short path. During this period, the long path carries no pheromone at all at the side closest to the food and the returning ants prefer the short path on their way back to the nest and deposit pheromone on it. This generates a positive feedback loop in favor of the short path. Thus, in most experiments, the great majority of ants follow the short path (See Fig. 1A). If both paths are of equal length, no time delay occurs. However, small stochastic differences between the paths occur at the beginning and positive feedback amplifies the path with an initially slightly higher concentration so that it is finally selected by most of the ants. Consequently, in most of the experiments symmetry breaking occurs and either one or the other path is preferred by the great majority of ants with equal probability (see Fig. 1B).

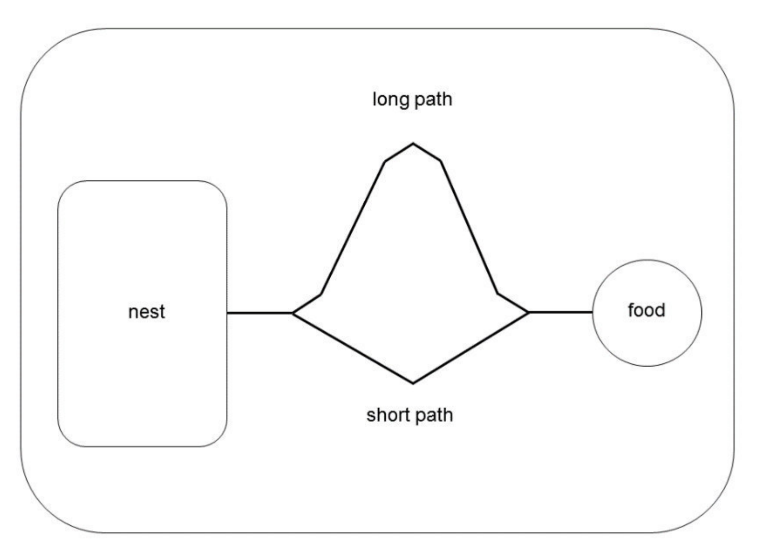
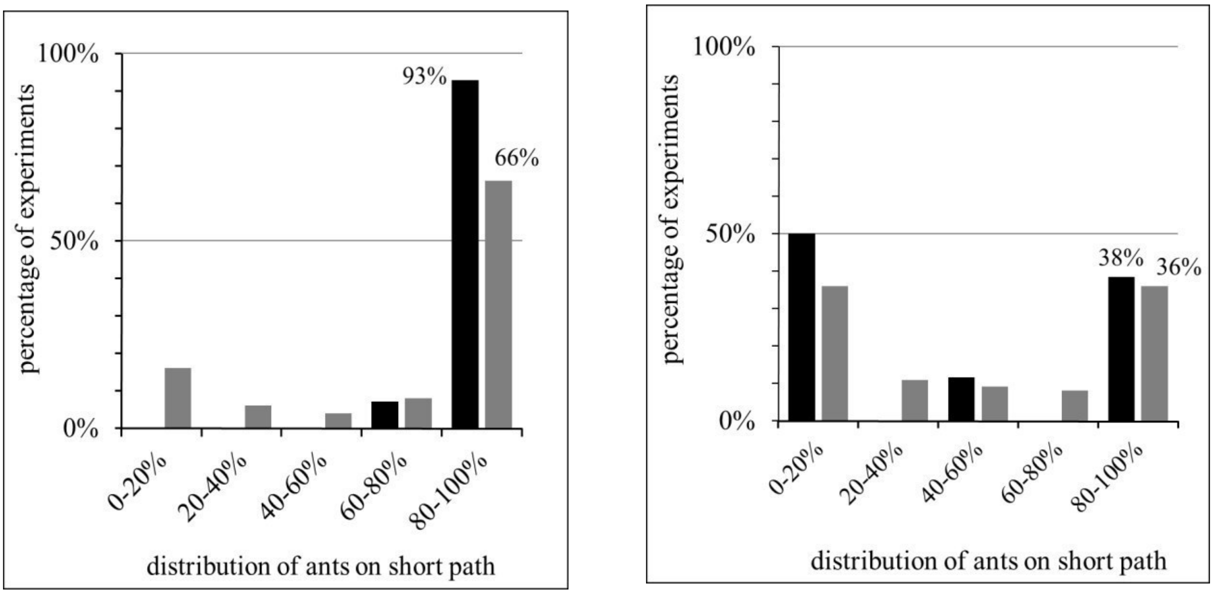


FIG. 2.–Schematic experimental setup of the shortest path experiments. In the majority of experiments, most of the ants took the short path between nest and food. Modified from Goss et al. (1989).



A **B**

FIG. 1.–Results of the original shortest path experiments, modified from Goss et al. (1989). The x-axis shows the distribution of ants that chose the short path (n=500); the y-axis shows the number of experiments or simulations in percent (n=1000). Black=original experimental results, grey=results of Monte Carlo simulations with parameters *b*=2, *k*=20. A: One path is twice as long as the other; in 13 out of 14 experiments (93%) more than 80% of the ants took the short path while this did not happen at all for the long path. B: Both paths (named “A” and “B”) are of equal length; in 10 out of 26 experiments (38%) more than 80% of the ants took path A and in 13 experiments (50%) this happened for path B.

The aim of this dissertation was to overcome these shortcomings. To achieve it, following tasks had been defined:

Task 1: Experimentally measure the exact dose-response relationship between pheromone concentration and ant response.

Task 2: Deduce all relevant parameter values of the DCF from these experiments.

Task 3: Find a new model based on a theory of perception that fits well to the experimental results and deduce all relevant parameter values for the underlying mathematical functions.

Task 4: Test if one of the experiments explained by the Deneubourg model can still be satisfactorily explained by using the DCF after adding the parameter values that had been deduced by the experiments before.

Task 5: Do the same test with the functions based on the new model.

Task 6: Apply the new model to an important aspect of ant colony behavior, the combined effect of trail pheromones and route memory.

Task 7: Apply the new model to a further important aspect of ant colony behavior, the combined effect of trail pheromones and motivation to forage.

For Task 1–3, three different ant species that are phylogenetically and ecologically clearly distinct were used: *Linepithema humile, Lasius niger* and *Euprenolepis procera*. As theory of perception (Task 3), *psychophysical theory* was investigated, which had already been successfully applied to humans and other animals in many studies, see Box 2 below. As mathematical tool to describe the relationship between pheromone concentration and response, a *psychometric functions (PF)* was used. As test for the models (Task 4 and 5), the results of the experiments from Goss et al. (1989) and Deneubourg et al. (1990) with *L. humile* were used. In these experiments ants selected the shorter of two paths (shortest path experiments). Like in the original publications, the experiments were simulated by computer and the simulated results were compared to the experimental results. The only difference was the use of realistic parameter values derived from the measurements of the dose-response relationship (Task 1). Finally psychophysical theory was applied to experiments in which the combined effect of pheromone and memory and the combined effect of pheromone and motivation on the behavior of ant colonies was tested (Tasks 6 and 7). The results of Task 1–3 are presented in chapter 1 (von Thienen et al. 2014), the results of Task 4 and 5 are presented in in chapter 2 (von Thienen et al. 2015) and the results of Tasks 6 and 7 are presented in chapter 3 (von Thienen et al. 2016).

Box 2: Psychophysical theory,

(modified from von Thienen et al. 2014; von Thienen et al. 2015)

Psychophysical theory was developed by G.T. Fechner (1860) based on the works of E.H. Weber (1834) to find a relationship between the strength of a physical stimulus and its sensory impressions (Klein 2001; Wichmann and Hill 2001; Kingdom and Prins 2010). It plays a major role in psychology and neurobiology. The main subjects of psychophysical theory are humans but it has also been successfully applied to perception and behavior in primates, birds and insects (Britten et al. 1992; Sarris 2006; Dyer et al. 2008; Chittka et al. 2009; Sasaki et al. 2013; Akre and Johnsen 2014) and in decision making processes (Kacelnik and Brito e Abreu 1998; Gold and Shadlen 2007). Weber (1834) discovered that the ability to discriminate between two stimuli depends on the ratio of the stimulus strengths, which is, within certain limits, independent of the absolute stimulus strengths (Weber's law). Fechner (1860) discovered later that the sensory impression of a physical stimulus is proportional to the logarithm of the stimulus strength, which is the reason why, for example, sound levels are measured on a logarithmic scale (decibel).

In the context of the psychophysical theory, a mathematical framework has been developed that relates physical stimuli to sensory impressions. This framework incorporates the effect of noisy backgrounds that influence the ability to detect a signal and it gives clear mathematical definitions of sensory thresholds. One of the most useful mathematical tools is the *psychometric function (PF)*, which describes the relationship between the probability of a positive response *p* to a stimulus and the stimulus strength *x* (see Fig. 3)

$$p(x) = \gamma + \left(1 - \lambda - \gamma\right) \cdot F(x) \, .$$

x – stimulus strength, λ – guess rate, γ – lapse rate, *F(x)* – function describing the probability to detect a stimulus by the underlying sensory mechanism (Kingdom and Prins 2010 p. 74). For F(x) a probability distribution like the Weibull distribution is applied.

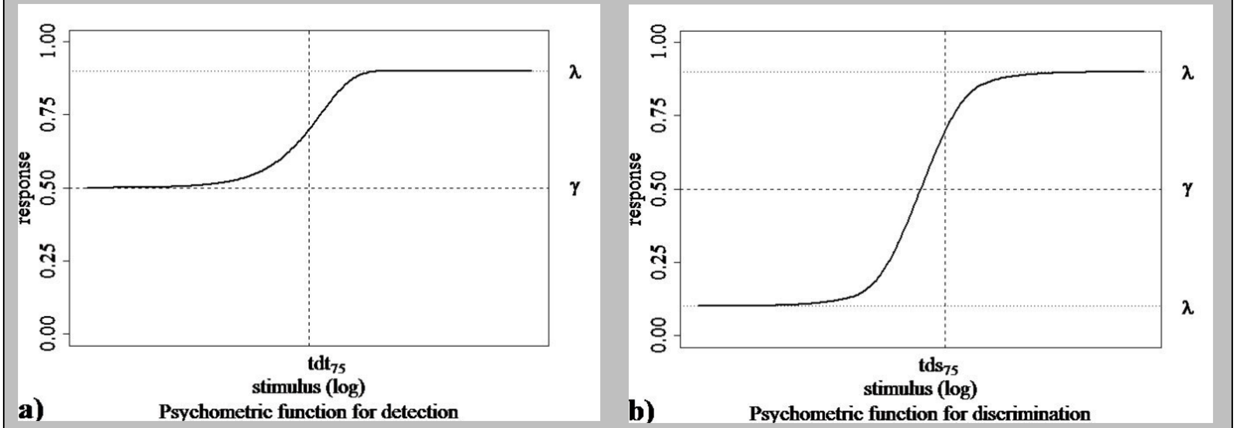


Fig. 3: Schematic examples of psychometric functions. In detection experiments (a) the psychometric function (PF) gives the probability that a stimulus is reported stronger (response) than a null-stimulus. It starts at 0.5 when both stimuli are zero or close to zero and cannot be distinguished. In discrimination experiments (b) the PF gives the probability that a stimulus is reported stronger (response) than a constant stimulus, which is larger than zero. Lapse rate (λ, dotted horizontal line), guess rate (γ, dashed horizontal line), 75%-detection threshold (tdt_{75}, dashed vertical line), 75%-discrimination threshold (tds_{75}, dashed vertical line). Note that the 75%-threshold is the point at half the distance between guess rate and upper asymptote, thus, it may not be exactly at p=0.75. Solid lines show the PF.

Psychophysical theory explains the probabilistic nature of the response to a stimulus by incorporating the influence of the random fluctuations of internal and external noise. It is assumed that a stimulus will only be clearly detected when the sensory impression produced by the stimulus exceeds a certain internal sensory threshold that is clearly above random noise. This assumption is called the *high threshold assumption*. The sensory threshold is usually defined as the stimulus strength that produces a correct response in 75% of the cases. This is assumed to be well above the influence of noise. In the context of psychophysical theory, other important explanations of the probabilistic nature of sensory impressions exist, for instance the *detection theory* (Kingdom and Prins 2010, p. 74ff).

There are two important classes of psychophysical experiments: 1) D*etection experiments* measure the response to a varying test stimulus compared to a null reference stimulus, while 2) *Discrimination experiments* measure the response to a varying test stimulus compared to a fixed reference stimulus. *Response* is defined as the probability to detect the stimulus as stronger than the reference (null or fixed) stimulus.

The PF is used to define and measure the 75%-*detection threshold* (tdt_{75}) and the 75%-*discrimination threshold* (tds_{75}), which are usually defined as the stimulus strength at which 75% of the responses are correct. In detection experiments, the *75%-detection threshold* is taken as a measure for the smallest detectable stimulus. Similarly, in discrimination experiments the *75%-discrimination threshold* is taken as a measure for the smallest noticeable difference or contrast. The *lapse rate* defines the errors that the test subjects make. According to psychophysical theory, the lapse rate is influenced by the motivation or attentiveness of the test subjects, by disturbances or by learning effects, which are caused by repeating the measurements with the same test subject. For further details see (von Thienen et al. 2014; von Thienen et al. 2015).

Chapter 1

Pheromone communication in ants: a detailed analysis of concentration dependent decisions in three species

Wolfhard von Thienen, Dirk Metzler, Dong-Hwan Choe, Volker Witte (2014)
Behavioral Ecology And Sociobiology 68:1611-1627

Behav Ecol Sociobiol (2014) 68:1611–1627
DOI 10.1007/s00265-014-1770-3

ORIGINAL PAPER

Pheromone communication in ants: a detailed analysis of concentration-dependent decisions in three species

Wolfhard von Thienen · Dirk Metzler ·
Dong-Hwan Choe · Volker Witte

Received: 18 March 2014 / Revised: 7 May 2014 / Accepted: 1 July 2014 / Published online: 16 July 2014

Abstract The collective behavior of ants and the emergence of self-organizing patterns in ant colonies have been explained with various theoretical approaches based on models of trail following behavior elicited by pheromones. Although existing models can explain collective behavior of ants, there is little empirical evidence on how ants precisely respond to various pheromone concentrations. Thus, important knowledge is lacking about how much realistic description of ant behavior can be provided by the models and their underlying mathematical functions. To fill in this gap, we conducted experiments with three different ant species to explore their responses to varying concentrations of pheromones that elicit ants' trail following behavior. We found that ants' decision making processes in trail following are best explained by psychophysical theory (PT), which describes the relationship between physical stimuli, sensory perception and decision making in humans, other primates, birds and insects. Furthermore, the theory provides clear definitions of biological parameters, such as detection- and discrimination thresholds. The species studied were distinctively different in the shape and parameters of their psychometric functions, which we attribute to specific adaptions to their environment. The observed differences are discussed in relation to their natural trail following behaviors. Our study opens a new perspective of understanding and explaining important aspects of collective ant behavior using a well-established theory of perception.

Keywords Ant behavior · Communication · Pheromones · Psychophysics · Weber's law

Introduction

Self-organization and pattern formation are important concepts in biology. The mathematician Alan Turing developed a model explaining how different kinds of complex biological structures could emerge from unstructured systems (Turing 1952). Grassé (1959) explained the building of complex nest structures by termites based on self-organizing mechanisms. Similar self-organizing mechanisms have been found in other social insects, particularly ants, which have become important model organisms for the study of self-organization.

Ants use different kinds of signals for communication (tactile, acoustical, chemical) and these signals often modulate each other (Hölldobler 1995). Yet, as Wilson had proposed (1958), their social behavior is mainly mediated by chemicals and a large amount of evidence has been found, showing that pheromones play an important role in the emergence of collective social behavior in ant colonies (Hölldobler and Wilson 1990, p. 227). Thus, to understand the social behavior of ants, it is important to understand how information is encoded and transmitted by pheromones and how this information is translated into specific behavior. It has been shown that ants, confronted with a binary choice of trails, prefer the trail with the higher pheromone concentration over that of lower concentration and that trail following fidelity increases with higher pheromone concentrations (Hangartner 1969; Van

---

Communicated by W. O. H. Hughes

Electronic supplementary material The online version of this article (doi:10.1007/s00265-014-1770-3) contains supplementary material, which is available to authorized users.

W. von Thienen (✉) · D. Metzler · V. Witte
Department Biologie II, Ludwig-Maximilians Universität München,
Großhaderner Str. 2, 82152 Planegg, Germany
e-mail: w@thienen.de

D.-H. Choe
Department of Entomology, University of California–Riverside,
Riverside, CA, USA

Vorhis Key and Baker 1982; Choe et al. 2012). This behavior resembles an analog communication system, which has the potential to transmit information about different states of the environment.

Importantly, the information content of continuous pheromone concentration is a central component of various models of self-organizing behavior of ant colonies, for example, how ants are able to find the shortest of two trails between their nest and a food source (Goss et al. 1989; Deneubourg et al. 1990). With a similar model, it was shown that the ability of ants to exploit the best quality food source in the environment depends on different pheromone concentrations deposited on the trails (Beckers et al. 1993). We refer to this class of models as *Deneubourg models*. To mathematically describe ant behavior, these models make use of the same or a slightly modified choice function, which we refer to as the *Deneubourg choice function* (see section "Deneubourg model").

Although it is possible to explain important aspects of collective ant behavior with these models, empirical evidence for crucial model assumptions concerning the precise decisions ants take depending on given pheromone concentrations is rare. The above-mentioned experiments of Hangartner, Van Vorhis Key and Baker, Choe et al. clearly revealed a relationship between the ants' decision and the concentration of the trail pheromones. However, their results applied only to two ant species and involved only up to six different pheromone concentrations, without knowing whether the concentration range matched natural conditions. Particularly low and high concentrations were not studied, which are presumably important in ant communication and thus also in models of self-organization. Consequently, there seems to be a lack of empirical data on the precise relationship between pheromone concentration and ant decision making.

The aim of this study was to fill in this gap by measuring the decisions that ants take depending on different pheromone concentrations. Another aim was to fit an appropriate choice function to the empirical data. We focused on three different ant species and on their glandular sources that had been shown in past studies to contain trail pheromones. For each, we conducted three different experiments. The experiments were carried out with phylogenetically distinct ant species to find out if there are differences in decision making that can be attributed to specific adaptations to a given environment. We compared the empirical data with the Deneubourg model and modified this model by considering a well-established general theory of perception, the *psychophysical theory* (see below). With this theory, the understanding of sensory perception in various animals and humans can be combined with knowledge about collective ant behavior to give deeper insight into the biology of decision-making processes in the trail following behavior of ants (see section "Applying the psychophysical theory to models of collective ant behavior").

The models

Understanding the Deneubourg model and the psychophysical theory are essential for the understanding of this article. Here, we give a short introduction for readers who are not familiar with these concepts. In addition, we describe our application of the psychophysical theory to the Deneubourg model.

Deneubourg model

The Deneubourg model was developed to explain important aspects of the collective behavior of ants that are based on pheromones (Deneubourg et al. 1990; Beckers et al. 1992). The model is based on the finding that ants encode information about the environment by dropping varying amounts of pheromones on their trails and this information is then used by other individuals for directional choices (Wilson 1962). The model describes the decision ants take at a bifurcation: the ants choose the trail with higher concentration in a probabilistic and nonlinear manner depending on the pheromone concentrations of the two trails given by the *Deneubourg choice function* with exponents ≈2:

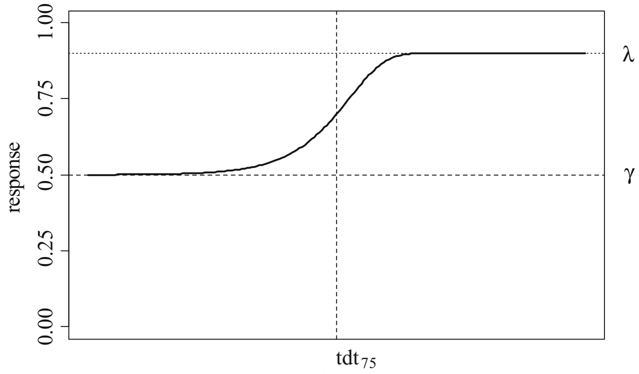
$$p = \frac{(k + c_{\mathrm{L}})^b}{(k + c_{\mathrm{L}})^b + (k + c_{\mathrm{R}})^b},$$

where p is the decision probability, c_{L} is the pheromone concentration of left branch, c_{R} is the pheromone concentration of right branch, b is the exponent, and k is a constant.

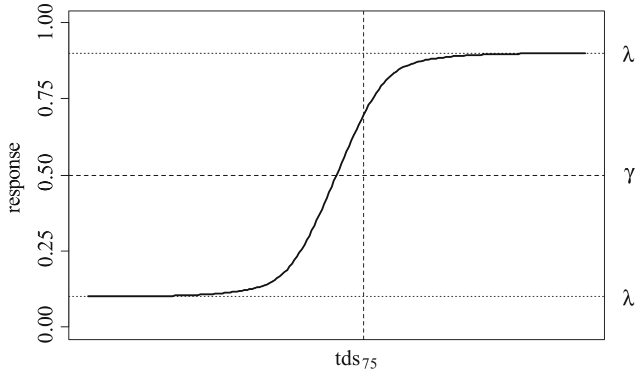
As more ants choose the trail with a higher concentration, more ants will deposit pheromones on that trail increasing its pheromone concentration and generating a positive feedback loop. Using the Deneubourg model, Goss et al. (1989) explained how *Linepithema humile* select the shortest of two paths to a food source by depositing pheromones on their way to and from the food (shortest path experiments). Various modifications of the model have also been developed to explain other types of collective ant behaviors, for instance the selection of the richest food source (Beckers et al. 1992), the division of labor by caste specific response thresholds to stimuli like pheromones (Bonabeau et al. 1996) or how ants form paths of minimal length between nest and food (Vela-Pérez et al. 2013). The key elements of the model, however, remain the same; the amount of pheromone deposited in the environment contains information about the quality of the environment, ants respond probabilistically to different pheromone concentrations according to the above-mentioned choice function or slight modifications of it, and a positive feedback loop attracts the ants to the favorable environmental condition.

Psychophysical theory

Psychophysical theory was developed by G.T. Fechner (1860) based on the works of E.H. Weber (1834) to find a relationship between the strength of a physical stimulus and its sensory impressions (Klein 2001; Wichmann and Hill 2001; Kingdom and Prins 2010). It plays a major role in psychology and neurobiology. Its main subjects are humans but it has also been successfully applied to perception and behavior in primates, birds and insects (Britten et al. 1992; Sarris 2006; Dyer et al. 2008; Chittka et al. 2009; Sasaki et al. 2013; Akre and Johnsen 2014) and in decision-making processes (Kacelnik and Brito e Abreu 1998; Gold and Shadlen 2007). Weber (1834) discovered that the ability to discriminate between two stimuli depends on the ratio of the stimulus strengths, which is within certain limits independent of the absolute stimulus strengths (Weber's law). Fechner (1860) discovered later that the sensory impression of a physical stimulus is proportional to the logarithm of the stimulus strength, which is the reason why, for example, sound levels are measured on a logarithmic scale (decibel).



a) Psychometric function for detection



b) Psychometric function for discrimination

Fig. 1 Schematic examples of psychometric functions. In detection experiments (**a**), the psychometric function (*PF*) gives the probability that a stimulus is reported stronger (response) than a null-stimulus. It starts at 0.5 (both stimuli are zero or close to zero and cannot be distinguished). In discrimination experiments (**b**), the PF gives the probability that a stimulus is reported stronger (response) than a constant stimulus, which is larger than zero. Lapse rate (λ, *dotted horizontal line*), guess rate (γ, *dashed horizontal line*), 75 % detection threshold (t_{dt75}, *dashed vertical line*), 75 % discrimination threshold (t_{ds75}, *dashed vertical line*). Note that the 75 % threshold is the point at half the distance between guess rate and upper asymptote; thus, it may not be exactly at $p = 0.75$. *Solid lines* show the PF

In the context of the psychophysical theory, a mathematical framework has been developed that relates physical stimuli to sensory impressions. This framework incorporates the effect of noisy backgrounds that influence the ability to detect a signal and it gives clear mathematical definitions of sensory thresholds. One of the most useful mathematical tools is the *psychometric function* (PF), which describes the relationship between the probability of a positive response p to a stimulus and the stimulus strength x

$$p(x) = \gamma + (1 - \lambda - \gamma) \cdot F(x),$$

where is x denotes stimulus strength, λ is the guess rate, γ is the lapse rate, and $F(x)$ is the function describing the probability to detect a stimulus by the underlying sensory mechanism (Kingdom and Prins 2010 p. 74). For $F(x)$, a probability distribution like the Weibull distribution is applied (see Online Appendix A).

The psychophysical theory explains the probabilistic nature of the response to a stimulus by incorporating the influence of the random fluctuations of internal and external noise. It is assumed that a stimulus will only be clearly detected when the sensory impression produced by the stimulus exceeds a certain internal sensory threshold that is clearly above random noise. This assumption is called the *high threshold assumption*. The sensory threshold is usually defined as the stimulus strength that produces a correct response in 75 % of the cases. This is assumed to be well above the influence of noise. In the context of the psychophysical theory, other important explanations of the probabilistic nature of sensory impressions exist, for instance the *detection theory* (Kingdom and Prins 2010, p. 74ff).

There are two important classes of psychophysical experiments: (1) In *detection experiments*, the response to a varying test stimulus compared to a null reference stimulus is measured and (2) in *discrimination experiments*, the response to a varying test stimulus compared to a fixed reference stimulus is measured. *Response* is defined as the probability to detect the stimulus as stronger than the reference (null or fixed) stimulus.

The parameters that define the PF are the *slope* (b), the *threshold* (t), the *lapse rate* (λ) and the *guess rate* (γ). The threshold defines the inflection point of the PF. In addition, the PF is used to define and measure the 75 % detection threshold (t_{dt75}) and the 75 % discrimination threshold (t_{ds75}), which are usually defined as the stimulus strength at which 75 % of the responses are correct. For a precise definition, see Online Appendix A. In detection experiments, the 75 % detection threshold is taken as a measure for the smallest detectable stimulus. Similarly, in discrimination experiments the 75 % discrimination threshold is taken as a measure for the smallest noticeable difference or contrast. The *lapse rate* defines the errors that the test subjects make. According to psychophysical theory, the lapse rate is influenced by the motivation or attentiveness of the test subjects, by disturbances or by learning effects, which are caused by repeating the measurements with the same test subject. The *guess rate* defines the response probability when the test subjects cannot detect the stimulus and have to guess.

It has been shown that Weber's law does not hold in regions of low and high stimuli. A simple way to describe this effect is to add a small constant k_{pf} to the stimulus strength (Stevens 1957). Other and more sophisticated modifications of the PF are known (e.g., Stevens 1957).

Applying the psychophysical theory to models of collective ant behavior

The Deneubourg model was able to explain important aspects of the collective behavior of ants by assuming that the decision of each single ant follows simple stochastic rules that can be described by the Deneubourg choice function. It did not give further biological explanations of the underlying stochastic nature of ant decisions. To fill in this gap, we propose that the perception of a stimulus (pheromone) by ants follows the principles of psychophysical theory and this determines their decisions. The theory provides a well-established framework for the stochastic nature of perception and behavior, which can be described by a PF. The function describes not only the relationship between pheromone concentrations and the resulting decision probabilities, but it also considers factors like noise, error rate and sensory and behavioral thresholds, and thus provides parameters which can be useful for understanding biological systems. For a more detailed interpretation of these parameters, see "Discussion" section.

The model can be tested experimentally by measuring trail following behavior of ants at a bifurcation of trails with varying pheromone concentrations. We hypothesized that the decision probability follows a PF and Weber's law is fulfilled, at least within certain concentration ranges.

Materials and methods

Species used

Experiments were conducted with queenright colonies of *Euprenolepis procera*, *Linepithema humile* and *Lasius niger*. The three species are distinct in several respects: *L. niger* and *E. procera* belong to different genera of the same subfamily Formicinae, but they have different habitats and life styles. *L. humile* belongs to the subfamily Dolichoderinae and again differs considerably in its habitat and life style.

E. procera (Formicinae) lives in the tropical rainforests of Malaysia and Indonesia. It is the only ant species known to feed entirely on mushrooms (Witte and Maschwitz 2008). Colonies are polygynous and consist of 500–20,000 individuals. The ants forage only at night and induce mass recruitment when they discover freshly grown mushrooms in their environment. They establish and maintain a trail system that leads from the nest to different mushroom sites and they follow these trails with high fidelity. Trail sections are abandoned when a food source is exhausted. However, when mushrooms regrow after some time at a previously exploited site, the ants reactivate abandoned trails with high fidelity (von Beeren et al. 2014). The trails can lead over distances of ca. 5 m through very uneven material (leaves, litter, wood, etc.), the ants do not clear the trails of obstacles as other ant species do and no light penetrates to the rainforest floor at night. Therefore, the reactivation of trails probably depends on long-lasting trail pheromones since optical and/or sensorimotor orientation is very unlikely. Ant colonies were collected at the field station in Ulu Gombak, Malaysia, and kept in plastic boxes (50×33×21 cm) under controlled conditions in a climate chamber (24 °C and humidity 85 %). Ant colonies were fed with half a mushroom (*Agaricus bisporus*) twice a week. Ants were starved 5 days prior to the experiments. We conducted experiments on three colonies that each contained about 5,000 individuals. Trail pheromone extracts were prepared from the rectum. In preceding experiments, the rectum was identified as the source of trail pheromone(s). In these tests, the trail effects of Dufour gland, poison gland and rectum extracts were tested with three different bioassays and results showed that only the extract from the rectum induced significant trail following (Witte, unpublished data).

L. humile (Dolichoderinae) originates from Argentina. It is invasive in most continents (Wetterer et al. 2009) and forms extensive polygynous supercolonies (Giraud et al. 2002). The ants maintain contact between different nests by frequently exchanging brood and workers and by internest recruitment (Holway and Case 2000). *L. humile* is a dietary generalist; it uses locally stable and persistent food sources like honeydew (which they gain via trophobiosis from aphids) but also distributed and short-lived food sources like insects, seeds or carrion (Mallis 1942; Suarez et al. 1998). Trail following of

L. humile depends mainly on pheromones and to a lesser extent on optical orientation (Aron et al. 1993). The ants were collected in Port Leucate, southern France. They were kept in tightly sealed plastic boxes (32×20×15 cm) under controlled conditions in a climate chamber (22 °C and 65 % humidity). Laboratory nests were supplied with a droplet of honey and half a cricket twice a week. Ants were starved 5 days prior to the experiments. We used five colonies each containing about 1,000 individuals. Trail pheromones were extracted from Pavan's gland. Various studies (Wilson and Pavan 1959; Robertson et al. 1980) as well as our own experiments (Witte, unpublished data) have shown that this gland is the source of pheromones that elicit a trail-following response. (*Z*)-9-Hexadecenal, a molecule produced in the Pavan's gland, has been suggested as a main component of the trail pheromone complex (Cavill et al. 1979, 1980; Van Vorhis Key and Baker 1982). However, (*Z*)-9-hexadecenal was analytically not detectable in natural trails, whereas two components of the pygidial gland (dolichodial and iridomyrmecin) were detectable and showed a trail following effect when the mixture was provided as a linear trail (Choe et al. 2012). We therefore included pygidial gland extract in one of our experiments with *L. humile*. In our analysis we also included the data of a behavioral assay done with ants from California (USA) using synthetic (*Z*)-9-hexadecenal (Choe et al. 2012).

L. niger (Formicinae) is one of the most common ants in Europe. It builds monogynous colonies with an average size of 14,000 individuals (Seifert 2007). The ants feed opportunistically on insects and they build long-lasting trails to trophobiosis sites, where they collect honeydew. They are active at day and night but in summer, their peak of activity is during the night (Seifert 2007). Besides pheromones, *L. niger* ants are reported to use memory and optical cues to a great extent for trail following (Aron et al. 1993; Evison et al. 2008; Czaczkes et al. 2011; Grüter et al. 2011). Parts of five colonies, each consisting of about 5,000 individuals, were collected close to the biocenter of the Ludwig Maximilians University in Planegg, Germany. They were kept in plastic boxes (32×20×15 cm) under controlled conditions in a climate chamber (22 °C and 65 % humidity). Laboratory nests where supplied with a droplet of honey and half a cricket twice a week. Five days prior to the experiments, the ants were starved. The trail pheromones were extracted from the rectum (Bestmann et al. 1992).

Experimental setup and behavioral assay

A plastic box containing one colony was brought into the experimental arena (see Online Appendix E). The box was connected via bridges to a food source. The bridges consisted of paper strips. After 20–30 min, the ants had usually discovered the food and established a trail between nest and food source. The food was half a mushroom for *E. procera* and a drop of honey and half a cricket for both *L. humile* and *L. niger*. For the experiment, a section of the trail was exchanged with a Y-shaped bridge carrying different concentrations of pheromones (see Online Appendix, Table B3). Ten ants altogether crossing the branches were counted. The Y-bridge was then exchanged for a new bridge to minimize influences due to pheromone deposition by ants. Usually ten bridges were used per treatment, resulting in 100 ant decisions. The proportion of ants crossing a branch gives a measure for the probability of the ants' decision. In all experiments, one trail was defined as *test trail* and the other as *reference trail*. We refer to the probability of the ants' decisions as p_{Dt} and p_{Dr} for test trail and reference trail, respectively; the pheromone concentration is referred to as c_{Pht} and c_{Phr}. A cardboard box was placed around the sides of the experimental arena to reduce the ants ability to orient and/or learn based on optical cues. In experiments with *E. procera*, light was dimmed to a minimum to promote activity since *E. procera* is only active during the night. To even out effects of optical and/or sensorimotor learning, the orientation of test trail and reference trail were switched each time a new Y-bridge was applied. We assumed that by altering the sides, possible memory effects were equally likely for test- and reference trail and thus eliminated each other. To make sure that only those ants were evaluated that were following the pheromone trail towards the food source and that were taking a clear choice, ants were only counted when they followed the trail for at least 2 cm before reaching the bifurcation and when they crossed the bridge completely. The level of ant travel on the bridge was controlled by temporarily removing a straight part of the bridge to slow down the ant flow so that it was always possible to follow individual ants' decisions. Each experiment started with pheromone concentration 0 as the control.

Preparation of pheromone and application

Ants were frozen at −20 °C for 7 min. Pheromones were then extracted by dissecting the pheromone sources in 50 μl dichloromethane (DCM, 99.9 %, Roth, Art.Nr. 7334.1). Following this step, the solution was separated from the gland tissue by extracting the liquid with a syringe (Hamilton Microliter glass syringe, 700 series, 100 μl). The liquid was further diluted with DCM to a standard solution. The number of glands extracted depended on the amount and concentration of pheromone needed for each experiment and was calculated in advance of each experiment. At least ten glands were extracted and diluted in 1 ml solvent for experiments with *E. procera* and *L. niger* and at least five glands were extracted and diluted in 2 ml solvent for experiments with *L. humile*. The standard solution was used to gain a dilution series with a constant solution factor of two, giving 125 μl of pheromone solution for each concentration in a dilution series.

The pheromone trails for the experiments were prepared by applying the pheromone solutions to Y-shaped bridges of paper (see Online Appendix E). The pheromone was applied on the straight part of the bridge and on one of the two other arms using a micropipette (10 μl, BRAND) to give a total trail length of 7 cm. The bridges were used for the experiments within 2–5 min after pheromone application. For each bridge, we used 4 μl of the solution. The measures used in this article are defined in Table 1.

Estimation of realistic concentration ranges

Physiologically realistic concentration ranges of pheromones were estimated prior to our experiments. This was done by comparing the ants' response to natural trails and artificial trails of different pheromone concentrations. Based on this estimation, we defined for each species a *base concentration* (*bc*) that we used for all our experiments. It was 0.0057 AE/cm for *E. procera* and *L. niger* and 0.0014 AE/cm for *L. humile* (for a detailed description, see Online Appendix F). All pheromone concentrations we report are given relative to the base concentration with 1 bc as unit. Note that we expect the pheromone concentration on natural trails to vary greatly within species (see, e.g., Beckers et al. 1992; Choe et al. 2012) and the bc is only an estimation that can be expected to be close to naturally occurring pheromone concentrations. All our experiments go far below and above the bc, and they are designed in a way that the results are independent of the absolute pheromone concentration of the bc. The 75 % detection threshold of the PF is the only exception since it is given in bc units. For (*Z*)-9-hexadecenal that had been previously investigated (Choe et al. 2012), we were able to give absolute quantities of pheromone per cm of trail.

Experiment 1: detection

In this experiment, we tested the ants' ability to detect different pheromone concentrations and we evaluated the 75 % detection thresholds. One side of the Y-bridges was always treated with pure DCM and without pheromone. The other side of the Y-bridges was treated with a series of pheromone concentrations.

For *L. humile* we used different pheromone sources: as described in section "Species used", the extract of Pavan's gland was reported to contain the trail pheromones of *L. humile*. We used this extract in our experiments. In addition, the compounds from the pygidial gland and the synthetic pheromone (*Z*)-9-hexadecenal have been reported to elicit trail following of *L. humile* (see section "Species used"). To determine if the pygidial gland extract shows a trail effect, we conducted the detection experiments with pygidial gland extract as well. To compare the results between Pavan's gland and (*Z*)-9-hexadecenal, we included the data from detection experiments of Choe et al. (2012) with *L. humile* and (*Z*)-9-hexadecenal.

Experiment 2: discrimination

In this experiment, we tested the ants' ability to discriminate between two trails of different concentrations and evaluated the 75 % discrimination thresholds. As in the detection experiment, one side of the Y-bridge was treated with a series of pheromone concentrations. The other side of the Y-bridge was used as reference trail, and applied with a constant pheromone concentration (1 bc).

Experiment 3: concentration dependency

The hypothesis that the decisions of the ants in discrimination experiments do not depend on the absolute pheromone concentration within certain concentration ranges (and thus fulfill Weber's law) was tested in three different ways (see below).

Experiment 3a: absolute concentration dependency of fixed concentration ratio

This experiment was similar to the discrimination experiment described above, but the concentration ratio between both trails was held constant at 0.5 over a wide range of different concentrations of up to 2^{14} bc units. It was not possible to test higher concentrations due to the high numbers of ants that would have to be dissected to accomplish this. The trail with the lower pheromone concentration was defined as test trail.

Experiment 3b: absolute concentration dependency of varying concentration ratios

We repeated the discrimination experiment of *E. procera* using a 2.5 times higher base concentration than before. In this way, we gained a second set of the same concentration ratios but with absolute concentrations 2.5 times higher. This experiment was conducted only with *E. procera*.

Experiment 3c: test of symmetry

We reevaluated the data of the discrimination experiment with a different method: we used the fact that the concentration ratios of the discrimination experiments are symmetric around 1. For instance, the ratio 0.25:1 between test- and reference trail in the lower half of the series is the same as the ratio 4:1 in the upper half of the series if test trail and reference trail are exchanged. In this way, we gained a series of pairs of the same concentration ratios at different absolute concentrations, which we compared.

Table 1 Measures used in this article

Measure	Symbol	Equation	Unit
Length of pheromone trail	t_l	–	cm
Volume of solvent	v_S	–	ml
Volume of pheromone solution	v_{Ph}	–	ml
Ant-equivalent	AE	–	gland
Pheromone concentration	c_{Ph}	$c_{Ph} = \frac{AE}{v_S}$	AE/ml
Trail concentration	T_c	$T_c = \frac{c_{Ph} \cdot v_{Ph}}{t_l}$	AE/cm

Statistical methods and model fitting

Software

We used the software "R", Version 2.14.2 (R Development Core Team 2013). For a list of R-libraries being used, see Online Appendix D.

Calculation of results and their confidence intervals

We fitted binomial distributions to the outcome of each experiment since the ants made binary decisions. The probability that the ants chose the test trail (p_{Dt}) and their 95 % confidence limits were estimated with the R-function binom.confint() using the Wilson method (Wilson 1927; Dorai-Raj 2009).

Model evaluation

To fit the parameters of the models that we evaluated, we used the optimize() function in R with the method "L-BFGS-B" (Byrd et al. 1995) which is able to fit more than one parameter at a time. To calculate the goodness-of-fit (GOF) of the models, we performed a log-likelihood ratio test between the model being evaluated and the full model (Hilborn and Mangel 1997, Chapter 7). In addition, we calculated the 95 % confidence limit of the model parameters, the predicted values and a 95 % prediction interval. These were evaluated using parametric bootstrapping with 1,000 simulated parameter values for each parameter (Efron and Tibshirani 1986; Kingdom and Prins 2010, p. 98 ff.).

Comparing models

We compared the PF and the Deneubourg choice function in their ability to fit our experimental data. Since these models are non-nested, we assessed the significance of differences in model fit by comparing the Akaike information criterion (AIC) of the models and by parametric bootstrapping (Efron and Tibshirani 1986; Hilborn and Mangel 1997 chapter 7; Kingdom and Prins 2010 chapter 8). For a detailed description, see Online Appendix G.

Applying the model to external data

To test repeatability and therefore the general value of the PF, we modeled data that had been collected for *L. humile* and synthetic (*Z*)-9-hexadecenal in another laboratory at a distant location (California, USA) (Choe et al. 2012). If the PF is species-specific, we expected similar parameter estimates regardless of location.

Results

General remarks

Due to the large amount of data, we present here only selected results. First, we present the data fitted to the PF with the detection experiments, followed by the discrimination experiments, followed by the concentration dependency experiments (Figs. 2, 3, 4, and 5). This part is followed by a section in which we present the results of a model comparison between the PF and the Deneubourg choice function. It includes the results of the detection and discrimination experiments fitted to the Deneubourg choice function (Fig. 6). Confidence limits are always given for *E. procera*, but they are omitted for the other species if this improved clarity of the figures (confidence limits of the other species are of similar magnitude). The online Appendix contains figures including confidence limits and detailed results for all experiments and species tested in the same order as above. Measurement series were usually made over a period of several days and with different colonies because foraging decreased after a certain time. Possible confounding effects were evaluated by including *date* and *colony* as factors in generalized linear models using the R-function glm() and drop1() and in generalized linear mixed effects models using glmer(). We found only minor side effects of the factors date and colony. For detailed results, see Online Appendix, Table B4.

Results of experiments

The results of the detection experiments and discrimination experiments show a clear relationship between pheromone

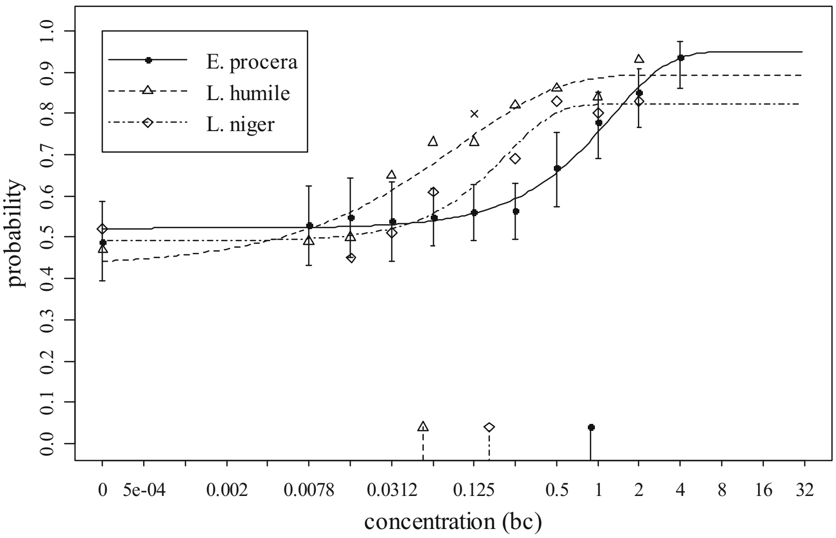


Fig. 2 Detection experiment fitted with the psychometric function. The symbols (*filled circle*, *empty triangle*, *empty diamond*) show the probability that ants take the test trail (response) and the bars show the 95 % confidence interval (only shown for *E. procera*). The *x*-axis shows the pheromone concentration of the test trail measured in base concentrations (*bc*) on a $\log_2$ scale. The *lines* show the fitted psychometric functions. The goodness-of-fit (*GOF*) was evaluated using a log likelihood ratio test, see section "Model evaluation". The symbols at the bottom indicate the 75 % detection thresholds. The *cross* (×) indicates an outlier for *Lasius niger.* *GOF* goodness-of-fit, *bc* base concentration, *b* slope, t_{dt75} 75 % detection threshold, λ lapse rate, *N* total sample size, *n* number of measurements per concentration (each measurement represents ten binomial ant decisions). *E. procera*: GOF=0.96, bc=0.0057 AE/cm, b=1.05 (range 0.72–1.63), t_{dt75}=0.89 bc (range 0.62–1.2 bc), λ (lapse rate)=0.05 (range 0.03–0.07), N=138, n=10/10/10/10/20/20/20/10/10/10/8 (from *left* to *right*). *L. humile*: GOF=0.25, bc=0.0014 AE/cm, b=0.6 (range 0.42–1.08), t_{dt75}=0.05 bc (range 0.04–1.11 bc), λ=0.11 (range 0.09–0.13), N=100, n=10 (each). *L. niger*: GOF=0.39, bc=0.0057 AE/cm, n=80, b=1.21 (0.6–3.86), t_{dt75}=0.16 bc (range 0.08–0.25 bc), λ=0.18 (range 0.15–0.2), N=80, n=10 (each)

concentration and response in all experiments ($p<0.001$, GLM) (see Online Appendix, Table B1). The fitted PFs as well as the Deneubourg choice functions gave a GOF >0.05 in all cases (see Online Appendix, Tables B1 and B2). The fitted

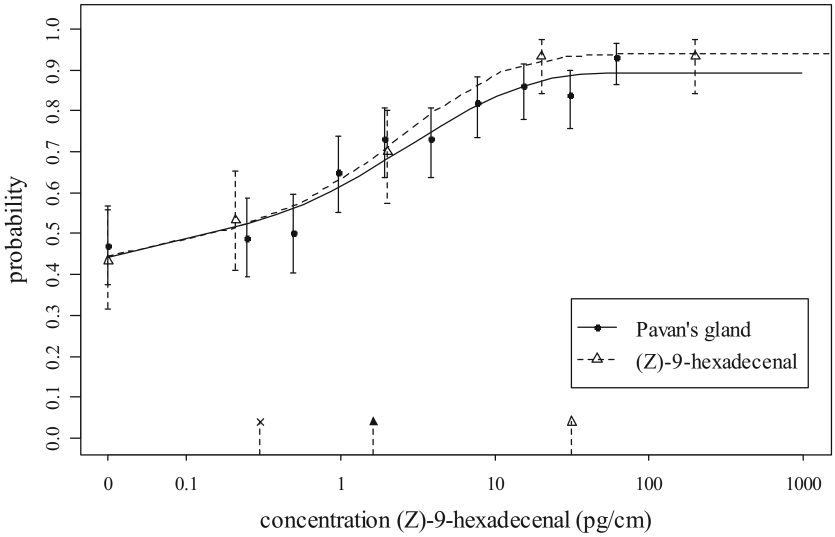


Fig. 3 Detection experiments with *L. humile* using Pavan's gland extract and (*Z*)-9-hexadecenal fitted with the psychometric function. The symbols (*filled circle*, *empty triangle*) show the probability (response) that ants take the test trail and the bars show the 95 % confidence interval. The *filled triangle* at bottom indicates the 75 % detection threshold that was set as equal for both experiments (see text). The *solid line* shows the fitted function for Pavan's gland. The *dashed line* shows the fitted function for synthetic (*Z*)-9-hexadecenal. The *cross* (×) at bottom shows the lower limit of the analytical method to detect (*Z*)-9-hexadecenal on trails, the *empty triangle* shows the bc of the measurement with Pavan's gland. The GOF between both fitted functions is 0.61. *GOF* goodness-of-fit, *bc* base concentration, *b* slope, t_{dt75} 75 % detection threshold, λ lapse rate, *N* total sample size, *n* number of measurements per concentration (each measurement represents ten binomial ant decisions for Pavan's gland and five decisions for (*Z*)-9-hexadecenal). Pavan's gland: GOF=0.25, bc=0.0014 AE/cm (=30.8 pg/cm), b=0.6 (range 0.42–1.08), t_{dt75}=0.05 bc (range 0.04–1.11 bc), λ=0.11 (range 0.09–0.13), N=100, n=10 (each). (*Z*)-9-Hexadecenal: GOF=0.64, b=0.68 (range 0.39–2.34), t_{dt75}=1.64 pg/cm (0.62–3.96 pg/cm), λ=0.06 (range 0.02–0.1), N=60, n=12 (each)

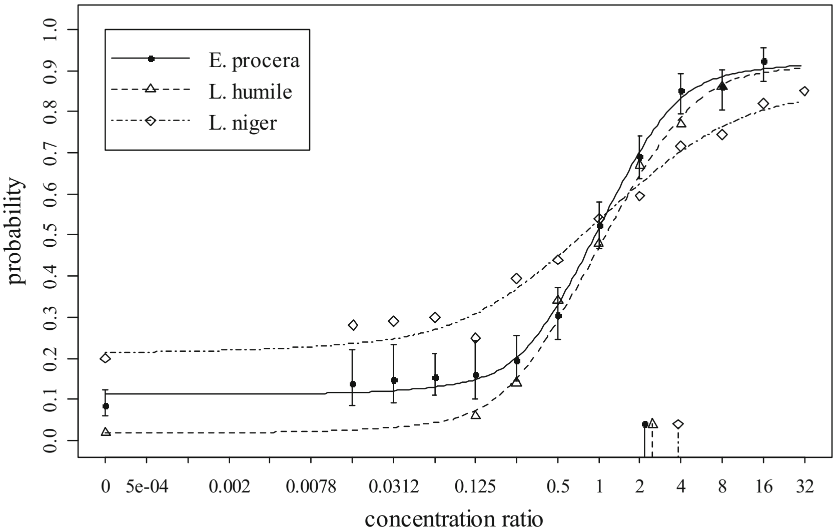
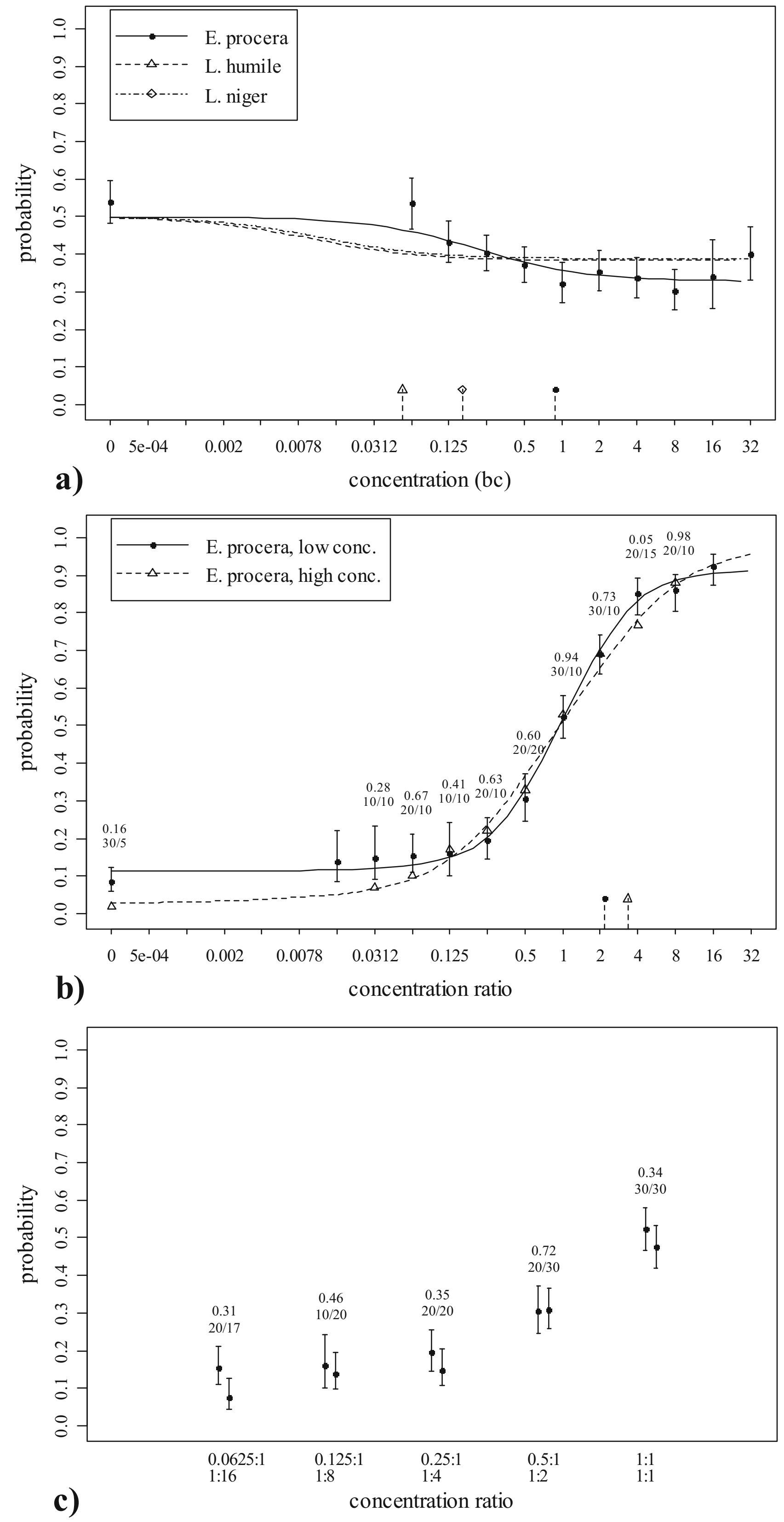


Fig. 4 Discrimination experiments fitted with the psychometric function. The symbols (*filled circle*, *empty triangle*, *empty diamond*) show the probability that ants take the test trail (response) and bars show the 95 % confidence interval (only shown for *E. procera*). The *lines* show the fitted psychometric functions. The x-axis shows the concentration ratio between test trail and reference trail on a $\log_2$ scale. The reference trail had a constant concentration of 1 bc. The test trail had varying concentrations measured in proportions of bc. The symbols at the bottom indicate the 75 % discrimination thresholds. *GOF* goodness of fit, *bc* base concentration, b slope, t_{ds75} 75 % discrimination threshold, λ lapse rate, N total sample size, n number of measurements per concentration (each measurement represents ten binomial ant decisions). *E. procera*: GOF=0.55, bc=0.0057 AE/cm, b=1.0 (range 0.82–1.23), t_{ds75}=2.14 (range 1.85–2.52), λ=0.11 (range 0.09–0.14), N=237, n=30/10/10/20/10/20/20/30/30/20/20/17 (from *left* to *right*). *L. humile*: GOF=0.86, bc=0.0014 AE/cm, b=0.87 (range 0.75–1.01), t_{ds75}=2.48 (range 2.16–2.85), λ=0.02 (range 0–0.05), N=80, n=10 (each). *L. niger*: GOF=0.57, bc=0.0057 AE/cm, b=0.56 (range 0.4–0.79), t_{ds75}=3.82 (range 2.59–6.49), λ=0.21 (range 0.15–0.25), N=210, n=20/10/10/10/20/20/20/20/20/20/20/10/10 (from left to right)

functions showed a typical S-shape with horizontal asymptotes at the lower and upper part. In detection experiments, the lower horizontal asymptotes lay at $\approx$0.5. At this concentration, the trails were taken randomly with equal chance. At higher concentrations, the fitted functions converged towards horizontal asymptotes that lay between 0.79 and 0.98. These asymptotes were especially low in *L. niger*. The 75 % detection thresholds for all species fell in a region in which the responses were rising. This indicates that our choices for the base concentrations were reasonable and that our measurements were in a region in which the ants showed a clear and differentiated response. In many cases, especially at lower concentrations, not only the fitted functions but also the data showed a horizontal asymptote. The thresholds and the positions of the curves parallel to the x-axis cannot be directly compared since we do not know absolute pheromone concentrations (see section “Model evaluation”). The differences between using Pavan’s gland as pheromone source for *L. humile* from Southern France and (*Z*)-9-hexadecenal as pheromone source for *L. humile* from USA were only minor (see Fig. 3). To make this comparison, we assumed that the t_{dt75} of the experiments with Pavan’s gland extract is equal to the t_{dt75} of (*Z*)-9-hexadecenal. By doing so, we were able to rescale the data for Pavan’s gland extract to absolute concentrations (1 bc=30.8 pg/cm). This assumption is reasonable since this pheromone is regarded as the active trail pheromone of *L. humile*, known to be produced in Pavan’s gland (see section “Species used”) and thus should produce similar responses. The assumption only holds if no other trail pheromones modulate or amplify the effect. In contrast to Pavan’s gland extract and (*Z*)-9-hexadecenal, the detection experiments with pygidial gland extract did not show trail following responses in *L. humile* (see Online Appendix, Fig. C1c).

In discrimination experiments (see Fig. 4), the fitted functions and data show horizontal asymptotes at lower concentrations. At these concentrations, the majority of ants followed the reference trail. When both trails carried equal amounts of pheromone, the decisions were taken with equal chance at $p_{Dt}\approx 0.5$. At higher concentrations, the fitted functions showed a horizontal asymptote below 1. As in detection experiments, it was lowest for *L. niger*. For *E. procera*, we repeated the same experiment at concentrations 2.5 times higher than before (see Fig. 5b). The results were similar, but the lower asymptote was closer to 0. In contrast to the detection experiments, the discrimination thresholds can be directly compared between the species. Comparing the thresholds between the species, we found that the threshold values were very similar for *L. humile* and *E. procera* while the threshold in *L. niger* was higher (see Fig. 4). Comparing the thresholds between the lower and the higher concentration series in *E. procera*, we found that the higher series shows a higher threshold (see Fig. 5b).

In detection and discrimination experiments, the fitted exponents were always <2 and in most cases $<\approx 1$ in the PF as


E. procera
L. humile
L. niger
probability
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
0 5e-04 0.002 0.0078 0.0312 0.125 0.5 1 2 4 8 16 32
a)
concentration (bc)
E. procera, low conc.
E. procera, high conc.
0.16 30/5
0.28 10/10
0.67 20/10
0.41 10/10
0.63 20/10
0.60 20/20
0.94 30/10
0.73 30/10
0.05 20/15
0.98 20/10
b)
concentration ratio
0.31 20/17
0.46 10/20
0.35 20/20
0.72 20/30
0.34 30/30
0.0625:1 1:16
0.125:1 1:8
0.25:1 1:4
0.5:1 1:2
1:1 1:1
c)
concentration ratio

◀ **Fig. 5** Concentration dependency experiments. The symbols (*filled circle*, *empty triangle*) show the probability that ants take the test trail (response) and bars show the 95 % confidence interval (only shown for *Euprenolepis procera*). **a** Absolute concentration dependency of fixed concentration ratio. The concentration ratio between test and reference trail was constant (0.5), while the absolute concentration varied. The concentration of the reference trail was always twice the concentration of the test trail. *Lines* show the fitted psychometric functions (for *Linepithema humile* and *Lasius niger*, they are shown only as *dashed lines* without symbols for data and confidence intervals). The symbols at the bottom (*filled circle*, *empty triangle*, *empty diamond*) indicate the 75 % detection thresholds from the detection experiments. *GOF* goodness of fit, *bc* base concentration, *b* slope, k_{pf} constant, *N* total sample size, *n* number of measurements per concentration (each measurement represents ten binomial ant decisions). *E. procera*: GOF=0.15, bc=0.0057 AE/cm, $b=0.8$ (range 0.65–1.0), $k_{\mathrm{pf}}=0.34$ (range 0.12–0.85), $N=306$, $n=30/20/30/40/40/30/30/30/28/10/18$ (from *left* to *right*). *L. humile*: GOF=0.34, bc=0.0014 AE/cm, $b=0.52$ (range 0.41–0.64), $k_{\mathrm{pf}}=0.02$ (range 0.0–0.06), $N=150$, $n=10$ (each). *L. niger*: GOF=0.08, bc=0.0057 AE/cm, $b=0.5$ (range 0.4–0.63), $k_{\mathrm{pf}}=0.02$ (range 0.0–0.09), $N=151$, $n=10/10/10/10/10/20/10/20/10/10/10/21$ (from left to right). **b** Absolute concentration dependency of varying concentration ratios for *E. procera*. The discrimination experiment was carried out twice with absolute concentrations that differed by a factor of 2.5 between both measurement series. By pairwise comparison of the same concentration ratios of the two series (e.g., 0.125), it can be tested, if the responses are different and thus depend on the absolute concentrations. *Solid line* shows the low concentration series (1 bc=0.0057 AE/cm). *Dotted line* shows the 2.5 times higher concentration series (1 bc=0.01425 AE/cm). The *number above bars* gives the *p* value and sample size of two-sample Wilcoxon test for pairs of equal concentrations. *GOF* goodness-of-fit, *bc* base concentration, *b* slope, t_{ds75} 75 % discrimination threshold, λ lapse rate, *N* total sample size. *E. procera*, low concentration: GOF=0.55, bc=0.0057 AE/cm, $b=1.0$ (range 0.82–1.23), $t_{\mathrm{ds75}}=2.14$ (range 1.85–2.52), $\lambda=0.11$ (range 0.09–0.14), $N=237$. *E. procera*, high concentration: GOF=0.89, bc=0.01425 AE/cm, $b=0.63$ (range 0.54–0.75), $t_{\mathrm{ds75}}=3.33$ (range 2.77–4.07), $\lambda=0.03$ (range 0.0–0.05), $N=110$. **c** Test of symmetry, *E. procera* (low concentration series). Shown are pairs of same concentration ratios from the results of the discrimination experiment. For example, the concentration ratio 0.25:1 is the same as the concentration ratio 1:4. Both differ by a factor of 4 in absolute concentrations. By comparing the pairs of equal concentration ratios, it can be tested, if the responses are different and thus depend on the absolute concentrations. *Number above bars* give *p* value and sample size of two-sample Wilcoxon test. For further details, see text

well as in the Deneubourg choice function (see Online Appendix, Tables B1 and B2).

The concentration dependency experiments (Fig. 5) showed that the response to pheromone concentration ratios at a bifurcation is independent from the underlying absolute pheromone concentrations within certain lower and upper concentration limits. In experiment 3a (Fig. 5a), we used constant concentration ratios of 0.5 and varied the absolute pheromone concentrations. The probability to choose the test trail started at $p_{\mathrm{Dt}}\approx 0.5$ at the lower concentration range. With rising concentrations it dropped and became asymptotic at $p_{\mathrm{Dt}}\approx 0.33$ (*E. procera*) and $p_{\mathrm{Dt}}\approx 0.39$ (*L. humile* and *L. niger*). For *E. procera*, the measurements showed similar results in a concentration range between 1 and 16 bc (0.0057–0.0912 AE/cm), spanning a region that differs by a factor of 16 between the lowest and highest concentrations. For *L. humile*, this region spans from 0.0156 to 32 bc (0.000022–0.5 AE/cm), differing by a factor of 2,048 with an outlier at 4 bc (see Online Appendix, Fig C3a). For *L. niger*, it spans from 0.0625 to 4 bc (0.00036–0.023 AE/cm), differing by a factor of 64 with two outliers at 0.5 and 2 bc (see Online Appendix, Fig C3b). We fitted the model with the additional parameter k_{pf} (see section "Psychophysical theory"). Note that, for all experiments, the perceptive range likely spans much further into regions of high concentrations, which could not be tested.

In experiment 3b, we compared two discrimination experiments with *E. procera* that differed in absolute concentrations by a factor of 2.5 (see Fig. 5b). We made a pairwise comparison between equal concentration ratios. The results of the different concentrations ratios from 0 and up to 8 were similar and showed no statistically significant difference ($p>0.05$, two-sided Wilcoxon test). Thus, the region of similar results spans a concentration region that spans a region from 0.03125 up to 8, which differ by a factor of 256. In contrast to experiment 3a, these results contain not only one single concentration ratio (0.5) but also other concentration ratios.

The test of symmetry (Experiment 3c) showed similar results. We made pairwise comparisons with concentration ratios of the lower and upper half of the discrimination experiment for *E. procera* (see Fig. 5b). Again, the pairs showed no statistically significant difference. The results for the other species are similar (see Online Appendix, Fig. C4).

Results of model evaluation

To explain our data, we investigated two models: one is based on Weber's law and the psychophysical theory with the PF (see section "Psychophysical theory") and the other one is the Deneubourg model with the Deneubourg choice function (see section "Deneubourg model").

Psychometric function

Figures 2, 3, 4, and 5b in the "Results" section contain the fitted PF as lines. The figures in Online Appendix C1 and C2 show the same for each species separately including prediction intervals. The PF fits the experiments with GOFs between 0.08 and 0.96. Table 2 shows the fitted parameters of the PF that provide important biological information. In addition, we define a measure for the *information capacity* (*IC*) an ant is able to transmit with the pheromone of a specific gland. We define it as the length of the trail an ant is able to mark with pheromone quantities that are detectable by other ants of the same species. The length of the detectable trail is equal to the amount of pheromone the ant carries in the gland divided by the amount of pheromone an ant needs to be able to detect it. This amount is proportional to the t_{dt75}. Thus the amount of

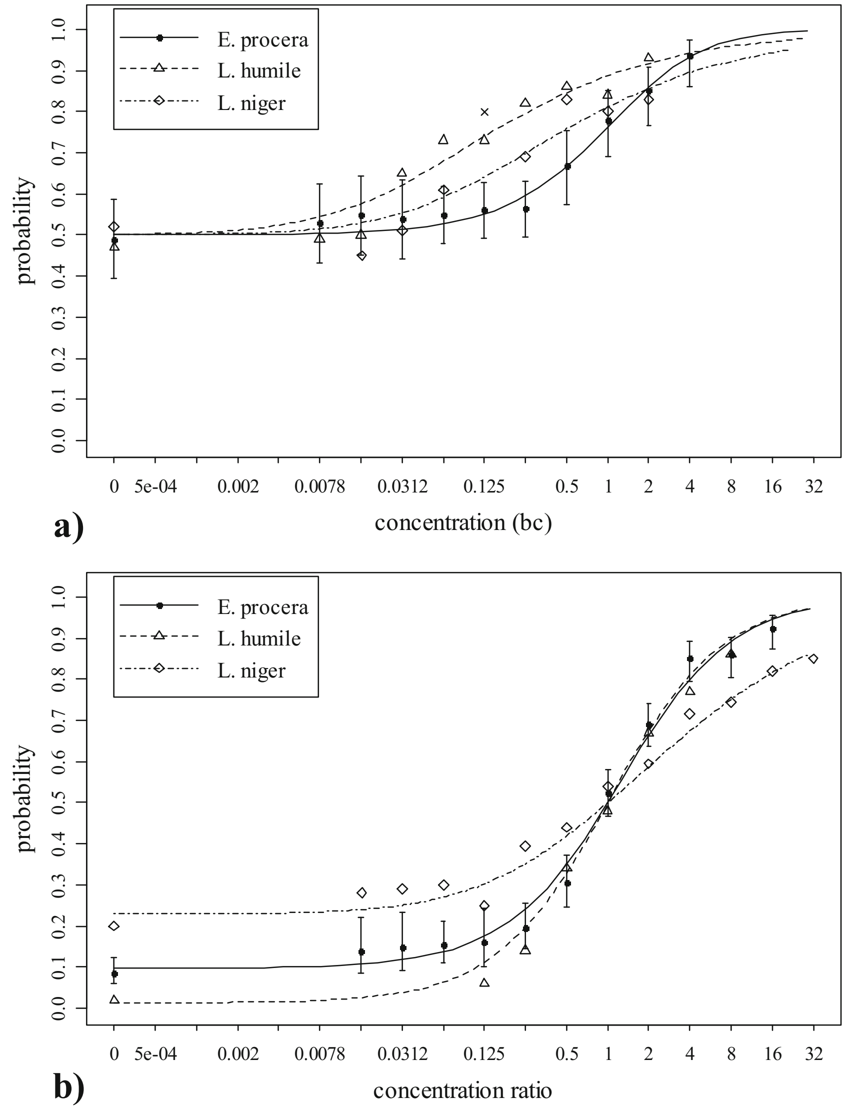


Fig. 6 Detection experiments (**a**) and discrimination experiments (**b**) for all three species fitted with the Deneubourg choice function. The *lines* show the fitted functions. For explanations, see Figs. 2 and 4. *GOF* goodness of fit, *bc* base concentration, *b* exponent, *k* constant, *N* total sample size, *n* number of measurements per concentration (each measurement represents ten binomial ant decisions). **a** *Euprenolepis procera*, detection: GOF=0.97, bc=0.0057 AE/cm, *b*=1.57 (range 1.16–2.54), *k*=0.91 (range 0.5–2.05), *N*=138, *n*=10/10/10/10/20/20/20/10/10/10/8 (from *left* to *right*). *Linepithema humile*, detection: GOF=0.41, bc=0.0014 AE/cm, *b*=0.52 (range 0.44–0.64), *k*=0.02 (range 0.01–0.04), *N*=100, *n*=10 (each). *Lasius niger*, detection: GOF=0.28, bc=0.0057 AE/cm, *b*=0.5 (range 0.4–0.65), *k*=0.06 (range 0.03–0.13), *N*=80, *n*=10 (each). **b** *E. procera*, discrimination: GOF=0.11, bc=0.0057 AE/cm, *b*=1.08 (range 0.96–1.23), *k*=0.15 (range 0.09–0.23), *N*=237, *n*=30/10/10/20/10/20/20/30/30/20/20/17 (from *left* to *right*). *L. humile*, discrimination: GOF=0.22, bc=0.0014 AE/cm, *b*=1.06 (range 0.96–1.19*)*, *k*=0.02 (range 0.0–0.06), *N*=80, *n*=10 (each). *L. niger*, discrimination: GOF=0.45, bc=0.0057 AE/cm, *b*=0.56 (range 0.49–0.65), *k*=0.13 (range 0.06–0.23), *N*=210, *n*=20/10/10/10/20/20/20/20/20/20/20/10/10 (from *left* to *right*)

information an ant is able to convey by depositing pheromone is inversely proportional to the t_{dt75}. In this way, we can use the t_{dt75} as a measure for the IC an ant is able to transmit. It is independent from the absolute concentration of the bc and thus a useful biological parameter (see Online Appendix A).

According to Bausenhart et al. (2012), the parameters of the PF may not depend solely on stimulus strength but also on additional factors like spatial orientation (left/right). One approach to account for this effect is to fit separate PFs and calculate the average of each parameter resulting in an averaged PF. There was a major difference between the averaged PFs and the pooled PFs only in the detection experiments with *E. procera* (averaged PF: t_{dt75}=0.58, IC=302) and *L. humile* (averaged PF: t_{dt75}=0.07, IC=9636).

Deneubourg model

We fitted the Deneubourg choice function to our data and calculated the GOF using the same methods as with the PF. The Deneubourg choice function fits our experiments with

Table 2 Psychometric measures of three ant species

Species	Experiment	75 % threshold	Information capacity (cm/gland)	Slope	Lapse rate
E. procera	Detection	0.89 bc	197	1.05	0.05
L. humile	Detection				
	Pavan's gland	0.05 bc	13,450	0.6	0.11
	Z9	1.64 pg/cm	11,185	0.68	0.06
L. niger	Detection	0.16 bc	1,094	1.21	0.18
E. procera	Discrimination				
	Low	2.1		1.0	0.11
	High	3.3		0.63	0.03
L. humile	Discrimination	2.5		0.87	0.02
L. niger	Discrimination	3.8		0.56	0.21

The 75 % threshold in discrimination experiments is dimensionless. In the case of *L. humile* and the pheromones (*Z*)-9-hexadecenal, we are able to give absolute concentrations in pg/cm for the 75 % detection threshold, in the other cases it is given in bc. Information capacity for *L. humile* with pheromones (*Z*)-9-hexadecenal are based on the average of two measurements of total quantity of pheromone per ant (see Choe et al. 2012)

GOFs between 0.08 and 0.97. Figure 6 gives the result for the detection and discrimination experiments. For further results, see Online Appendix, Table B2 and Figs. C5–C7.

Comparing models

Testing the alternative hypothesis, that the PF fitted the data better than the Deneubourg choice function gave no clear result if we compared the seven experiments separately. In three experiments, the PF showed a lower AIC with a maximum difference of 5.8, indicating a better fit. In four experiments the Deneubourg choice function showed a lower AIC with a maximum difference of 3.0. The parametric bootstrapping yielded a similar result with the PF showing a significantly better fit in three experiments.

Discussion

In our experiments, the ants clearly reacted to different pheromone concentrations and their decisions could be described both by the PF as well as the Deneubourg choice function. However, we propose using the PF as a decision rule in models on collective ant behavior since it gives the possibility for discussing the biological meaning of specific model parameters like sensory thresholds, lapse rate and IC. In addition, psychophysical theory provides a well-established framework for the explanation of the stochastic nature of responses by noise generated within the nervous system. By using the PF, the principles of the Deneubourg model, such as the explanation of the shortest path experiments, need not be changed. The underlying mechanisms remain valid, and only the calculation of choice probabilities changes. In this respect, our findings support the general approach of the Deneubourg model. An important finding is that the magnitude of nonlinearity expected by the Deneubourg model with exponents ≈2 could not be confirmed.

The comparison between the results for Pavan's gland extract tested with *L. humile* from southern France and synthetic (*Z*)-9-hexadecenal tested with *L. humile* from California (USA) shows that these two chemicals were similar in their influence on ant behavior. Furthermore, it demonstrates the repeatability and the general value of the psychophysical approach to describe ant behavior by showing that a PF based on *L. humile* colonies from one location is capable of predicting the behavior of other colonies of the same species even if they are clearly separate in time and space.

The results of experiments 3a (see Fig. 5a and Online Appendix Figs. C7b and c) show that the fitted functions follow a horizontal line at concentrations above the detection threshold. This shows that the ability of ants to discriminate different pheromone concentrations is independent of the underlying absolute pheromone concentrations within certain lower and upper concentration limits. The lower and upper limits differ by a factor of at least 16 in experiment 3a for *E. procera*, and up to a factor of at least 2,048 for *L. humile*. This shows that Weber's law is fulfilled within these limits in our experiments with three ant species. The very low concentrations were probably below the perception threshold so that the ants were not able to detect the trails and hence took the trails with equal chance ($p=0.5$). The fact that Weber's law is only valid within certain limits is well documented in psychophysics and can be described by adding a small constant (k_{pf}) to the pheromone concentrations (see section "Psychophysical theory"). With this modification, we obtain a GOF between 0.08 and 0.34 for the whole concentration series. Biologically, fulfilling Weber's law makes sense for ants since absolute pheromone concentrations may vary considerably due to collective pheromone deposition and environmental effects (temperature, rain, dew, substrate, etc.). Due to Weber's law, the information about different states of the environment, encoded in concentration ratios, remains stable and reliable even if the underlying absolute concentrations vary.

Recently, it has been shown in *L. humile* that the angle ants turn away from their current heading depends on the number of ants that had been close left and right to their current position in the past (Perna et al. 2012). This relationship also follows Weber's law. The authors assume that the pheromone concentration at a certain point is proportional to the number of ants passing that point. It follows that the response to concentration differences is independent from absolute concentrations and follows Weber's law (Perna et al. 2012). Our results confirm the assumptions made in that study.

To further discuss our findings with respect to differences between the biology of the three species, we first attempt a biological interpretation of the psychometric measures we defined, and then discuss these interpretations in relation to the ecology and specific behavior of the three species. According to psychophysical theory, the *lapse rate* defines the errors test subjects make due to low motivation, disturbances or learning. In classical psychophysical experiments, these factors are considered to bias the experiments and are kept as low as possible. In the case of ants, the lapse rate may provide important biological information since it can serve as a direct measure for trail fidelity. Ants might show a varying motivation to follow a trail due, for example, to different levels of starvation (Mailleux et al. 2011). Furthermore, they might incorporate other information into their decision process like visual orientation and learning. Such effects might influence the trail following fidelity and thus the lapse rate. The lapse rate in pheromone detection can be interpreted as a measure for the ants' ability to explore new solutions, avoid getting stuck and adapt to changing environments. Importantly, due to species-specific differences in learning ability, visual orientation and other traits, characteristic differences in lapse rates can be expected. The *discrimination threshold* gives a measure for the ability of ants to discriminate between trails of different pheromone concentrations. As described in "The models" section, the collective behavior of ants greatly depends on information encoded in different concentration ratios of the trail pheromones. In this way, the discrimination threshold can again be interpreted as a measure for the ability of ants to act collectively. While lapse rate and discrimination threshold are measures for the collective response of ants to pheromone trails, the *information capacity* can be interpreted as a measure of the ability of individual ants to change the collective behavior of the colony. If the IC is high, a single ant can deposit more information units in its environment and thereby modify the behavior of the colony to a greater extent compared to ants with a lower IC. The IC can also be taken as a measure for the collectiveness of trail laying. If ants have a low IC, it takes more ants to establish a trail system compared to ants that have a high IC. However, the IC cannot be interpreted on its own since the amount of pheromone an ant needs for conveying a message also depends on the volatility of the pheromone. Pheromones with a high volatility allow the ants to react to short-term changes in the environment but the ants need a comparatively high IC. On the other hand, long-lasting pheromones might tend to build up in higher concentrations on the trail as ants continue trailing. Under such circumstances, individual ants need higher amounts of pheromone (i.e., a higher IC) to lead other ants away from well-established trails and change the collective behavior. Here it is of interest to note that *E. procera* and *L. humile* responded to artificial pheromone trails even if they were 22 h old, while *L. niger* showed no significant reaction if the trails were more than 160 min old (W. von Thienen, unpublished data). Beckers et al. (1993) measured a mean lifetime of trail pheromone of ca. 47 min for *L. niger*; however, Evison et al. (2008) report that trails of *L. niger* in the presence of optical cues had a longer mean lifetime. The *detection threshold* gives a measure for the lowest level of pheromone concentration to which ants are responding. Since we do not know the absolute pheromone concentrations, the detection threshold cannot be compared between different species. As we gave a psychometric definition of the detection threshold, it is important to show how this definition may relate to definitions given in other studies. For instance, Choe et al. (2012) used the term "subthreshold concentration" to describe the lowest of a series of tested pheromone concentrations that gave a different response than no pheromone (Choe et al. 2012). In another study, the threshold was defined as the pheromone concentration which 50 % of the ants follow on a trail of a certain length (Van Vorhis Key and Baker 1982). The psychometric detection threshold represents the inflection point of the PF in which the slope reaches its maximum. Thus, it is the point on the curve that can be defined with the highest statistical certainty and its definition follows clear mathematical conventions that can be applied to data of different studies. However, thresholds given by different studies can only be compared if same or at least similar assays had been used and the measures for concentrations can be related. Since these requirements were met, we were able to calculate the 75 % threshold of synthetic (*Z*)-9-hexadecenal from data of (Choe et al. 2012) and relate it to the 75 % threshold of Pavan's gland.

In the following section, we will discuss the ecology and specific behavior of the three species while considering the interpretation of the psychometric measures given before. *E. procera* showed comparatively high discrimination abilities, a low lapse rate, a low IC and long-lasting trails. It thus may trigger fine-tuned collective reactions with high trail fidelity. The trail system thereby has to be maintained by many ants and each individual is limited in triggering a collective reaction on its own. These findings are consistent with the biology of *E. procera*: these ants establish and maintain a trail system that leads from the nest to the different mushroom sites, and they follow these trails with high fidelity. If a food source is exhausted, the ants stop foraging. After some time, the mushrooms grow again at the same site and the ants reactivate the abandoned trail, probably by orienting on long-lasting trail pheromones (see section "Species used"). Thus, *E. procera* needs only low information capacities since it probably uses a long-lasting pheromone on long-lasting trails and the trail system can be established and maintained by many ants together.

The results of the experiments with the Pavan's gland for *L. humile* showed high discrimination abilities, low lapse rate and a very high IC together with long-lasting trails. Thus, this species is able to trigger very fine-tuned collective reactions even by single ants. Despite long-lasting pheromones, a single ant will still be able to trigger a group response and recruit other ants to new locations due to its high IC. Thus, *L. humile* is able to establish trails by individual ants as well as collectively. Due to its low lapse rate, it shows a high trail fidelity, which is consistent with the finding that it mainly depends on pheromones and less on optical orientation (Aron et al. 1993). *L. humile* is able to share information about slight changes in the available food supply of the environment and react to it while maintaining long-lasting trails to stable food sources as well. Thus, it is able to use all kinds of food in its environment ranging from long-term food sources like aphid farming to short-term sources like seed, insects or carrion. This might be one cause for its ecological success.

Based on chemical analyses of natural trails deposited by *L. humile* workers from California (USA), Choe et al. (2012) estimated that the rate of (*Z*)-9-hexadecenal on the trails could not exceed 0.3 pg/cm. Conversely, our estimation for the natural trail concentration in *L. humile* of Southern France was 1.5 AE/ml (range 0.8–2.7 AE/ml) which is equivalent to 18.5 pg/cm (range 9.9–33.3 pg/cm) and about 30–100 times higher (see Online Appendix, Table F1). This discrepancy warrants further research.

L. niger showed low discrimination abilities and a high lapse rate compared to the other two species. This can be explained by the fact that it uses optical orientation as well as memory to a great extent (Aron et al. 1993; Evison et al. 2008; Czaczkes et al. 2011; Grüter et al. 2011). Thus, its collective behavior is not governed by pheromones alone, leaving the ants a relatively high degree of freedom to explore the terrain beyond the pheromone trails, find new food sources and adapt to changing environments. The IC of *L. niger* is 11 times lower than that of *L. humile* but six times higher than that of *E. procera*. This has to be interpreted with care: the amount of pheromone the ants need is comparatively high since the pheromone is short lived compared to the other two species. On the other hand, the ants reduce pheromone deposition as soon as they have learned to localize a food source optically, resulting in the need for lower pheromone amounts.

Our results also show that at least one parameter differs within a single species when measured under different conditions. The 75 % discrimination thresholds in the low and high concentration series of *E. procera* differ in the low (t_{ds75}=2.1) and high (t_{ds75}=3.3) concentration series. A possible explanation is that response thresholds in ants vary according to physiological state and environmental conditions. Fixed and adaptive response thresholds in fact play a major role in models designed to explain polyethism in ants (Detrain and Pasteels 1991; Bonabeau et al. 1996; Theraulaz et al. 1998; Gautrais et al. 2002; Diwold et al. 2009). The measurement of PFs in ant colonies under variable conditions may give more insight into the role of adaptive thresholds.

Our findings provide a base for quantitatively evaluating, verifying and improving the models of ant behavior by comparing these models with the parameters deduced from experiments. With psychophysical methods, we are able to quantify and compare parameters like thresholds, noise and errors and might be able to measure effects of motivation and learning. Thus, psychophysical theory and related methods provide a biological framework for stimulating further studies of the social behavior of ants and their chemical communication system and establish a relationship between the properties of the sensory apparatus of ants and their social and collective behavior.

Acknowledgments We thank Rolf Ulrich and Karin Bausenhart from the Universität Tübingen for valuable advice on psychophysical theory. We thank C. v. Beeren, T. Czaczkes, Hannah Fried-Petersen, Magdalena Mair, Angelika Pohl and Deborah Schweinfest for reading the manuscript and giving valuable advice. We thank Sandra Franz and Kai von Szadkowski for help with the experimental work.

Ethical standards The authors declare that the experiments comply with the current law of the country in which they were performed.

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

References

Akre KL, Johnsen S (2014) Psychophysics and the evolution of behavior. Trends Ecol Evol 29:291–300

Aron S, Beckers R, Deneubourg JL, Pasteels JM (1993) Memory and chemical communication in the orientation of two mass-recruiting ant species. Insect Soc 40:369–380

Bausenhart KM, Dyjas O, Vorberg D, Ulrich R (2012) Estimating discrimination performance in two-alternative forced choice tasks: routines for MATLAB and R. Behav Res Methods 44: 1157–1174

Beckers R, Deneubourg J, Goss S (1992) Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). Insect Soc 39:59–72

Beckers R, Deneubourg JL, Goss S (1993) Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. J Insect Behav 6:751–759

Bestmann HJ, Kern F, Schafer D, Witschel MC (1992) 3,4-Dihydroisocoumarins, a new class of ant trail pheromones. Angew Chem Int Ed 6:795–796

Bonabeau E, Theraulaz G, Deneubourg JL (1996) Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. Proc R Soc Lond B Biol 263:1565–1569

Britten KH, Shadlen MN, Newsome WT, Movshon JA (1992) The analysis of visual motion: a comparison of neuronal and psychophysical performance. J Neurosci 12:4745–4765

Byrd RH, Lu P, Nocedal J, Zhu C (1995) A limited memory algorithm for bound constrained optimization. SIAM J Sci Comput 16:1190–1208

Cavill GWK, Robertson PL, Davies NW (1979) An Argentine ant aggregation factor. Experientia (Basel) 35:989–990

Cavill GWK, Davies NW, McDonald JF (1980) Characterization of aggregation factors and associated compounds from the Argentine ant, *Iridomyrmex humilis*. J Chem Ecol 6:371–384

Chittka L, Skorupski P, Raine NE (2009) Speed–accuracy tradeoffs in animal decision making. Trends Ecol Evol 24:400–407

Choe D-H, Villafuerte DB, Tsutsui ND (2012) Trail pheromone of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae). PLoS One 7(9):e45016

Czaczkes TJ, Grüter C, Jones SM, Ratnieks FLW (2011) Synergy between social and private information increases foraging efficiency in ants. Biol Lett 7:521–524

Deneubourg JL, Aron S, Goss S, Pasteels JM (1990) The self-organizing exploratory pattern of the Argentine ant. J Insect Behav 3:159–168

Detrain C, Pasteels JM (1991) Caste differences in behavioral thresholds as a basis for polyethism during food recruitment in the ant, *Pheidole pallidula* (Nyl.) Hymenoptera: Myrmicinae). J Insect Behav 4:157–176

Diwold K, Merkle D, Middendorf M (2009) Adapting to dynamic environments: polyethism in response threshold models for social insects. Adv Complex Syst 12:327–346

Dorai-Raj S (2009) Binomial confidence intervals for several parameterizations. In: R package version 1.0-6/r2

Dyer AG, Spaethe J, Prack S (2008) Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. J Comp Physiol A 194:617–627

Efron B, Tibshirani R (1986) Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Stat Sci 1:54–77

Evison SEF, Petchey OL, Beckerman AP, Ratnieks FLW (2008) Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. Behav Ecol Sociobiol 63:261–267

Fechner G (1860) Elemente der Psychophysik. Breitkopf und Härtel, Leipzig

Gautrais J, Theraulaz G, Deneubourg JL, Anderson C (2002) Emergent polyethism as a consequence of increased colony size in insect societies. J Theor Biol 215:363–373

Giraud T, Pedersen JS, Keller L (2002) Evolution of supercolonies: the Argentine ants of southern Europe. Proc Natl Acad Sci U S A 99: 6075–6079

Gold JI, Shadlen MN (2007) The neural basis of decision making. Annu Rev Neurosci 30:535–574

Goss S, Aron S, Deneubourg JL, Pasteels JM (1989) Self-organized shortcuts in the Argentine ant. Naturwissenschaften 76:579–581

Grassé PP (1959) La reconstruction du nid et les coordinations interindividuelles chez *Bellicositermes natalensis* et *Cubitermes* sp. La théorie de la stigmergie: Essai d'interpretation du comportement des termites constructeurs. Insect Soc 6:41–80

Grüter C, Czaczkes TJ, Ratnieks FL (2011) Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav Ecol Sociobiol 65:141–148

Hangartner W (1969) Orientierung von *Lasius fuliginosus* Latr. an einer Gabelung der Geruchspur. Insect Soc 16:55–60

Hilborn R, Mangel M (1997) The ecological detective: confronting models with data. Princeton University Press, Princeton

Hölldobler B (1995) The chemistry of social regulation: multicomponent signals in ant societies. Proc Natl Acad Sci U S A 92: 19–22

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge

Holway DA, Case TJ (2000) Mechanisms of dispersed central-place foraging in polydomous colonies of the Argentine ant. Anim Behav 59:433–441

Kacelnik A, Brito e Abreu F (1998) Risky choice and Weber's law. J Theor Biol 194:289–298

Kingdom FAA, Prins N (2010) Psychophysics, 1st edn. Elsevier, London

Klein A (2001) Measuring, estimating, and understanding the psychometric function: a commentary. Percept Psychophys 63(8):1421–1455

Mailleux A-C, Buffin A, Detrain C, Deneubourg JL (2011) Recruitment in starved nests: the role of direct and indirect interactions between scouts and nestmates in the ant *Lasius niger*. Insect Soc 58:559–567

Mallis A (1942) Half a century with the successful Argentine ant. Sci Mon 55:536–545

Perna A, Granovskiy B, Garnier S, Nicolis SC, Labedan M, Theraulaz G, Fourcassie V, Sumpter DJ (2012) Individual rules for trail pattern formation in Argentine ants (*Linepithema humile*). PLoS Comput Biol 8:e1002592

R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Robertson PL, Dudzinski ML, Orton CJ (1980) Excocrine gland involvement in trailing behaviour in the argentine ant (Formicidae: Dolichoderinae). Anim Behav 28:1255–1273

Sarris V (2006) Relational psychophysics in humans and animals. Psychology Press, New York

Sasaki T, Granovskiy B, Mann RP, Sumpter DJT, Pratt SC (2013) Ant colonies outperform individuals when a sensory discrimination task is difficult but not when it is easy. Proc Natl Acad Sci U S A 110: 13769–13773

Seifert B (2007) Die Ameisen Mittel- und Nordeuropas. Lutra Verlags- und Vertriebsgesellschaft, Tauer

Stevens SS (1957) On the psychophysical law. Psychol Rev 64(3):153

Suarez AV, Bolger DT, Case TJ (1998) Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79:2041–2056

Theraulaz G, Bonabeau E, Deneubourg JL (1998) Response threshold reinforcement and division of labour in insect societies. Proc R Soc Lond B Biol 265:327–332

Turing AM (1952) The chemical basis for morphogenesis. Philos Trans R Soc B 237:37–72

Van Vorhis Key SE, Baker TC (1982) Trail-following responses of the Argentine ant, *Iridomyrmex humilis* (Mayr) to a synthetic trail pheromone component and analogs. J Chem Ecol 8:3–14

Vela-Pérez M, Fontelos MA, Velásquez JJL (2013) Ant foraging and geodesic paths in labyrinths: analytical and computational results. J Theor Biol 320:100–112

von Beeren C, Lizon à l'Allemand S, Hashim R, Witte V (2014) Collective exploitation of a temporally unpredictable food source: mushroom harvesting by the ant *Euprenolepis procera*. Anim Behav 89:71–77

Weber EH (1834) De Pulsu, resorptione, auditu et tactu. Annotationes anatomicae et physiologicae. In: Koehler (ed) Leipzig

Wetterer JK, Wild AL, Suarez AV, Roura-Pascual N, Espadaler X (2009) Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Myrmecol News 12: 187–194

Wichmann FA, Hill NJ (2001) The psychometric function: I. Fitting, sampling, and goodness of fit. Percept Psychophys 63(8):1293–1313

Wilson EB (1927) Probable inference, the law of succession, and statistical inference. J Am Stat Assoc 22:209–212

Wilson EO (1958) A chemical releaser of alarm and digging behavior in the ant *Pogonomyrmex badius* (Latreille). Psyche 65:41–51

Wilson EO (1962) Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). Anim Behav 10:134–164

Wilson EO, Pavan M (1959) Glandular sources and specifity of some chemical releasers of social behavior in dolichoderine ants. Psyche 66:70–76

Witte V, Maschwitz U (2008) Mushroom harvesting ants in the tropical rainforest. Naturwissenschaften 95:1049–1054

Chapter 2

Modelling shortest path selection of the ant Linepithema humile using psychophysical theory and realistic parameter values

Wolfhard von Thienen, Dirk Metzler, Volker Witte (2015)
Journal of Theoretical Biology 372:168-178

Journal of Theoretical Biology 372 (2015) 168–178

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Modeling shortest path selection of the ant *Linepithema humile* using psychophysical theory and realistic parameter values

Wolfhard von Thienen*, Dirk Metzler, Volker Witte

Fakultät für Biologie, Department Biologie II, Ludwig-Maximilians Universität München, Großhaderner Str. 2, D-82152 Planegg, Germany

HIGHLIGHTS

- The Deneubourg model explaining how ants are able to find the shortest of two paths between nest and food source has been tested based on parameters deduced from experiments.
- The Deneubourg model could be confirmed.
- However, the model uses a mathematical choice function describing the decision of ants depending on pheromone concentrations. This choice function has to be exchanged by a psychometric function for the model to satisfactorily explain the shortest path experiments.
- The finding of Aron et al. (1989) that ants modulate their pheromone deposition depending on their direction to or from the nest is important for the selection of the shortest path.
- For the first time, psychophysical theory has been successfully applied to the pheromone based social behavior of insects.

ARTICLE INFO

Article history:
Received 12 July 2014
Received in revised form
23 February 2015
Accepted 28 February 2015
Available online 11 March 2015

Keywords:
Ant behavior
Pheromones
Psychophysics
Ant colony optimization (ACO)
Weber's law

ABSTRACT

The emergence of self-organizing behavior in ants has been modeled in various theoretical approaches in the past decades. One model explains experimental observations in which Argentine ants (*Linepithema humile*) selected the shorter of two alternative paths from their nest to a food source (*shortest path experiments*). This model serves as an important example for the emergence of collective behavior and self-organization in biological systems. In addition, it inspired the development of computer algorithms for optimization problems called *ant colony optimization* (ACO). In the model, a *choice function* describing how ants react to different pheromone concentrations is fundamental. However, the parameters of the choice function were not deduced experimentally but freely adapted so that the model fitted the observations of the shortest path experiments. Thus, important knowledge was lacking about crucial model assumptions. A recent study on the Argentine ant provided this information by measuring the response of the ants to varying pheromone concentrations. In said study, the above mentioned choice function was fitted to the experimental data and its parameters were deduced. In addition, a *psychometric function* was fitted to the data and its parameters deduced. Based on these findings, it is possible to test the shortest path model by applying realistic parameter values. Here we present the results of such tests using Monte Carlo simulations of shortest path experiments with Argentine ants. We compare the choice function and the psychometric function, both with parameter values deduced from the above-mentioned experiments. Our results show that by applying the psychometric function, the shortest path experiments can be explained satisfactorily by the model. The study represents the first example of how psychophysical theory can be used to understand and model collective foraging behavior of ants based on trail pheromones. These findings may be important for other models of pheromone guided ant behavior and might inspire improved ACO algorithms.

1. Introduction

1.1. Background

Pheromones play an important role in the emergence of collective social behavior in ants (Hölldobler and Wilson, 1990 p. 227). Importantly, it has been shown that ants, confronted with a binary choice of

---

* Corresponding author. Tel.: +49 8233 31286.
E-mail addresses: w@thienen.de (W. von Thienen), metzler@bio.lmu.de (D. Metzler), witte@bio.lmu.de (V. Witte).

http://dx.doi.org/10.1016/j.jtbi.2015.02.030
0022-5193/

trails of different pheromone concentrations, prefer the trail with the higher concentration. Furthermore, trail following fidelity increases with pheromone concentrations (Choe et al., 2012; Hangartner, 1969; Van Vorhis Key and Baker, 1982). This resembles a communication system, which has the potential to transmit continuous information about different states of the environment.

1.2. Models of ant behavior

The information content of continuous pheromone concentration levels is a central component in models of self-organizing behavior of ant colonies, for example the way in which Argentine ants (*Linepithema humile*) are able to find the shorter of two alternative paths between their nest and a food source and in case of equal path lengths, they collectively select only one of the two paths (Deneubourg et al., 1990; Goss et al., 1989). In the following, we refer to these experiments for simplicity as *shortest path experiments*. This model had great influence by serving as an important example of collective behavior and self-organization of biological systems (Camazine et al., 2001) and inspired the development of optimizing processes called *ant colony optimization* (ACO) in the field of bionics and informatics (Dorigo and Stützle, 2004; Dorigo et al., 1996). Using a similar model, it was shown that the ability of ants to exploit the best quality food source in the environment depends on pheromone concentrations deposited on the trails (Beckers et al., 1992, 1993). The above-mentioned models make use of the same or a slightly modified choice function to describe the stochastic behavior of the ants. We refer to this class of models as the *Deneubourg model* and to the choice function as the *Deneubourg choice function* (DCF) (see Section 2.1 Deneubourg model). The DCF is not only used by ACO and the above mentioned models, but also in other models of ant behavior, for instance in explaining the influence of noise (Dussutour et al., 2009a), the path efficiency in artificial networks (Vittori et al., 2006), the symmetry breaking in foraging behavior (Lanan et al., 2012), the role of multiple pheromones (Dussutour et al., 2009b) and foraging in dynamic environments (Bandeira de Melo and Araújo, 2011; Ramsch et al., 2012).

According to the Deneubourg model, ants decide between two trails at a bifurcation in a probabilistic manner, which depends on the ratio of pheromone concentrations and is described by a choice function. Monte Carlo simulations of the shortest path experiments based on the Deneubourg model produced results similar to experiments conducted with real Argentine ants (Deneubourg et al., 1990; Goss et al., 1989). However, the function parameters were freely adapted to fit the results of the shortest path experiments, since experimental evidence on exact reaction of Argentine ants to different pheromone concentration was rare. In later experiments, Perna et al. (2012) and Vittori et al. (2006) deduced the parameters of the DCF for *L. humile* experimentally and found different values for the function parameters, especially for the exponent. They did not measure pheromone concentrations directly but assumed that the pheromone concentration at a certain point is proportional to the number of ants that had passed that point before. To our knowledge, the parameters of the DCF have never been deduced experimentally based on controlled pheromone concentrations, despite the fact that they are used by many models. Thus, crucial model assumptions have not been tested. A previous study attempted to fill this gap in knowledge (von Thienen et al., 2014) by measuring the response of Argentine ants to varying pheromone concentrations at a bifurcation by using gland extract as well as synthetic pheromone. In the same study, the DCF as well as the *psychometric function* (see Section 2.2 Psychophysical theory) were fitted to the experimental data and their parameters had been deduced. A major finding was that the amount of nonlinearity proposed by the Deneubourg model was much lower than expected and that the response to different pheromone concentrations follows Weber's law (see Section 2.2 Psychophysical theory).

1.3. Aim of this study

In the study presented here, we used the previously deduced parameters for computer simulations of shortest path experiments. Thereby, the data from the previous experimental study allowed, for the first time, computer simulations based on realistic model parameter values, so as to verify the Deneubourg model and the underlying mathematical functions. Thus, based on empirical data, we were able to verify a model of ant behavior that serves as an important example for collective behavior and self-organization in biological systems (Camazine et al., 2001). Here we present and compare the results of these computer simulations and discuss their implications for the understanding of collective ant behavior.

2. The models

An understanding of the Deneubourg model and psychophysical theory are essential for the comprehension of this article. Here, we provide a short introduction into these concepts.

2.1. Deneubourg model

The Deneubourg model was developed to explain important aspects of the collective ant behavior that are based on pheromones (Deneubourg et al., 1990; Goss et al., 1989). The model is based on the finding that ants encode information about the environment by dropping varying amounts of pheromones on their trails and that this information is used by other individuals for directional choices (Wilson, 1962). The model was able to partly explain experiments in which ants have the choice between alternative paths on their way from the nest to the food and back (see Fig. 1) (Aron et al., 1989; Deneubourg et al., 1990; Goss et al., 1989). In such an experiment, a bridge with two branches was placed between the ants' nest and a food source. After an exploration phase, the ants discovered the food and started foraging. Later, the majority of ants preferred one of the two alternative paths. Experimental repetition showed that the path selected in the majority of experiments depended on the difference in length between both paths. If the paths were of equal length, the choice was either left or right with equal probability (see Fig. 2B). If one path was shorter than the other, the shorter path was selected by most of the ants in most of the experiments (see Fig. 2A). Such effects in the foraging behavior of insects have been referred to as symmetry breaking (De Vries and Biesmeijer, 2002; Lanan et al., 2012) and can be seen in the asymmetric distribution along the x-axis in Fig. 2A and

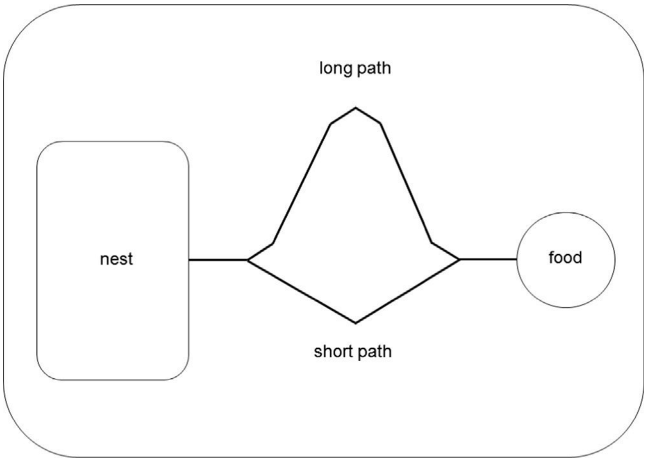


Fig. 1. Schematic experimental setup of the shortest path experiments. In the majority of experiments, most of the ants took the short path between nest and food. Modified from Goss et al. (1989).

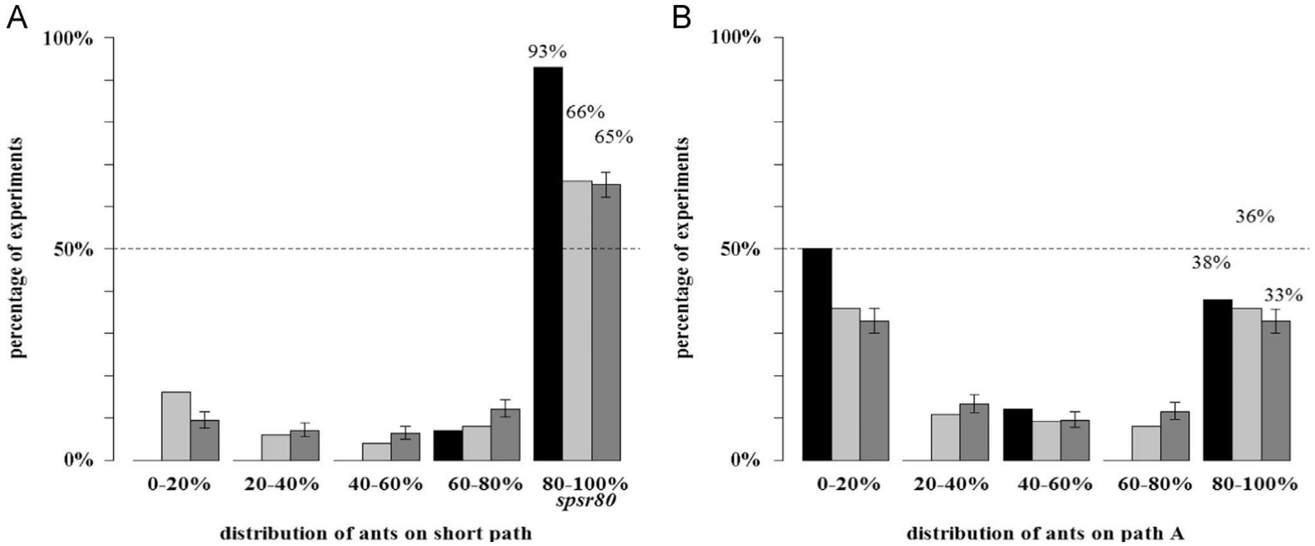


Fig. 2. Results of the original shortest path experiments, modified from Goss et al. (1989). The *x*-axis shows the distribution of ants that chose the short path ($n=500$); the *y*-axis shows the number of experiments or simulations in percent ($n=1000$). Black=original experimental results, light grey=results of Monte Carlo simulations with parameters $b=2$, $k=20$, dark grey=our simulations, reproducing the original model and parameter values. Error bars give 95%-binomial confidence intervals of our simulations. A: One path is twice as long as the other; in 13 out of 14 experiments (93%) more than 80% of the ants took the short path while this did not happen at all for the long path. B: Both paths (named "A" and "B") are of equal length; in 10 out of 26 experiments (38%) more than 80% of the ants took path A and in 13 experiments (50%) this happened for path B.

B, where most of the experiments and simulations fell in the range 0–20% and 80–100%. It happened in experiments and simulations with equal path lengths as well as in experiments and simulations with unequal path lengths.

According to the model, ants at bifurcations decide to follow the two paths in a probabilistic manner depending on the pheromone concentration of the trails given by the *Deneubourg choice function*

$$p_S = \frac{(k+c_S)^b}{(k+c_S)^b + (k+c_L)^b}, \tag{1}$$

where p_S and $p_L = 1 - p_S$ are the probabilities to follow the short or the long trail, c_S respectively c_L are the pheromone concentrations on the short respectively long trail, b is the exponent, and k is a constant. Please note, for reasons of simplicity, we refer to one of the paths as "short" and the other as "long" even if both paths are of equal length.

The model explains the outcome of the experiments by initial differences between the pheromone concentrations of the two paths. If the paths are of different length, there is a time delay between the ants arriving at the food via the long compared to the short path. During this period, the long path carries no pheromone at all at the side closest to the food and the returning ants prefer the short path on their way back to the nest and deposit pheromone on it. This generates a positive feedback loop in favor of the short path. Thus, in most experiments, the great majority of ants follow the short path (see Fig. 2A). If both paths are of equal length, no time delay occurs. However, small stochastic differences between the paths occur at the beginning and positive feedback amplifies the path with an initially slightly higher concentration so that it is finally selected by most of the ants. Consequently, in most of the experiments either one or the other path is preferred by the great majority of ants with equal probability (see Fig. 2B). The model only works, if the ants deposit pheromone while heading in both directions. It is not known whether all ant species show such trailing behavior, but *L. humile* does (Aron et al., 1989). In addition, after finding food, *L. humile* workers deposit more pheromone on their return to the nest than on their way out (Aron et al., 1989); an important fact that had not been incorporated in the model of the shortest path experiments so far. The results of the experiments were partly reproduced by Monte Carlo simulations (see grey columns in Fig. 2). However, the outcome of simulations is highly dependent on the parameters used for the DCF and it is possible to generate various kinds of distribution patterns depending on the exponent b and the constant k. von Thienen et al. (2014) found that the expected amount of non-linearity defined by the exponent ($b \approx 2$) was much lower than expected ($b=1.06$). Another important detail ignored in the literature so far is the fact that, according to the experiments, the ants nearly exclusively decided for the short path while even under best model assumptions, such a high value was not reached by the model: In 13 out of 14 experiments (93%) more than 80% of the ants chose the short path while this happened in only ca. 66% of the model simulations (see Fig. 2A). The same holds for other experiments with a different path length ratio of 1.4, which we do not report here. Thus, the model was able to give a general explanation of how ants are able to select the shortest path and explain the occurring phenomenon of symmetry breaking, but it could not explain the detailed experimental results.

2.2. *Psychophysical theory*

Psychophysical theory has been developed by Fechner (1860) based on works of Weber (1834) to find a relationship between the strength of a physical stimulus and its sensory impression (Kingdom and Prins, 2010; Klein, 2001; Wichmann and Hill, 2001). It plays a major role in psychology and neurobiology. Its main subjects are humans but the part of psychophysics that deals with animals (comparative psychophysics) is a fast growing field of research with work done on primates, birds and insects (Akre and Johnsen, 2014; Britten et al., 1992; Chittka et al., 2009; Dyer et al., 2008; Sarris, 2006; Sasaki et al., 2013). An important discovery in psychophysics is that the ability of a test subject to discriminate between two stimuli depends on the ratio of the stimuli strengths and is independent of the absolute stimuli strengths within certain limits (Weber's law). There is increasing evidence that Weber's law is very common in animals and relevant for the understanding of decision making based on sensual perception like hearing, vision, chemoreception etc. (Akre and Johnsen, 2014). Another important discovery (Fechner, 1860) is that the sensory impression of a physical stimulus is proportional to the logarithm of the stimulus strength. This is the reason why, for example, sound levels are measured on a logarithmic scale (decibel). Psychophysical theory explains the probabilistic nature of the response to a stimulus by the influence of the random fluctuations of internal and external noise. It is assumed that a stimulus will only be clearly detected when the sensory impression produced by the stimulus exceeds a certain internal sensory threshold that is clearly above random noise. This assumption is called the *high threshold assumption*. The threshold is usually defined as the stimulus intensity, which produces a correct response in 75% of the cases (75%-threshold). This is assumed to be well above the influence of noise. Another threshold often used is the stimulus intensity at which 50% of the responses are correct (50%-threshold). This is the case when the stimulus is too low to be distinguished from background noise and thus the test subjects have to guess. Two classes of psychophysical experiments are important: (1) In *detection experiments* the response to a test stimulus compared to a background with no stimulus is measured. (2) In *discrimination experiments* the response to a test stimulus compared to a fixed reference stimulus is measured.

To describe these experiments, a framework has been developed that relates physical stimuli to sensory impressions. This framework incorporates the effect of noisy backgrounds that influence the ability to detect a signal and gives clear mathematical definitions of sensory thresholds. One of the major mathematical tools is the *psychometric function* (PF). In detection experiments, it describes the ability to detect a stimulus of intensity x against a background with no stimulus

$$p = \gamma + (1 - \lambda - \gamma) \cdot F(x, \mathrm{tdt}_{75}), \tag{2}$$

where p is the probability to detect the stimulus, tdt_{75} is the 75%-detection threshold, λ is the *guess rate*, γ is the *lapse rate* and $F()$ is "a function, describing the probability of detecting a stimulus by the underlying sensory mechanism" (Kingdom and Prins, 2010 p. 74).

In *discrimination experiments* the PF describes the ability to discriminate a stimulus of intensity x compared to a constant reference stimulus of intensity s_r

$$p = \lambda + 2 \cdot (\gamma - \lambda) \cdot F(x, s_r). \tag{3}$$

where p is the probability do discriminate the stimulus from the reference stimulus. If both stimuli are equal, test subjects guess with equal probability $p = 0.5$ and the magnitude of these stimuli represent the 50%-discrimination threshold tds_{50} with $s_r = \mathrm{tds}_{50}$.

The *lapse rate* defines the errors that the test subjects make. According to psychophysical theory, the lapse rate is influenced by disturbances or by the motivation or attentiveness of the test subjects and by learning effects, which are caused by repeating the measurements with the same test subject. The *guess rate* defines the response probability when the test subjects cannot detect the stimulus and have to guess. Thus, in experiments with two possible choices, the guess rate equals 0.5. The PF is consistent with Weber's law, independent of the slope parameter and the lapse rate, as long as the guess rate equals 0.5.

Usually p is measured by presenting the test subjects the test stimulus and recording their ability to detect it. p is given by the sum of all positive responses divided by the number of measurements. After measuring p experimentally, all parameters of the PF can be deduced by fitting the function to the data (see von Thienen et al., 2014). The PF has a sigmoidal shape on a logarithmic x-scale (see Fig. 3). The sigmoidal part of the PF for detection defined by $F()$ lies in the region between $p = \gamma$ and $p = 1 - \lambda$. For discrimination, it lies in the region from λ to $2 \cdot (\gamma - \lambda)$.

For $F()$ a cumulative probability function, like the Weibull function, is used, which has the general form

$$W(x) = 1 - e^{\left(\frac{x}{s}\right)^b}, \tag{4}$$

where s is the scale parameter and b is the form parameter. By applying the Weibull function, we get the PF for detection:

$$p_t = \gamma + (1 - \lambda - \gamma) \cdot \left[1 - e^{\ln(1-\gamma) \cdot \left(\frac{s_t}{\mathrm{tdt}_{75}} \right)^b} \right], \tag{5}$$

where p_t is the probability to detect the test stimulus of intensity s_t, tdt_{75} is the 75%-detection threshold, λ is the lapse rate, γ is the guess rate and b is the slope.

For the discrimination between different stimuli, we get the PF for discrimination:

$$p_t = \lambda + 2 \cdot (\gamma - \lambda) \cdot \left[1 - e^{\ln(1-\gamma) \cdot \left(\frac{s_t}{\mathrm{tds}_{50}} \right)^b} \right], \tag{6}$$

where p_t is the probability to discriminate the test stimulus of intensity s_t from a reference stimulus of strength tds_{50}, which represents the 50%-discrimination threshold.

According to von Thienen et al. (2014), the Weibull function was chosen in experiments with ants, because in preliminary studies it gave a better fit compared to other functions and it is reported to give a good fit for the PF in other contexts (Klein, 2001, p. 1430). It was formulated in a way, that threshold and slope are independent and the threshold does not change the shape of the function, it only results in a parallel shift along the x-axis.

To apply the PF to bifurcation experiments with ants, we take the pheromone concentrations as test- and reference stimuli and apply

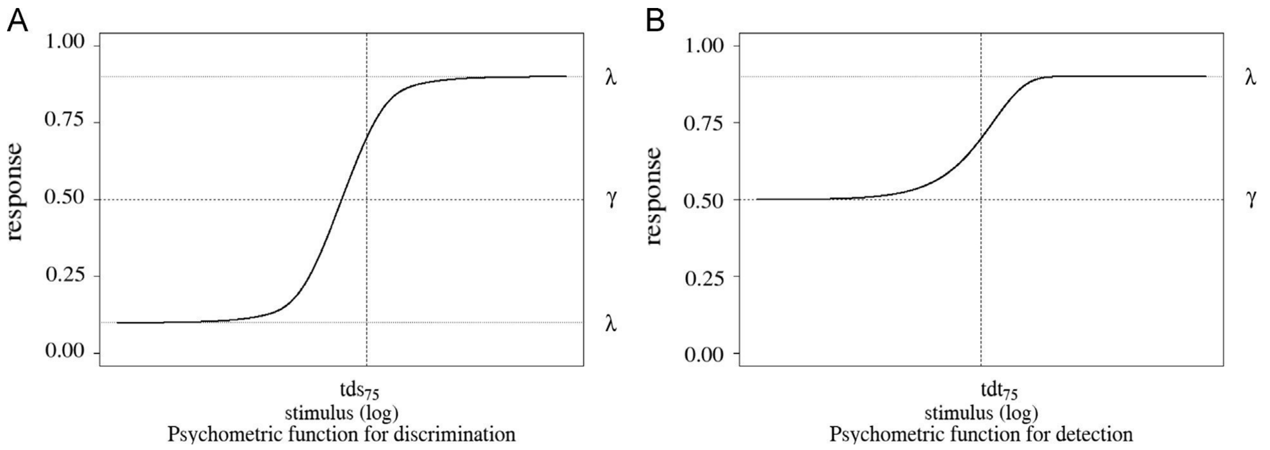


Fig. 3. PF for discrimination (A) and detection (B). The PF for discrimination gives the response defined as the probability that a stimulus is reported stronger than a constant reference stimulus which is different from zero. In case of detection, the reference stimulus equals zero. Lapse rate (λ, dotted horizontal line), guess rate (γ, dashed horizontal line), 75%-discrimination threshold ($\mathrm{tds}_{75,}$ dashed vertical line), 75%-detection threshold ($\mathrm{tdt}_{75,}$ dashed vertical line).

following Eqs. (7) and (8b) (von Thienen et al., 2014, modified) depending if only one trail carries pheromone or both trails carry pheromone. If only one trail carries pheromone, we apply the PF for detection

$$p_{S|L} = \gamma + (1-\lambda-\gamma) \cdot \left[1 - e^{\ln(1-\gamma) \cdot \left(\frac{c_{S|L}}{\mathrm{tdt}_{75}} \right)^{b}} \right], \tag{7}$$

where $p_{S|L}$ is the probability to follow the trail that carries the pheromone, which can be either the short or the long trail. $p_{L|S} = 1 - p_{S|L}$ is the probability to follow the trail with no pheromone, $c_{S|L}$ is the pheromone concentration of the trail that carries the pheromone, tdt_{75} is the pheromone concentration at the 75%-detection threshold.

If both trails carry pheromone, we apply the PF for discrimination

$$p_S = \lambda + 2 \cdot (\gamma - \lambda) \cdot \left[1 - e^{\ln(1-\gamma) \cdot \left(\frac{c_S}{c_L} \right)^{b}} \right], \tag{8a}$$

where p_S is the probability to follow the short trail. $p_L = 1 - p_S$ is the probability to follow the long trail, c_S is the pheromone concentration on the short trail and c_L is the pheromone concentration on the long trail.

The PF for discrimination shows two asymmetries depending on which branch of the bifurcation is defined as reference stimulus.

1. Two different PFs yield results that are slightly different, depending on which trail is the reference stimulus with a maximum difference of 0.06. This is corrected in the fitting procedure of von Thienen et al. (2014) by averaging the exponential terms. The resulting function lies in the middle of the two alternative functions.

$$\begin{aligned} p_S &= \lambda + 2 \cdot (\gamma - \lambda) \cdot \left[1 - \left(e^{\ln(1-\gamma) \cdot \left(\frac{c_S}{c_L} \right)^{b}} + 1 - e^{\ln(1-\gamma) \cdot \left(\frac{c_L}{c_S} \right)^{b}} \right) \cdot 0.5 \right] \\ &= \lambda + (\gamma - \lambda) \cdot \left[1 - e^{\ln(1-\gamma) \cdot \left(\frac{c_S}{c_L} \right)^{b}} + e^{\ln(1-\gamma) \cdot \left(\frac{c_L}{c_S} \right)^{b}} \right]. \end{aligned} \tag{8b}$$

   where $p_L = 1 - p_S$ is the probability to follow the long trail.
2. The second asymmetry occurs, because the guess rate was fitted at $\gamma = 0.47$ for *L. humile* (von Thienen et al., 2014). This represents a bias of the PF towards the reference stimulus and reflects the situation that Weber's law is not exactly fulfilled at low concentrations (von Thienen et al., 2014). Thus, to simulate ant behavior at a bifurcation, we have to take into account that the results differ slightly, depending on which stimulus is defined as reference stimulus. In our simulations, we let the virtual ants randomly decide which trail they take as reference (see Section 3.1).

3. Materials and methods

3.1. Simulations

Table A1 and Table A2 in Appendix A provide the parameters of the PF and the DCF deduced from previous experiments (von Thienen et al., 2014). We repeated the Monte Carlo simulations of Goss et al. (1989) using the DCF and the parameters of Table A1. In addition, we did the same simulations with the PF and the parameters of Table A2. For the simulations, we used the same model assumptions as reported by Goss et al. (1989) and Deneubourg et al. (1990):

- 1000 simulations with 1000 iterations each,
- 500 ants are counted between the 501th and 1000th iteration,
- short-trail-length = 20 units, long-trail-length = 40 units,
- ants walk 1 unit in length per iteration,
- 1 ant leaving the nest per iteration, after reaching the food, they immediately return,
- per trail passage, an ant deposits 0.5 *pheromone units per ant* (phpa) at each end of the short respectively long arm of the y-bridge, altogether one pheromone unit for each trail passage heading towards the food or heading towards the nest,
- the first ant chooses the branch randomly by a binomial decision with $p = 0.5$ and $n = 1$.

The ants' choice at the two bifurcations was simulated as a random decision. The probability that it takes the short branch is given by the ratio of pheromone concentrations of the two branches according to the model formula. For the DCF, Eq. (1) is used, while for the PF, Eq. (7) is used if one trail carries no pheromone (detection). If both trails carry pheromone, Eq. (8b) is used (discrimination). In case of the DCF, we also distinguished between detection and discrimination. The equation is the same in both cases, but the fitted exponent is different (see Table A1). To handle the above described asymmetry of the PF, depending on which trail is reference stimulus, we assume that both trails are randomly chosen as reference trail with equal probability. We also tested the possibility to take the average of the two alternatives, which did not affect the results. Simulations were programmed in C++ (gcc 4.4.5). Random numbers were generated using a method from Park and Miller (1988) cited by Press et al. (1992), p. 279.

3.2. Analysis

In each simulation-run, we performed 1000 simulations. In each simulation, we counted the number of ants out of 500 taking the short path and the number of ants taking the long path (starting with the 501st ant, similar to Goss et al. (1989). In the first step, we tested whether either paths had been selected more often by the virtual ants in the majority of simulations within a simulation-run. We counted the number of simulations S, in which the path defined as "short" was selected more often than the path defined as "long". We tested the null hypothesis that S is binomially distributed with $p = 0.5$ and $n = 1000$. In case of equal path lengths, we applied a two-sided binomial test. In case of different path lengths, our test was one-sided.

In the majority of the original experiments, one path was collectively selected by the great majority of ants (symmetry breaking), even if both paths were of equal length. Consistent with the original work of Goss et al. (1989), we measured the strength of this effect by counting the proportion of simulations in which more than 400 (80%) of the ants took the short path. We defined it as the *80%-short path selection rate* (spsr80) and calculated its 95%-binomial confidence interval. Similarly, we calculated the *80%-long path selection rate* (lpsr80). The spsr80 is equivalent to the 80–100% range of the original experiments in Goss et al. (1989) (see Fig. 2) and the lpsr80 is equivalent to the 0–20% range. The 0.9999-quantile of a binomial distribution with $n = 500$ and $p = 0.5$ lies at 59% ($n = 500$, $p = 0.5$). Thus, results $> 59\%$ and certainly results $> 80\%$ have to be considered as highly significant with $p \ll 0.0001$. Since the distribution in the original experiments nearly exclusively fell in the region of the spsr80 or lpsr80, we focus on these rates when we compare the distribution of the original experiments to our simulations.

For all tests, we used R version 3.0.1 (2013-05-16) (R Development Core Team, 2013). For binomial testing, we used *binom.test*(), (library *stats*) (Mailleux et al., 2000). For calculating binomial confidence limits we used *binom.confint*(), (library *binom*) Dorai-Raj (2009), with the method "wilson" (Wilson, 1927). All these packages are freely available at the CRAN repository.

4. Results

Figs. 2, 4–6 show the results of the simulations. They are presented in a format similar to Goss et al. (1989) and show the distributions of ants taking the short path. The outcome of each simulation is grouped into one of five ranges (*x*-axis). Each range specifies the percentage of ants taking the short path in a single simulation. The *y*-axis gives the percentage of experiments or simulations in which the outcome fell into the specified range. For example Fig. 2A shows that in 67% of all simulations, between 80% and 100% of the ants took the short path. Please note that the range 80–100% is equivalent to our definition of the 80%-short path selection rate (spsr80) (see Section 3.2) which is presented on top of the figure bars. Detailed results with confidence intervals are given in Table A3 in the appendix. We first present results of simulations that repeat the original simulations of Goss et al. (1989), then we focus on simulations with different path lengths followed by simulations of equal path lengths.

4.1. *Repetition of original simulations of Goss et al. (1989)*

Our simulations of the DCF using the original parameter values from Goss et al. (1989) gave results similar to their simulations. If both paths were of equal length (see Fig. 2B) the short path was taken by most of the ants in 49% of the simulations, which was not significant ($p=0.36$, two sided binomial test, $n=1000$). The spsr80 (33%) and the lpsr80 (33%) were nearly equal. If one path was twice as long as the other (see Fig. 2A) the short path was taken by most of the ants in 81% of the simulations, which was significant ($p<0.0001$, one sided binomial test, $n=1000$). The spsr80 was 65%.

4.2. *Results with fitted parameters and different path lengths*

When more realistic parameters deduced from previous experiments were applied to the DCF (see Fig. 4, dark grey bars), the simulations showed that the short path was preferred in 57% of the simulations, which was significant ($p<0.0001$, one sided binomial test, $n=1000$), but low compared to 100% in the original experiments.

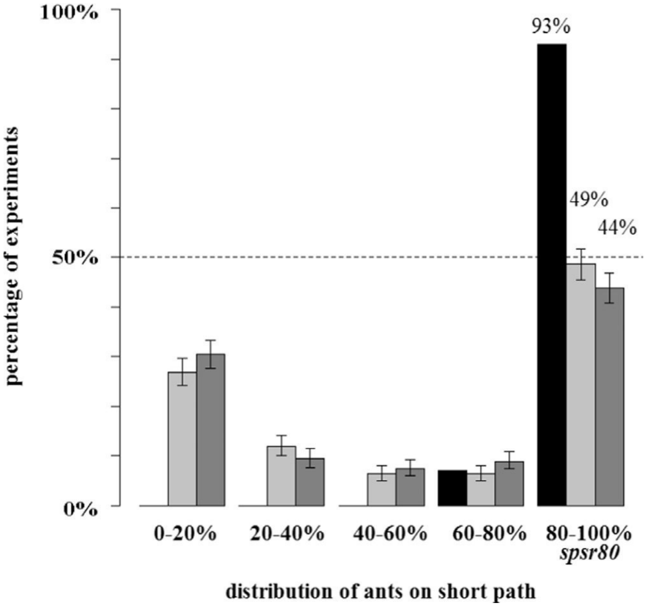


Fig. 4. Monte Carlo simulations of different path lengths with the DCF and the PF with realistic parameter values. Parameters for the DCF: $b=1.06$, $k=0.02$ (discrimination) and $b=0.52$, $k=0.02$ (detection). Parameters for the PF: $\gamma=0.47$, $\lambda=0.02$, $b=0.87$ (discrimination) and $\gamma=0.43$, $\lambda=0.11$, $b=0.6$, $tdt_{75}=0.053$ (detection). The *x*-axis shows the distribution of ants that chose the short path ($n=500$). The *y*-axis shows the number of experiments ($n=14$) or simulations ($n=1000$) in percent. Black=original experimental results modified from Goss et al. (1989), dark grey=simulation of the DCF, light grey=simulation of the PF. Error bars give 95%-binomial confidence intervals of the simulations.

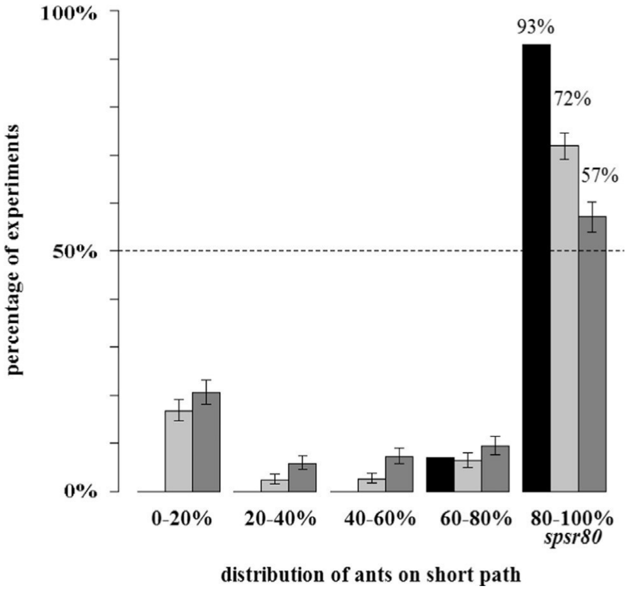


Fig. 5. Monte Carlo simulations of different path lengths and pheromone modulation with the DCF and the PF. The assumption was made that ants deposit four times more pheromone on their way back from food to nest. The *x*-axis shows the distribution of ants that chose the short path ($n=500$). The *y*-axis shows the number of experiments ($n=14$) or simulations ($n=1000$) in percent. Black= original experimental results modified from Goss et al. (1989), light grey=simulation of the PF, dark grey=simulation of the DCF. Error bars give 95%-binomial confidence intervals of the simulations. Parameters were set within their confidence limits to give a distribution pattern as close as possible to the original experimental results. Parameters for the DCF: $b=1.19$, $k=0.02$ (discrimination) and $b=0.52$, $k=0.02$ (detection). Parameters for the PF: $\gamma=0.48$, $\lambda=0$, $b=1.02$ (discrimination) and $\gamma=0.43$, $\lambda=0.13$, $b=1.1$, $tdt_{75}=0.04$ (detection).

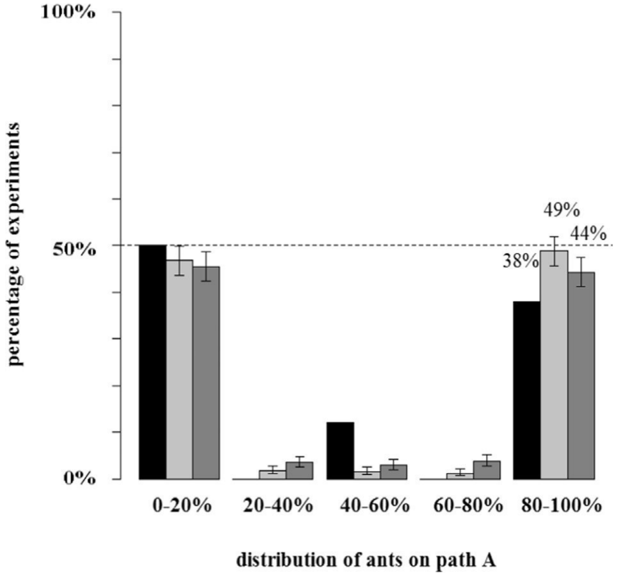


Fig. 6. Monte Carlo simulations as in Fig. 5, but with both paths of equal length (20 units). The *x*-axis shows the distribution of ants that chose the short path ($n=500$). The *y*-axis shows the number of experiments ($n=26$) or simulations ($n=1000$) in percent. Black=original experimental results modified from Goss et al. (1989), light grey=simulation of the PF, dark grey=simulation of the DCF. Error bars give 95%-binomial confidence intervals of the simulations. For further details and parameter settings please refer to Fig. 5.

The spsr80 was 44% and much below the *sprs80* of the original experiments (93%). Applying the PF and realistic parameter values (see Fig. 4, light grey bars), the simulations showed similar results to the DCF. In 58% of the 1000 simulations, the short path was taken by most of the ants, which was significant ($p < 0.0001$, one sided binomial test, $n = 1000$) and the spsr80 was 49%.

4.3. Results with pheromone modulation and different path lengths

It is known that Argentine ants returning from the food to the nest lay more pheromone than when heading out from the nest (Aron et al., 1989). In this study, the ants made 2-times longer streaks, which were 2-times less distant from each other if they returned to the nest compared to their behavior in the opposite direction. From these data we deduced that the pheromone amount deposited on the way back to the nest is 1.6 to 4 times higher than on the way to the food (see Table S3, Supplemental content).

We included the assumption that ants modulate the pheromone amount by factor of four into the model with the PF and set the parameters to the confidence limits which gave the highest short path selection rate. In 80% of the simulations the short path was taken by most of the ants, which was significant ($p < 0.0001$, one sided binomial test, $n = 1000$). The spsr80 was 72%, which is 21% below the original experimental results (93%), but 6% higher than the original simulations of (Goss et al., 1989) (66%). By applying the DCF, the short path was taken by most of the ants in 71% of the simulations, which was significant ($p < 0.0001$, one sided binomial test, $n = 1000$). The spsr80 was 57%, which is 36% below the original simulations.

4.4. Difference between DCF and PF

To evaluate why the PF showed a higher short path selection rate than the DCF in experiments with different path lengths, we tested the hypothesis that the lapse rate, a parameter unique for the PF, is responsible for the difference. This parameter describes the tendency of ants to show no response to the trail pheromone and can be interpreted as a measure for the independence of ants from pheromone trails (see von Thienen et al., 2014). In the initial phase of the experiment, it usually occurs that one trail carries pheromone while the other carries none. By applying the PF, ca. 86% of the ants follow the path with pheromone, while in case of the DCF, nearly all ants follow the pheromone trail (96%). This difference is mainly contributed to the lapse rate of 0.11 of the PF for detection. It is important in the initial phase of the experiment and causes an asymmetry between short and long path selection. The first ant randomly selects the short or the long path with equal probability. Due to the pheromone deposited by the first ant, the majority of subsequent ants will follow this selection. However, a small fraction of ants will also follow the other trail and in some cases reverse the initial selection. This small fraction is higher for the PF (14%) than for the DCF (ca. 4%), due to the lapse rate of the PF. If the short path is selected by the first ant, it is very unlikely that the final outcome will be reversed towards the long trail, since the positive feedback described by the Deneubourg model already favors the short path. In this case, we do not expect a major difference between PF and DCF, the rate for short path selection will be close to 100%. However, if the long path is selected by the first ant, the small fraction of following ants taking the short trail will enhance the general preference for the short path and thus be able to reverse the initial selection of the long path towards the short path. This tendency to reverse the initial selection of the long path towards the short path will depend on the amount of subsequent ants taking the short path, which is much higher in case of the PF. Thus, we expect the probability for short path selection to be greater for the PF than for the DCF. To test this hypothesis, we made a simulation-run in which the first ant always selected the long path. In the same way, we made a simulation-run in which the first ant always selected the short path. By doing so, we were able to compare the ability of the two functions to reverse this initial choice as the experiment progressed. Settings were the same as described in Fig. 5. The results are presented in Table 1 and confirm the hypothesis: If the first ant selects the short path, this selection is usually also the final result and the spsr80 is similar for both functions (95% and 92%). If the long path is initially selected, the PF reaches a higher spsr80 (51%) compared to the DCF (26%). If the lapse rate of the PF is set to 0, the spsr80 drops to 0%.

4.5. Test of additional assumptions

The results of the simulations of different path lengths with the PF are of slightly better quality than the original simulations of Goss et al. (1989), showing an 8% higher spsr80. However, there is still a discrepancy between experiment and model since the model does not reach the high spsr80 that occurred in the experiments (experiment: 93%, model PF 72% and model DCF 57%, see Fig. 5). Obviously, real ants decided exclusively for the short path, while the virtual ants did not. Therefore, we tested additional assumptions and reached in simulations with the PF higher values for the spsr80 that reproduced the original experiments very well (model: 89%, experiment: 93%). These results are presented in the Supplement S1.

4.6. Simulation of equal path length

We repeated all simulations with equal path lengths (named “A” and “B”). They showed very similar results to the original experiments of Goss et al. (1989) (see Fig. 2B) with no significant preference for either path but with the symmetry breaking effect. An example, which is representative for all simulations with equal path lengths, is given in Fig. 6. Results for the DCF were as follows: In 50% of the simulations either path A or path B was taken by most of the ants, which was not significant ($p = 0.92$, two sided binomial test, $n = 1000$). The selection rate in the range 80%–100% and 0%–20% were nearly equal (44% and 46%) and show that in 90% of the simulations one single path was collectively selected. Results for the PF: In 51% of the simulations path A and in 49% path B was taken by most of the ants, which is not significant ($p = 0.59$, two sided binomial test, $n = 1000$). The selection rate in the range 80%–100% and 0%–20% were nearly equal (49% and 47%) and show that in 96% of the simulations one single path was collectively selected.

4.7. Sensitivity of the PF and DCF to the pheromone scale

The pheromone scale is proportional to the amount of pheromone a single ant releases at the beginning and end of the trail each time it passes these points (*pheromone unit per ant*—phpa). Since we have no knowledge about the pheromone amounts single ants drop, it was arbitrarily set to 0.5 units in our simulations. Like in the simulations of Goss et al. (1989), this gives one pheromone unit per trail passage in

Table 1
Results of simulations with 1st ant selection fixed.

1st ant	Function		spsr80 (%)
Long path	PF	$\lambda = 0.11$	51
		$\lambda = 0$	0
	DCF		26
Short path	PF	$\lambda = 0.11$	95
		$\lambda = 0$	100
	DCF		92

Notes: The simulations of Fig. 5 had been repeated with the first ant forced to select the long path for 1000 simulations. This was repeated for the short path for another 1000 simulations. In case of the PF, we present results for the lapse rate $\lambda = 0.11$ and $\lambda = 0$.

each direction. Since phpa is not known, the underlying pheromone scale is unknown and the parameters of the model that depended on the pheromone scale might have influenced the result of the simulations depending on the pheromone scale being used. For the DCF, this means that it is independent of the pheromone scale, as long as the parameter k is kept proportional to the scale. Thus, since absolute pheromone amounts were unknown, k was a free parameter. Similarly, the PF for detection is independent of the pheromone scale, as long as the detection threshold (tdt_{75}) is kept proportional to the scale. Therefore, tdt_{75} was also a free parameter. However, this happens only in a few iterations during the initial phase of the simulations, in which the PF for detection is applied. Due to Weber's law, the PF for discrimination, which was applied in most iterations, is independent from the pheromone scale. A detailed analysis of the whole parameter space of both functions showed that the DCF was much more sensitive to the pheromone scale than the PF (see S2, Supplemental content). By systematically varying these parameters, we found that $k=0.02$ and $tdt_{75}=0.04$ gave the best approximations to the distribution found in the experiments of Goss et al. (1989). Please note that von Thienen et al. (2014) measured pheromone concentrations in a different scale based on an estimation of concentrations occurring in natural trails (*base concentration*, bc) which is proportional to the scale we used in our simulations. Since we do not know the amount of pheromone ants drop, the factor of proportionality between these scales is unknown.

4.8. Parameter space

To get a more detailed picture of how the two functions differed with respect to the experiments with different path lengths, we systematically varied the parameters of the functions, repeated 100 simulations for each parameter value and calculated the spsr80. These simulations confirmed the results from above and show that both functions were only able to explain the results of the shortest path experiments by assuming that ants deposit more pheromone on their way returning to the nest than coming from it. While both functions fail to reach the high spsr80 of the original experiments, the simulations with the PF reached higher values for the spsr80 over the whole parameter space and over the confidence region of the fitted parameters compared to simulations with the DCF. By assuming a higher exponent/slope in the initial phase, simulations with the PF were consistent with the experimental results, while simulations with the DCF were not. For details see S2, Supplemental content.

5. Discussion

Until the recent study by von Thienen et al. (2014), we knew of no empirical evidence based on controlled pheromone concentrations for the specific parameter values used in the Deneubourg model and in other models that use the DCF. Three studies by Deneubourg et al. (1990), Perna et al. (2012) and Vittori et al. (2006) deduced these parameters for *L. humile* experimentally. They measured pheromone concentrations indirectly assuming that the concentration at a certain point is proportional to the number of ants that had passed that point before. The parameter b (exponent of the Denenbourg choice function) was fitted to different values: 1.06 (Perna et al., 2012), 2 (Deneubourg et al., 1990) and 4 (Vittori et al., 2006). The parameters had been fitted to discrimination tasks while parameters for detection were not evaluated. In contrast to these experiments, von Thienen et al. (2014) measured the response of ants to controlled pheromone concentrations with gland extracts and with the synthetic pheromone (*Z*)-9-hexadecenal. They deduced the model parameters for detection as well as for discrimination tasks ($b=1.06$ for discrimination and $b=0.52$ for detection). In addition, von Thienen et al. (2014) showed that the ants' response in discrimination experiments is independent from absolute concentrations and Webers' law is fulfilled. When applying the DCF, this is only possible with $b\approx 1$, see also Perna et al. (2012). By applying such realistic parameter values, we attempted to reproduce the experimental results of the shortest path experiments by Monte Carlo simulations.

In our simulations with equal path lengths, the experimental results could be reproduced very well by using either the DCF or the PF. The results were independent from the specific function and the parameters being used. This shows that a simple positive feedback mechanism, which is common to both functions, is sufficient to explain the results of experiments with equal path lengths.

In contrast to experiments with equal path lengths, we can expect that simulations of different path lengths are very sensible to slight changes in the model assumptions, due to the high dynamic of the whole process and thus reveal subtle differences between models and model parameters. This can be clearly seen in the results of our simulations of different path lengths, which show great differences between the functions and parameters being used. By applying the DCF and a realistic value for the exponent b, a significant but low preference of the short path could be reproduced (spsr80 = 44%, lpsr80 = 30%), although the parameter k was free and set to its optimum for short path selection. By assuming a pheromone modulation by a factor of four, the short path was selected significantly more often than the long path (spsr80 = 57%, lpsr80 = 21%), but not as often as in the experiments (spsr80 = 93%, lpsr80 = 0%). The reason for the discrepancy between the original simulations of Goss et al. (1989) and our simulations of the DCF is the low value of the exponent b, which was expected to be twice as high ($b\approx 2$) as it actually turned out to be ($b=1.06$). As mentioned before, it is important to note that the DCF is only consistent with Weber's law if the exponent is close to one.

By applying the PF and using realistic parameter values, the result was slightly better than the DCF (spsr80 = 49%, lpsr80 = 27%). By assuming a pheromone modulation by a factor of four, a highly significant short path selection rate resulted (spsr80 = 72%, lpsr80 = 17%). This result reproduced the original experiments even better than the original simulations of Goss et al. (1989) (spsr80 = 66%, lpsr80 = 16%). This shows that under the assumption of pheromone modulation, the PF was closer to the experimental results than the DCF.

We evaluated the differences of both functions with respect to their ability to reproduce the shortest path experiments. The lapse rate, a parameter that is only part of the PF, was responsible for this difference. It allowed simulations with the PF to reproduce higher values of the short path selection rate compared to simulations with the DCF. The lapse rate can be interpreted biologically as a measure for the independence of ants from pheromone trails (von Thienen et al., 2014). It is responsible for an asymmetry between the selection of the short and long path by enhancing the tendency to reverse an initial selection of the long path towards the short path and thus enhancing the selection of the short path. This asymmetry had never been described before and adds an additional mechanism that favors short path selection to the Deneubourg model. It shows that the initial phase of the experiment is very important and that a certain degree of freedom from pheromone guided behavior is an important feature of short path selection, which only the PF describes.

Although the PF reproduced the shortest path experiment better than the DCF, there was still a gap between simulations and experimental results. In the experiment, the ants exclusively selected the short path (spsr80 = 93%), while in the model this could not be fully reproduced (57% DCF and 72% PF). Thus, real ants were better in selecting the short path than virtual ants, indicating other factors that influence ant behavior not incorporated in the model so far. By including the additional assumption that ants show a higher responsiveness to pheromones for a short time after they have found food and in the beginning of the recruitment, we were able to nearly fully reproduce the experiments by applying the PF (spsr80 = 89%), see S1 in the supplement for details. This was not possible by applying the DCF. These differences between PF and DCF can be explained by an enhancement of the tendency to reverse an initial long path selection due to the lapse rate of the PF. As a possible biological explanation, we assume that in the beginning, only highly motivated ants or specialized ants like scouts may be involved in foraging and we assume that these ants express a higher slope of the PF. This assumption has not been tested for *L. humile*, but we know from experiments with Pharao's ants that 20% of individual ants involved in foraging show a significantly higher response to pheromone trails (Jackson and Châline, 2006). Other factors that we had not tested could be important too. For example, it was shown that the trail following behavior in *L. humile* is a complex system influenced by other pheromones from different glandular sources as well by different forms of physical contact between the ants (Cavill et al., 1980; Choe et al., 2012; Robertson et al., 1980; Van Vorhis Key and Baker, 1981, 1982). Factors, such as food quality and learning, influence the ants' response to pheromones (Aron et al., 1993; Beckers et al., 1993; Czaczkes et al., 2011; Evison et al., 2008; Grüter et al., 2011). Thus, single pheromone models of ant behavior may be too simple to describe the complex nature of ant behavior. To refine our theoretical understanding of these processes, we would need more empirical evidence on communication in *L. humile*. However, even if our models of ant behavior need to incorporate a higher complexity of ant systems, it is important to know exactly how ants respond to different kinds of stimuli and give a mathematical and biological description for this relationship, which can be achieved by using the PF.

Besides the reason that the PF fits well to experimental evidence, there are other, maybe more important, reasons for PF's suitability as tool to describe important aspects of collective ant behavior. It is part of the psychophysical theory with a well-established practical and theoretical toolset to measure and evaluate the relation between stimuli, sensory perception and behavior. It is consistent with Weber's law, which is fundamental to many aspects of sensory perception and behavior in animals. It provides clear definitions of biological parameters, like sensory and behavioral thresholds, which are important for the understanding of the collective behavior of ants. Thus, our results may be of great importance for the development of realistic models of collective ant behavior. In addition, applying psychophysical theory to collective ant behavior opens the field of behavioral and comparative psychophysics to collective behavior in general, especially to the collective behavior of social insects like bees, bumble bees, wasps, termites and ants by exactly measuring their behavioral responses to physical stimuli and signals.

For ACO algorithms, we do not expect a generally better performance by using the PF since the equations are more complex than the DCF. However, certain aspects of PF might lead to better performing ACO algorithms. For instance, the concept of the lapse rate might prevent algorithms to run into stagnation and show a better convergence to an optimal solution. Further concepts like depositing higher pheromone amounts at the return path or higher slopes in the beginning might lead to a faster or better convergence towards the optimal solution.

5.1. Conclusions

For the first time, psychophysical theory has been successfully applied to pheromone-based social behavior of insects. The Deneubourg model for the explanation of the shortest path experiments could be confirmed based on parameters deduced from new experiments. However, the Deneubourg choice function should be exchanged by a psychometric function for the model to better explain the data of the shortest path experiments. The finding of Aron et al. (1989) that ants modulate their pheromone deposition depending on their direction to or from the nest is important for the selection of the shortest path. In addition, we found that a certain degree of freedom in the initial phase of the experiment, allowing some ants to ignore the trail pheromone, is important for the final outcome of the experiment. Further, although empirical data are missing, our simulations suggest that in the initial phase specialized ants with an increased response to pheromone might be important to fully explain the experimental results of the shortest path experiments.

Disclosure

All authors have approved the article.

Appendix A

See Tables A1–A3.

Table A1
Fitted parameters of the DCF for *Linepithema humile.*

Experiment	b	k	GOF
Discrimination	*0.96*	*0.0 bc*	0.22
	1.06	0.02 bc	
	1.19	*0.06 bc*	
Detection	*0.44*	*0.01 bc*	0.41
	0.52	0.02 bc	
	0.64	*0.04 bc*	

From von Thienen et al. (2014).
Notes: Values for fitted parameters are given in the order lower confidence limit, fitted value, and upper confidence limit from top to bottom with confidence limits in italics. Confidence limits were calculated at 95%-level by parametric bootstrap ($n = 1000$). GOF was calculated by a log-likelihood ratio test between the model being evaluated and the full model.
b—Exponent.
k—Constant.
GOF—Goodness of fit.
bc—Base concentration (0.0014 AE/cm (ant equivalent/centimeter) ≙ 30.8 pg/cm). Please note that the bc resembles the pheromone concentration that can be expected on a naturally laid trail according to von Thienen et al. (2014). It is not equivalent to the pheromone amount a single ant drops (phpa).

Table A2
Fitted parameters of the PF for *Linepithema humile.*

Experiment	b	γ	λ	tdt$_{75}$	GOF
Discrimination	*0.75*	*0.46*	*0*	–	0.86
	0.87	0.47	0.02		
	1.02	*0.48*	*0.05*		
Detection	*0.42*	*0.35*	*0.08*	*0.04 bc*	0.25
	0.6	0.43	0.11	0.053 bc	
	1.1	*0.53*	*0.13*	*0.11 bc*	

From von Thienen et al. (2014).
Notes: Values for fitted parameters are given in the order lower confidence limit, fitted value, and upper confidence limit from top to bottom with confidence limits in italics. Confidence limits were calculated at 95%-level by parametric bootstrap ($n = 1000$). GOF was calculated by a log-likelihood ratio test between the model being evaluated and the full model.
b—Slope.
γ—Guess rate.
λ—Lapse rate.
tdt*75*—75%-Detection threshold.
GOF—Goodness of fit.
bc—Base concentration (0.0014 AE/cm (ant equivalent/cm) ≙ 30.8 pg/cm). Please note that the bc resembles the pheromone concentration that can be expected on a naturally laid trail according to von Thienen et al. (2014). It is not equivalent to the pheromone amount a single ant drops (phpa).

Table A3
Detailed results of simulations.

Description		Simulations preferring short path		n	p	spsr80		lpsr80	
Original simulations repeated, equal path length, Fig. 2B	DCF	*454*	49%	1000	0.36	*300*	33%	*299*	33%
		485				328		327	
		516				*358*		*357*	
Original simulations repeated, different path length, Fig. 2A	DCF	*780*	81%	1000	< 0.0001	*622*	65%	*77*	9%
		806				652		93	
		829				*681*		*113*	
Fitted parameters, different path length, Fig. 4	DCF	*535*	57%	1000	< 0.0001	*408*	44%	*275*	30%
		566				438		303	
		596				*469*		*332*	
	PF	*546*	58%	1000	< 0.0001	*455*	49%	*241*	27%
		577				486		267	
		607				*517*		*295*	
Pheromone modulation, different path length, Fig. 5	DCF	*676*	71%	1000	< 0.0001	*540*	57%	*181*	21%
		705				571		205	
		732				*601*		*231*	
	PF	*769*	80%	1000	< 0.0001	*690*	72%	*146*	17%
		795				719		168	
		819				*746*		*192*	
Higher slope/exponent in the beginning and pheromone modulation, different path length, Fig. 6	DCF	*477*	51%	1000	0.38	*477*	51	*461*	49%
		508				508		492	
		539				*539*		*523*	
	PF	*872*	89%	1000	< 0.0001	*872*	89%	*89*	11%
		893				893		107	
		911				*911*		*128*	
Pheromone modulation, equal path length, Fig. 6	DCF	*467*	50%	1000	0.92	*412*	44%	*424*	46%
		498				443		455	
		529				*474*		*486*	
	PF	*478*	51%	1000	0.59	*457*	49%	*437*	47%
		509				488		468	
		540				*519*		*499*	

Notes: Values in italics give 95%-binomial confidence intervals.
n—Number of simulations.
p—pa-Value from binomial test (equal path length: two sided, different path length: one-sided).
spsr80—80%-Short path selection rate.
lpsr80—80%-Long path selection rate.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jtbi.2015.02.030.

References

Akre, K.L., Johnsen, S., 2014. Psychophysics and the evolution of behavior. Trends Ecol. Evol. 29, 291–300.

Aron, S., Pasteels, J.M., Deneubourg, J.L., 1989. Trail-laying behaviour during exploratory recruitment in the argentine ant, *Iridomyrmex humilis* (Mayr). Biol. Behav. 14, 207–217.

Aron, S., Beckers, R., Deneubourg, J.L., Pasteels, J.M., 1993. Memory and chemical communication in the orientation of two mass-recruiting ant species. Insect Soc. 40, 369–380.

Bandeira de Melo, E.B., Araújo, A.F., 2011. Modelling foraging ants in a dynamic and confined environment. Biosystems 104 (1), 23–31.

Beckers, R., Deneubourg, J., Goss, S., 1992. Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). Insect Soc. 39, 59–72.

Beckers, R., Deneubourg, J.L., Goss, S., 1993. Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. J. Insect Behav. 6, 751–759.

Britten, K.H., Shadlen, M.N., Newsome, W.T., Movshon, J.A., 1992. The analysis of visual motion: a comparison of neuronal and psychophysical performance. J. Neurosci. 12, 4745–4765.

Camazine, S., Deneubourg, J.-L., Franks, N.R., Sneyd, J., Theraulaz, G., Bonabeau, E., 2001. Self-Organization in Biological Systems. Princeton University Press.

Cavill, G.W.K., Davies, N.W., McDonald, J.F., 1980. Characterization of aggregation factors and associated compounds from the Argentine ant, *Iridomyrmex humilis*. J. Chem. Ecol. 6, 371–384.

Chittka, L., Skorupski, P., Raine, N.E., 2009. Speed-accuracy tradeoffs in animal decision making. Trends Ecol. Evol. 24, 400–407. http://dx.doi.org/10.1016/j.tree.2009.02.010.

Choe, D.-H., Villafuerte, D.B., Tsutsui, N.D., 2012. Trail pheromone of the Argentine Ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae). PLoS One 7 (9).

Czaczkes, T.J., Grüter, C., Jones, S.M., Ratnieks, F.L.W., 2011. Synergy between social and private information increases foraging efficiency in ants. Biol. Lett. 7, 521–524. http://dx.doi.org/10.1098/rsbl.2011.0067.

De Vries, H., Biesmeijer, J.C., 2002. Self-organization in collective honeybee foraging: emergence of symmetry breaking, cross inhibition and equal harvest-rate distribution. Behav. Ecol. Sociobiol. 51, 557–569.

Deneubourg, J.L., Aron, S., Goss, S., Pasteels, J.M., 1990. The self-organizing exploratory pattern of the Argentine ant. J. Insect Behav. 3, 159–168. http://dx.doi.org/10.1007/BF01417909.

Dorai-Raj, S., 2009. Binomial Confidence Intervals For Several Parameterizations. R Package Version 1.0–6/r2.

Dorigo, M., Stützle, T., 2004. Ant Colony Optimization. MIT Press, Cambridge, Mass.

Dorigo, M., Maniezzo, V., Colorni, A., 1996. Ant system: optimization by a colony of cooperating agents. IEEE Trans. Syst. Man Cybern. B Cybern. 26, 29–41. http://dx.doi.org/10.1109/3477.484436.

Dussutour, A., Beekman, M., Nicolis, S.C., Meyer, B., 2009a. Noise improves collective decision-making by ants in dynamic environments. Proc. Biol. Sci. 276, 4353–4361. http://dx.doi.org/10.1098/rspb.2009.1235.

Dussutour, A., Nicolis, S.C., Shephard, G., Beekman, M., Sumpter, D.J., 2009b. The role of multiple pheromones in food recruitment by ants. J. Exp. Biol. 212, 2337–2348. http://dx.doi.org/10.1242/jeb.029827.

Dyer, A.G., Spaethe, J., Prack, S., 2008. Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. J. Comp. Physiol. A 194, 617–627. http://dx.doi.org/10.1007/s00359-008-0335-1.

Evison, S.E.F., Petchey, O.L., Beckerman, A.P., Ratnieks, F.L.W., 2008. Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. Behav. Ecol. Sociobiol. 63, 261–267.

Fechner, G., 1860. Elemente der Psychophysik. Breitkopf und Härtel.

Goss, S., Aron, S., Deneubourg, J.L., Pasteels, J.M., 1989. Self-organized shortcuts in the Argentine ant. Naturwissenschaften 76, 579–581. http://dx.doi.org/10.1007/BF00462870.

Grüter, C., Czaczkes, T.J., Ratnieks, F.L., 2011. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav. Ecol. Sociobiol. 65, 141–148.

Hangartner, W., 1969. Orientierung von Lasius fuliginosus Latr. an einer Gabelung der Geruchspur. Insect Soc. 16, 55–60. http://dx.doi.org/10.1007/BF02224462.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Harvard University Press, Cambridge, Mass.

Jackson, D.E., Châline, N., 2006. Modulation of pheromone trail strength with food quality in Pharao's ant, Monomorium pharaonis. Anim. Behav. 74, 463–470.

Kingdom, F.A.A., Prins, N., 2010. Psychophysics. Elsevier, London.

Klein, A., 2001. Measuring, estimating, and understanding the psychometric function: a commentary. Percept. Psychophys. 63 (8), 1421–1455.

Lanan, M.C., Dornhaus, A., Jones, E.I., Waser, A., Bronstein, J.L., 2012. The trail less traveled: individual decision-making and its effect on group behavior. PLoS One 7, e47976. http://dx.doi.org/10.1371/journal.pone.0047976.

Mailleux, A.C., Deneubourg, J.L., Detrain, C., 2000. How do ants assess food volume? Anim. Behav. 59, 1061–1069. http://dx.doi.org/10.1006/anbe.2000.1396.

Park, S.K., Miller, K.W., 1988. Commun. ACM 31, 1192–1201.

Perna, A., Granovskiy, B., Garnier, S., Nicolis, S.C., Labedan, M., Theraulaz, G., Fourcassie, V., Sumpter, D.J., 2012. Individual rules for trail pattern formation in Argentine ants (*Linepithema humile*). PLoS Comput. Biol. 8, e1002592. http://dx.doi.org/10.1371/journal.pcbi.1002592.

Press, W.H., Teukolsky, S.A., Vetterling, W.T., Flannery, B.P., 1992. Numerical Recipes in C, The Art of Scientific Computing. Cambridge University Press, New York.

R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsch, K., Reid, C.R., Beekman, M., Middendorf, M., 2012. A mathematical model of foraging in a dynamic environment by trail-laying Argentine ants. J. Theor. Biol. 306, 32–45. http://dx.doi.org/10.1016/j.jtbi.2012.04.003.

Robertson, P.L., Dudzinski, M.L., Orton, C.J., 1980. Excocrine gland involvement in trailing behaviour in the argentine ant (Formicidae: Dolichoderinae). Anim. Behav. 28, 1255–1273.

Sarris, V., 2006. Relational Psychophysics in Humans and Animals. Psychology Press, New York.

Sasaki, T., Granovskiy, B., Mann, R.P., Sumpter, D.J.T., Pratt, S.C., 2013. Ant colonies outperform individuals when a sensory discrimination task is difficult but not when it is easy. Proc. Natl. Acad. Sci. U.S.A. 110, 13769–13773. http://dx.doi.org/10.1073/pnas.1304917110.

Van Vorhis Key, S.E., Baker, T.C., 1981. Effects of gaster trail extract trail concentration on the trail following behaviour of the Argentine ant, *Iridomyrmex humilis* (Mayr). J. Insect Physiol. 27, 363–370.

Van Vorhis Key, S.E., Baker, T.C., 1982. Trail-following responses of the Argentine ant, *Iridomyrmex humilis* (Mayr) to a synthetic trail pheromone component and analogs. J. Chem. Ecol. 8, 3–14. http://dx.doi.org/10.1007/BF00984000.

Vittori, K., Talbot, G., Gautrais, J., Fourcassie, V., Araujo, A.F., Theraulaz, G., 2006. Path efficiency of ant foraging trails in an artificial network. J. Theor. Biol. 239, 507–515. http://dx.doi.org/10.1016/j.jtbi.2005.08.017.

von Thienen, W., Metzler, D., Choe, D.-H., Witte, V., 2014. Pheromone communication in ants: a detailed analysis of concentration dependent decisions in three species. Behav. Ecol. Sociobiol. 68, 1611–1627.

Weber, E.H., 1834. De Pulsu, resorptione, auditu et tactu. Annotationes anatomicae et physiologicae. In: Koehler, (Ed.), Leipzig.

Wichmann, F.A., Hill, N.J., 2001. The psychometric function: I. Fitting, sampling, and goodness of fit. Percept Psychophys. 63 (8), 1293–1313.

Wilson, E.B., 1927. Probable inference, the law of succession, and statistical inference. J. Am. Stat. Assoc. 22, 209–212.

Wilson, E.O., 1962. Chemical communication among workers of the fire ant Solenopsis saevissima (Fr. Smith). Anim. Behav. 10, 134–164.

Chapter 3

How memory and motivation modulate the responses to trail pheromones in three ant species

Wolfhard von Thienen, Dirk Metzler, Volker Witte (2016)
Behavioral Ecology and Sociobiology 70:393-407

Behav Ecol Sociobiol (2016) 70:393–407
DOI 10.1007/s00265-016-2059-5

ORIGINAL ARTICLE

How memory and motivation modulate the responses to trail pheromones in three ant species

Wolfhard von Thienen[1] · **Dirk Metzler**[1] · **Volker Witte**

Received: 4 April 2015 / Revised: 4 January 2016 / Accepted: 11 January 2016 / Published online: 21 January 2016

Abstract Ants are able to modulate their behavior according to private and collective information. Collective information is coded in different concentrations of pheromone deposited in the environment, especially on ant trails, whereas private information is learned and memorized by individual ants. It has been shown that both kinds of information act synergistically on the collective trail-following behavior of ant colonies. Another important factor influencing ant behavior is their motivation to follow pheromone trails. Here, we show how private information and motivation modulate the response to collective information. We investigate these effects using a recently proposed approach that employs psychophysical methods to measure the response to varying pheromone concentrations. We studied the effect of private information (route memory) in the species *Lasius niger*, *Euprenolepis procera*, and *Linepithema humile*. Additionally, the effect of motivation was studied in the species *E. procera* and *L. humile*. Using psychophysical methods, we quantified these effects for important biological parameters like behavioral thresholds and error rates. The differential changes in these parameters between the three species imply specific adaptations to their environment.

Keywords Ant behavior · Pheromones · Memory · Motivation · Starvation

Introduction

Ants use different kinds of signals to communicate (pheromones, tactile, and acoustical signals), of which trail pheromones are of great importance for the emergence of collective behavior (Wilson 1958; Hölldobler and Wilson 1990, p. 227; Hölldobler 1995; Hölldobler 1999). The response to the pheromone signal may be altered by route memory acquired by visual learning (Aron et al. 1988; Aron et al. 1993; Czaczkes et al. 2011; Grüter et al. 2011; Czaczkes et al. 2013). Different ant species show considerable differences in the extent to which they use visual learning and trail pheromones as source of information if both are available. *Linepithema humile* ants mainly respond to trail pheromone and less to visual learning while ants of the species *Temnothorax unifasciatus* and *Lasius niger* show a much higher response to visual learning compared to trail pheromone (Aron et al. 1988; Aron et al. 1993). These differences between species can be explained by *L. humile* mainly depending on collective information while *L. niger* and *T. unifasciatus* use individual information to a great extent besides collective information (Aron et al. 1993).

The response of *L. humile* to a combination of visual learning and trail pheromone depends on how often the training for visual learning was repeated implying that the strength of visual learning influences the response to the pheromone (Aron et al. 1993). In *L. niger*, results are controversial. Aron et al. (1993) and Grüter et al. (2011) found only a minor influence of repeated training, while Evison et al. (2008)

Communicated by M. Giurfa

Significance statement Collective organization in ants is significant to environmental adaptation. Worker foraging may be guided by private (memory) and social (pheromonal) information, and the relative reliance on these information sources should vary interspecifically. Memory and motivation were found to vary in their influence on response thresholds to trail pheromones and error rates in three ant species. This variation among species underscores specific ecological adaptations.

✉ Wolfhard von Thienen
w@thienen.de

[1] Department Biologie II, Ludwig-Maximilians Universität München, Großhaderner Str. 2, 82152 Planegg, Germany

showed visual learning enhances the response to trail pheromone at all concentration levels in *L. niger.* This discrepancy might be explained through differences in bioassays and pheromone concentrations used between the studies.

When ants are able to use visual learning and pheromones as two independent information sources, the error rate in trail following is reduced, thus increasing trail-following speed. Consequently, the availability of two independent information sources increases the foraging efficiency of ants (Grüter et al. 2011; Czaczkes et al. 2013).

Although the above-mentioned studies revealed crucial information about the social communication in ants, there were some aspects that they did not consider.

1. All of the above-mentioned studies used only up to four pheromone concentrations, which were only measured indirectly. Thus, we do not know the exact relationship between route memory, pheromone concentration and route following.
2. The time gap between training and testing was short, since the training of the ants was done immediately before testing their behavior. Only in the study of Evison et al. (2008), the time gap between training and testing was up to 120 min which might be an alternative explanation of the discrepancy of their results with those of Aron et al. (1993) and Grüter et al. (2011).
3. Visual learning can influence the response to trail pheromone in two different ways. First, if the information provided by pheromone and visual learning is congruent, both information sources act synergistically and may for instance increase the foraging efficiency. On the other hand, if information from the two different sources are not congruent, they cause a conflict between social and private information and may, for instance, increase the errors ants make in following a pheromone trail (Czaczkes et al. 2011; Grüter et al. 2011). Both ways are of different biological importance and may show characteristic differences between species. However, the studies on ants mentioned so far investigated either the synergistic situation or the conflicting situation but not both which may be another cause for the differences in their findings.

Besides private information and pheromones, the nutritional status of a colony influences the foraging behavior of an ant colony. In *Myrmica sabuleti* starvation increases the individual motivation to forage and the communication among individuals by lowering the response threshold to recruitment signals (de Biseau and Pasteels 2000). When *L. niger* ants are experiencing increased levels of starvation, individuals inside the nest show a growing tendency to aggregate around the nest entrance, ready to take in food from returning ants by trophallaxis, while ants close to the entrance show an increased tendency to leave the nest in search of food. This behavior can be explained by the high motivation of starved colonies to exploit new food sources. By aggregating close to the entrance, the ants speed up the process of food intake. Ants gathering close to the entrance are assumed to have a lower response threshold to the recruitment signals (trail pheromone and/or tactile signals) and thus show a higher tendency to leave the nest and follow the pheromone trail towards the food source (Mailleux et al. 2011).

In our experiments, we aimed to go further than the above-mentioned studies by investigating the relationship between pheromone concentration and trail-following behavior, including the effect of private information (route memory) and motivation (starvation) on this relationship. In our investigation of route memory, we included the effects of conflicting as well as synergistic information sources. By using clearly phylogenetically and ecologically distinct species, we tested whether differences between species can be attributed to specific adaptations to a given environment.

To evaluate the relationship between ants' response and pheromone concentration under the influence of memory or starvation, we used a previously established psychophysical method to assess the pheromone-dependent responses in ants (von Thienen et al. 2014) and fitted *psychometric functions (PF)* to the data (see Appendix for an introduction into psychophysics).

Based on the above-mentioned studies, we hypothesized that the addition of private information or motivation would alter the collective behavior of the ants. Further, these changes would reflect in the parameters of the PFs. Due to the high sensitivity of psychophysical methods, we expected to gain a deeper insight into factors like thresholds and error rates that influence ant behavior. In detail, we expected to find following effects:

Hypothesis 1

The effects of memory should be lower in a species that mainly depends on collective information compared to a species that uses private information as well. Thus, we expect *L. humile* to show smaller effects of memory than *L. niger.* For *E. procera*, we could not make such a prediction since data about the extent of their collective and private information use are not available.

Hypothesis 2

Memory should alter the response to the trail pheromone.

Hypothesis 3

Remembering an existing food source should lower the detection threshold if the trail leads towards the food source since pheromone and memory effects should amplify each other.

Hypothesis 4

a) If two independent information sources (pheromone and memory) are providing contradictory information, the lapse rate (a measure for the error rate) should increase due to an increased uncertainty.
b) In the opposite case, where pheromone and memory provide congruent information, the lapse rate should decrease.

Hypothesis 5

Starvation should alter the response to the trail pheromone.

Hypothesis 6

Starved ants will be eager to get food and thus should have a higher attentiveness to the pheromone resulting in a lower detection threshold.

Hypothesis 7

Starved ants will be eager to get food and thus have a very low tendency to ignore a pheromone trail, resulting in a low lapse rate.

Hypothesis 8

Starvation should alter the discrimination response (the response if two pheromones sources of different concentration are offered to the ants).

Materials and methods

We used the same species, pheromone sources, experimental setups, statistical methods, and fitting procedures as described in a previous study (von Thienen et al. 2014). Here, we give a summary.

Species and pheromones used

Experiments were conducted with laboratory colonies of the species *L. humile*, *E. procera*, and *L. niger*. *L. humile (Dolichoderinae)* originates from Argentina, but is now invasive on most continents (Wetterer et al. 2009), where it forms extensive polygynous supercolonies (Giraud et al. 2002). Brood and workers are frequently exchanged between the nests (Holway and Case 2000). *L. humile* uses locally stable food sources like honeydew, but also distributed and short-lived food sources like insects, seeds or carrion (Mallis 1942; Suarez et al. 1998). As trail pheromone, we used synthetic (*Z*)-9-hexadecenal (Bedoukian Research, Danbury/USA, product no. P4100-90), a molecule from the Pavan's gland which was identified as the main component of the trail pheromone complex (Wilson and Pavan 1959; Cavill et al. 1979; Cavill et al. 1980; Robertson et al. 1980; Van Vorhis Key and Baker 1982). *E. procera* (*Formicinae*) lives in the tropical rainforests of Malaysia and Indonesia. It feeds mainly on mushrooms (Witte and Maschwitz 2008). Colonies are polygynous and consist of 500–20,000 individuals. The ants forage only at night and maintain an extensive trail system, in which individual trails to different mushroom patches can be abandoned and reactivated (von Beeren et al. 2014). As source for the trail pheromone, we used extracts from the rectum, which had been identified as the source of the trail pheromone (Witte, unpublished data, cited in von Thienen et al. 2014). Lastly, *L. niger* (*Formicinae*) builds monogynous colonies with an average of 14,000 individuals (Seifert 2007). The ants feed on insects and build long-lasting trails to trophobiosis sites, where they collect honeydew. They are active during the day and at night (Seifert 2007). We used extract from the rectum as source for trail pheromone (Bestmann et al. 1992). For each species, the colonies used for our experiments were of similar size (1500–2000 workers including queen(s)).

Pheromones were prepared and dissolved in dichloromethane (DCM) as described by von Thienen et al. (2014). Pheromone concentrations for *E. procera* and *L. niger* are given in *ant equivalents per cm (AE/cm)*. An ant equivalent corresponds to the pheromone content of one rectum. For *L. humile*, we give absolute concentrations in pg/cm. All concentrations reported here are given in units relative to a *base concentration (bc)*, which is 0.0057 AE/cm for *E. procera* and *L. niger* and 30.8 pg/cm for *L. humile*. The base concentration was similar to that of naturally formed trails, see von Thienen et al. (2014).

Detection experiments

To test the ants' ability to detect different pheromone concentrations, we conducted bifurcation experiments in which ants had to cross a y-shaped bridge between their nest and a food source. One branch of the y-bridge was marked with varying concentrations of pheromone forming an artificial trail of length 7 cm and width 2 mm. We refer to this branch as *test trail*. The other branch, the *reference trail*, did not carry any pheromone. The probability that the ants took the test trail—calculated as the proportion of ants crossing the test trail—was taken as a measure of the ants' response to the pheromone. For each bridge, the first 10 ants were counted. The orientation of test trail and reference trail were switched each time a new y-bridge was applied. We used at least 10 bridges with 100 tested ants in total for each concentration. In a few cases, ants stopped foraging before reaching this number. In another few cases, ants were foraging very actively so that we could test more than 100 ants.

Discrimination experiments

To test the ants' ability to discriminate different pheromone concentrations, we conducted bifurcation experiments similar to those above. However, the reference trail was marked with pheromone of constant concentration (1 base concentration) instead of being unmarked.

Behavioral assay for memory

Prior to the experiments, ants had been deprived of food for 3 days to motivate them to forage. In a first step, ants were conditioned to the left side of the y-bridge by offering food ad libitum at the end of the left side bridge and allowing the ants to forage for 60 min. To enhance the orientation, the left side was lit with a microscopic fiber optic source. After about 20 min, the ants had discovered the food and established a constant stream of foraging ants between the nest and the food. The subsequent training phase lasted another 40 min, so that most of the active foragers should have visited the food source at least once. We conducted detection experiments 24 h after the training phase. The environment of the test arena, including the lighting, remained constant. As a control, we tested untrained ants with the same behavioral assay. It is possible that differences in the foraging strategies of the species result in differences of how often individual ants visit the food source. The bioassay measures the behavior at colony level, not at individual level. However, since colonies were of similar size (1500–2000 workers) and the ants were continuously foraging, we do not expect great differences between the species in individual visiting frequency.

With these experiments, we measured different combinations of memory and pheromone treatments: The effect of pheromone without memory was measured using untrained ants (control). We refer to these measurements as *pheromone alone (P-alone)*. If pheromone was placed at the same side that the ants had been conditioned to (left side), the test looked at the combined effect of pheromone and positive conditioning. We refer to these treatments as *pheromone plus memory (P+M)*. If pheromone was placed at the opposite side (right side), the test investigated the effect of pheromone contradicting conditioning. We refer to these treatments as *pheromone* versus *memory (PvsM)*. Measurements of *P+M* and *PvsM* are considered as simultaneous since the orientation of test trail and reference trail were switched each time a new y-bridge was applied. Therefore, effects of timing and order can be excluded. To test the effect of memory in the absence of pheromone, we evaluated the response at concentration 0 bc. *P+M* measurements tested the effect of positive conditioning without pheromone. *PvsM* tested the effect of opposite conditioning without pheromone. Finally, *P-alone* represented the null effect of neither pheromone nor memory. In this case, we expected the ants to select either side by chance with equal probability and used this as additional control. Since ants stopped foraging after a certain time, we used up to three colonies for each treatment. The treatments of a single colony always included all pheromone concentrations. For the treatments of *P+M* and *PvsM*, the same colonies were used, while for *P-alone*, the colonies were distinct.

Behavioral assay for motivation

To investigate the influence of motivation, we used ants of the species *E. procera* and *L. humile* in detection experiment, as described above. Since no method exists to measure the ants' motivation directly, we used the following indirect method. We assumed that by depriving ants of food for several days, their motivation to forage would increase. Thus, we took the time without fresh food as an indirect measure for the motivational state of the ants. Prior to the behavioral experiment, *L. humile* was starved for 6 or 9 days by removing the food supply of the laboratory colonies. *E. procera* was starved for 3 or 6 days. In preliminary experiments, these starvation periods showed clear effects. As a control, the same experiments were conducted with ants that had not been starved at all (0 days). We ran an additional control in the absence of pheromone (concentration 0 bc) whereby we expected that ants would select either side with equal probability. To test the effect of motivation on the ants' ability to discriminate between two pheromone sources, we performed a discrimination experiment on *E. procera* only. For each species, we used the same colony for all experiments. Please note that it is likely the ants were not without food right from the time the food supply was removed because an unknown amount of food was stored in their crops. Thus, we do not know the exact time at which all food supplies were completely exhausted and the ants began to starve. However, our aim was to study a motivation effect qualitatively, rather than quantitatively which would depend on the exact starvation period (if this is possible at all).

Statistical methods and model fitting

As results of our experiments, we calculated the ants' responses to different pheromone concentrations, fitted PFs to the data and evaluated the parameters of the PFs. This was done according to von Thienen et al. (2014). As statistical software we used "R", Version 2.14.2 (R Development Core Team 2013). We estimated the probability of ants choosing the test trail (response) and its 95 % confidence interval using the function binom.confint() from the package binom (Dorai-Raj 2009) with the Wilson-method (Wilson 1927).

To test hypothesis 1, we compared the effect of memory in the treatments *P+M* and *P-alone* for the species *L. humile* and *L. niger* using a generalized linear model (GLM) to predict the responses of the ants. The model included the numerical covariable *pheromone* *concentration*, the factors *species* (two levels: *L. humile*, *L. niger*), *training* (two levels: trained and untrained) and the interaction between *species* and *training*. A species was considered as showing a significantly higher effect of memory, if the GLM-estimate of the interaction between *species* and *training* was positive and yielded a *p* value below significance level ($\alpha = 0.05$). We used the R-functions glm() with the option family="quasibinomial" and the default link function, such that a logistic regression analysis was performed (R Development Core Team 2013).

To test hypothesis 2, we pairwise compared the results of the three treatments (*P+M*, *PvsM*, *P-alone*) for each species. We applied a similar GLM as before including the numerical covariable *pheromone concentration* and the factor *treatment* for each comparison (two levels for each comparison). A species was considered as showing a significant memory effect if factor *treatment* showed a *p* value below significance level in at least one of the comparisons ($\alpha_{\mathrm{Bf}} = 0.0017$, Bonferroni adjusted for three comparisons). The memory effect was considered to increase the ants' response to the pheromone if the GLM-estimate of the treatment *P+M* was positive and the *p* value was below the significance level ($\alpha = 0.05$).

To test hypotheses 5 and 8, we applied a similar GLM including the numerical covariable *pheromone concentration* and the factor *hunger* (three levels: 0/6/9 days for *L. humile* and 0/3/6 days for *E. procera*). The effect of starvation was considered as significant if the significance level for the factor *hunger* was below the significance level ($\alpha = 0.05$).

To compare the parameter values of the PFs (hypotheses 3 and 4), we pairwise compared their confidence intervals between the different treatments (*P+M*, *PvsM* and *P-alone*). If they did not overlap, the PF parameter values were considered as significantly different ($\alpha_{\mathrm{Bf}} = 0.017$, Bonferroni adjusted for three comparisons). The confidence intervals were evaluated by parametric bootstrap with 1000 iterations. In a similar way, we compared the parameters of the starvation treatments to the control (hypotheses 6 and 7) ($\alpha_{\mathrm{Bf}} = 0.025$, Bonferroni adjusted for two comparisons).

To compare different treatments in the absence of pheromone (at 0 bc), we used a proportion tests with the R-function prop.test() which is based on a χ^2 test (R Development Core Team 2013).

Statement concerning blindness of the study: The counting of the ants had been done by two students who did not know the hypotheses. The students knew the pheromone concentration, training, and/or starvation period of the ants. Additionally, they knew that ants respond in a probabilistic manner to pheromone trails of different concentrations in bifurcation experiments.

Results

Table 1 and Figs. 1, 2, and 3 show the results of the memory experiments. Table 2 and Figs. 4, 5, and 6 show the results of the motivation experiments. Table 3 shows a summary of the comparisons of hypothetical and actual results.

Comparison of memory effect between *L. niger* and *L. humile*

The generalized linear model gave a positive and significant estimate for the interaction between training and the species *L. niger* (Estimate = 0.55, $t = 2.86$, $p = 0.0045$) showing that the effect of visual memory in *L. niger* was significantly higher than in *L. humile*. These results are consistent with hypothesis 1.

Memory experiments—*L. humile*

1. Pairwise comparison of the three treatments by GLM showed no significant difference (Fig. 1, Table 1). This is not consistent with hypothesis 2.
2. The detection threshold of the PF was higher but not significantly higher for *P+M* compared to *P-alone*. This is not consistent with hypothesis 3.
3. The lapse rate was not significantly higher in *PvsM* compared to *P-alone* which is not consistent with hypothesis 4a. The lapse rate in *P+M* was significantly lower which is consistent with hypothesis 4b.
4. The response in the absence of pheromone at 0 bc for the treatment *P+M* was not significantly different from 0.5, showing no significant effect ($p = 0.76$, two-sided proportion test, $n = 100$). In contrast, the response for *PvsM* was significantly lower than 0.5 ($p = 0.03$, one-sided proportion test, $n = 100$). The response for *P-alone* at 0 bc was, as expected, not significantly different from 0.5 ($p = 0.62$, two-sided proportion test, $n = 100$).

Memory experiments – E. procera

1. The comparison of treatments *P+M* and *PvsM* as well as the comparison between *PvsM* and *P-alone* showed significant higher GLM-estimates which is consistent with hypothesis 2 (Fig. 2, Table 1). The other comparison was not significant.
2. The detection thresholds in *P+M* and *P-alone* showed no significant differences, which is not consistent with hypothesis 3.
3. The lapse rate of *PvsM* was significantly higher compared to *P-alone* and the lapse rate of *P+M* was significantly lower, which is consistent with hypothesis 4a and b.

Table 1 Memory experiments—pairwise comparison of different treatments

Species	Treatment	Detection threshold	Lapse rate	GLM		
				Estimate	t	p
L. humile	P+M	*0.15*–0.21–*0.29*	***0.0***–**0.02**–***0.03***	0.23	1.5	0.13
	P-alone	*0.11*–0.15–*0.2*	***0.08***–**0.10**–***0.12***			
	PvsM	***0.05***–**0.08**–***0.11***	*0.07*–0.09–*0.11*	0.07	0.44	0.66
	P-alone	***0.11***–**0.15**–***0.2***	*0.08*–0.10–*0.12*			
	P+M	***0.15***–**0.21**–***0.29***	***0.0***–**0.02**–***0.03***	0.16	1.2	0.25
	PvsM	***0.05***–**0.08**–***0.11***	***0.07***–**0.09**–***0.11***			
E. procera	P+M	*0.19*–0.25–*0.33*	***0.0***–**0.0**–***0.01***	0.16	1.29	0.2
	P-alone	*0.14*–0.2–*0.28*	***0.04***–**0.06**–***0.08***			
	PvsM	*0.16*–0.24–*0.42*	***0.17***–**0.21**–***0.24***	**−0.39**	**−3**	**0.003**
	P-alone	*0.14*–0.2–*0.28*	***0.04***–**0.06**–***0.08***			
	P+M	*0.19*–0.25–*0.33*	***0.0***–**0.0**–***0.01***	**0.55**	**4.13**	**5.9e-5**
	PvsM	*0.16*–0.24–*0.42*	***0.17***–**0.21**–***0.24***			
L. niger	P+M	***0.03***–**0.06**–***0.1***	***0.06***–**0.06**–***0.1***	**0.44**	**−4.14**	**4.1e-5**
	P-alone	***0.1***–**0.15**–***0.27***	***0.1***–**0.14**–***0.16***			
	PvsM	***0.31***–**0.6**–***1.04***	***0.17***–**0.21**–***0.24***	**−0.62**	**−6.8**	**4.7e-11**
	P-alone	***0.1***–**0.15**–***0.27***	***0.1***–**0.14**–***0.16***			
	P+M	***0.03***–**0.06**–***0.1***	***0.06***–**0.06**–***0.1***	**1.05**	**8.8**	**1.0e-15**
	PvsM	***0.31***–**0.6**–***1.04***	***0.17***–**0.21**–***0.24***			

The ants were trained for 40 min to associate the left side with food 24 h before the experiment. Parameter values are given with their confidence limits in italics, calculated by parametric bootstrap with 1000 iterations ($\alpha_{\mathrm{Bf}}=0.0017$, Bonferroni adjusted for 3 comparisons). Parameter values are considered as significantly different if their confidence intervals did not overlap. *GLM* gives the results (*Estimate*, *t* and *p* value) for the factor *treatment* of a generalized linear model estimating the ants' response by including the numerical covariable *pheromone concentration* and the factor *treatment* with two levels. The upper treatment shows significantly higher responses if *Estimate* is positive and *p* value is below significance level ($\alpha_{\mathrm{Bf}}=0.0017$, Bonferroni adjusted for 3 comparisons). Bolded results are significant. The 75 %-detection threshold is given in units relative to the base concentration (bc), which is 0.0057 AE/cm for *E. procera* and *L. niger* and 30.8 pg/cm for *L. humile*

P+M pheromone plus memory, *PvsM* pheromone versus memory, *P-alone* pheromone alone

4. The response in the absence of pheromone at 0 bc for the treatment *P+M* was not significantly different from 0.5, showing no significant effect of memory ($p=0.76$, two-sided proportion test, $n=100$). The same holds for *PvsM* ($p=0.76$, two-sided proportion test, $n=100$). The response for *P-alone* at 0 bc was, as expected, not significantly different from 0.5 ($p=0.76$, two-sided proportion test, $n=100$).

Memory experiments—*L. niger*

1. The comparison of treatment *P+M* to the other treatments showed significantly higher GLM-estimates which is consistent with hypothesis 2 (Fig. 3, Table 1).
2. The detection threshold in *P+M* was significantly lower compared to *P-alone*, which is consistent with hypothesis 3.
3. The lapse rate of *PvsM* was significantly higher compared to *P-alone* and the lapse rate of *P+M* was significantly lower, which is consistent with hypothesis 4a and b.
4. The response in the absence of pheromone at 0 bc for the treatment *P+M* was not significantly different from 0.5, showing no significant effect of memory ($p=0.4$, two-sided proportion test, $n=110$). The response for *PvsM* was lower than 0.5 but with significance level slightly above 0.05 ($p=0.076$, one-sided proportion test, $n=110$). The response for *P-alone* at 0 bc was, as expected, not significantly different from 0.5 ($p=0.58$, two-sided proportion test, $n=160$).

Motivation experiments—*L. humile*

The treatments showed a significant effect in the GLM, which is consistent with hypothesis 5 (Fig. 4, Table 2). The thresholds after 6 and 9 days were significantly lower than the control, which is consistent with hypothesis 6. The results were not consistent with hypothesis 7 since the lapse rates were not significantly lower. As expected, the effect of the absence of pheromone at 0 bc was not significant (0 days $p=0.92$, 6 days $p=0.92$, 9 days $p=1$, two-sided proportion test, $n=100$).

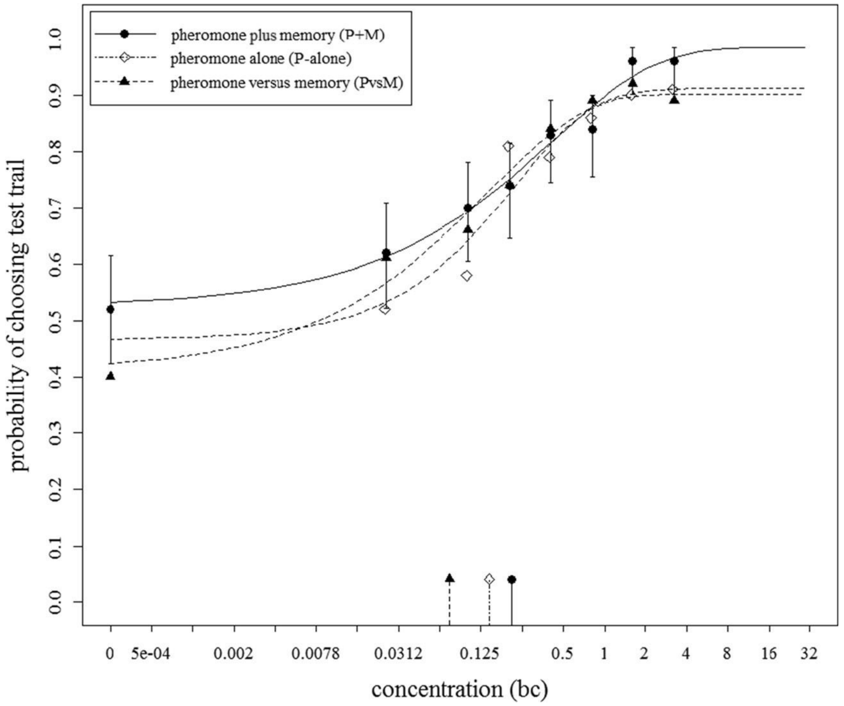


Fig. 1 Influence of memory in detection experiments with *L. humile*. Results are presented for untrained ants as control (*P-alone*), trained ants with pheromone placed on the side to which the ants had been conditioned to (*P+M*) and trained ants with pheromone on the opposite side (*PvsM*). The *y*-axis shows the probability (response) of ants taking the test trail with the pheromone. The *symbols* (•, ▲, ◊) represent the results and the *bars* show the 95 %-confidence interval. For clarity reasons, confidence intervals are only given for *P+M*, other CIs are of similar magnitude. The *x*-axis shows the pheromone concentration on the test trail with synthetic (*Z*)-9-hexadecenal measured in base concentrations (1 bc = 30.8 pg/cm) on a $\log_2$-scale. The reference trail carried no pheromone. The *symbols at the bottom* indicate the 75 %-detection thresholds. *Lines* show fitted PFs. Number of measurements for each treatment at each concentration = 10 (each measurement represents 10 binomial ant decisions)

Motivation experiments – E. procera

The treatments showed a significant effect in the GLM which is consistent with hypothesis 5 (Fig. 5, Table 2). Consistently with hypothesis 6, the threshold of the PF was significantly lower after 6 days compared to the control. However, the lapse rate was significantly higher, falsifying hypothesis 7. The measurements after 3 days hunger showed no major differences from the control. As expected, the effect of the absence of pheromone was not significant (0 days $p = 0.92$, 3 days $p = 0.92$, 6 days $p = 0.76$, two-sided proportion test).

E. procera was the only species in which we tested the effect of starvation in discrimination experiments. The comparison between the treatments by GLM was significant which is consistent with hypothesis 8 (Fig. 6, Table 2). The difference was mainly caused by the high lapse rate of sated ants (control) while the discrimination thresholds were not significantly different. Since the pheromone stimuli at both branches were equal at 1 bc, we expected the ants to respond by chance with a probability of 0.5 for either branch after 0 as well as after 6 days. This expectation was met when the responses were not significantly different from 0.5 (0 days $p = 0.76$, 6 days $p = 0.92$, two-sided proportion test).

Discussion

Memory and motivation showed clear effects on the collective pheromone guided behavior of the ant species we investigated. These effects are reflected in the parameters of the psychometric function and show that memory and motivation modulate the process by which the perception of trail pheromones is transformed into behavior. The effects differ between the species and reflect different ecological needs. Of all three species under investigation, *L. niger* showed the strongest effects of memory. Their response to trail pheromone of various concentration was clearly enhanced, the detection threshold was much lower and the lapse rate sank. *L. humile*, on the other hand, showed only small effects of memory. The responses to the trail pheromone showed no significant differences. Only the lapse rate of *P+M* was significantly lower compared to *P-alone*. Comparing the results between *L. niger* and *L. humile* with a generalized linear model showed that the effect of memory is significantly greater in *L. niger* than in *L. humile*. This is consistent with our first hypothesis that memory has a lower effect on the response to trail pheromones in a species that mainly depends on

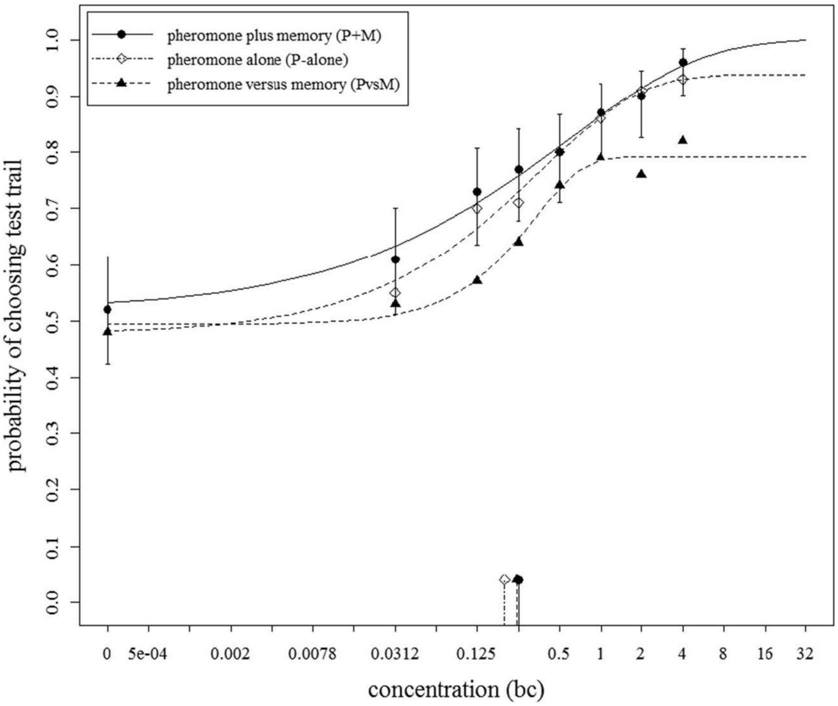


Fig. 2 Influence of memory in detection experiments with *E. procera*. The *x*-axis shows the pheromone concentration on the test trail with extract from the rectum measured in base concentrations (1 bc = 0.0057 AE) on a $\log_2$-scale. The reference trail carried no pheromone. Number of measurements for each treatment at each concentration *n* = 10 (each measurement represents 10 binomial ant decisions). For further explanations, please refer to Fig. 1

collective information compared to one that uses private information as well. *E. procera* is positioned somewhere between the other two species. The ants showed a significant effect of memory, but only when memory and pheromone gave contradictory information. This manifested in a much lower lapse rate while the detection thresholds showed no significant differences. As we have stated, there are no studies on the extent of the use of collective and private information for *E. procera*. For this reason, we could not rank them like Aron et al. (1993) did for *L. humile* and *L. niger*. At this point, it is important to mention that Aron et al. (1993) did not give an exact quantitative definition of collectiveness relating it instead to factors like colony size, short- or long-lived food sources or emigration strategies. Other information sources besides pheromones and vision that the ants might

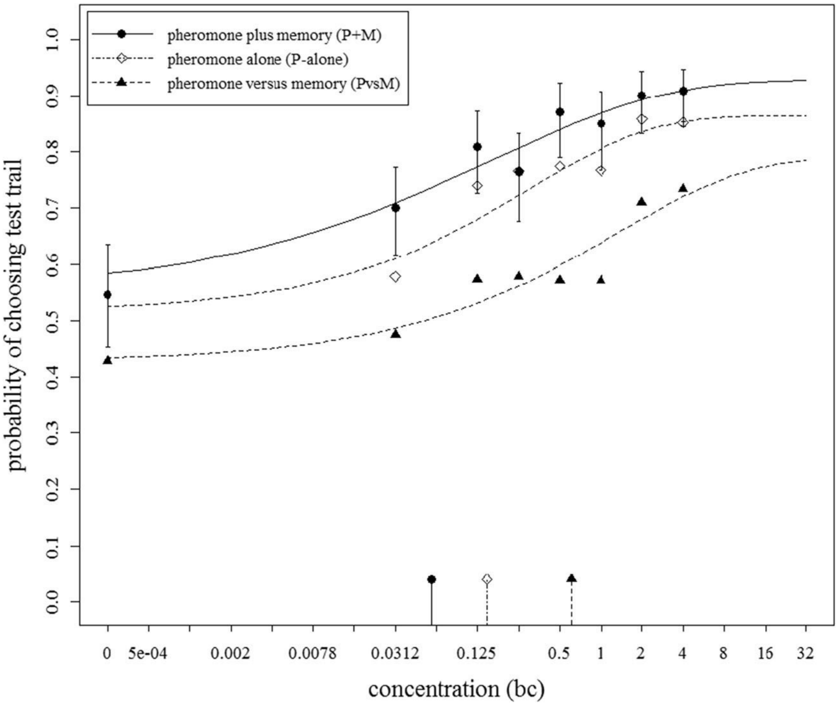


Fig. 3 Influence of memory in detection experiments with *L. niger*. The *x*-axis shows the pheromone concentration on the test trail with extract from the rectum measured in base concentrations (1 bc = 0.0057 AE) on a $\log_2$-scale. The reference trail carried no pheromone. The *symbols at the bottom* indicate the 75 %-detection thresholds. *Lines* show fitted PFs. Number of measurements at each concentration = 11/11/53, 13/12/50, 11/11/20, 11/9/20, 10/10/31, 10/10/54, 12/10/52, 13/12/56 (from left to right, *P+M*/*PvsM*/*P-alone*). Each measurement represents 10 binomial ant decisions. For further explanations, please refer to Fig. 1

Table 2 Motivation experiments—pairwise comparison of different treatments

Species/experiment	Hunger	threshold	Lapse rate	GLM		
				Estimate	t	p
L. humile	0 days	*0.06*–0.08–*0.11*	*0.0*–0.01–*0.02*	**0.042**	**2.02**	**0.043**
detection	6 days	***0.03*–0.04–*0.05***	***0.04*–0.05–*0.06***			
	9 days	***0.01*–0.02–*0.02***	*0.0*–0.01–*0.02*			
E. procera	0 days	*1.34*–1.65–*1.95*	*0.0*–0.0–*0.003*	**0.058**	**2.36**	**0.02**
detection	3 days	*0.98*–1.19–*1.42*	*0.0*–0.003–*0.012*			
	6 days	***0.33*–0.4–*0.48***	***0.048*–0.065–*0.086***			
E. procera	0 days	*1.65*–2.0–*7.0*	***0.41*–0.42–*0.44***	**−0.05**	**−2.6**	**0.009**
discriminxation	6 days	*1.66*–1.9–*2.4*	***0.23*–0.25–*0.26***			

Threshold gives the 75 %-detection threshold or the 75 %-discrimination threshold, respectively. Parameter values are given with their confidence limits in italics, calculated by parametric bootstrap with 1000 iterations (for detection $\alpha_{Bf} = 0.025$, Bonferroni adjusted for 2 comparisons, for discrimination $\alpha = 0.05$). Parameter values are considered as significantly different if their confidence intervals did not overlap. The comparison is made between *x*-days hunger and 0 days hunger. *GLM* gives the results (*Estimate*, *t*, and *p* value) for the factor *hunger* of a generalized linear model estimating the ants' response by including the numerical covariable *pheromone concentration* and the factor *hunger* with three levels. The treatment shows significantly higher responses if *Estimate* is positive and *p* value is below significance level ($\alpha = 0.05$). Bolded results are significant. The 75 %-detection threshold is given in units relative to the base concentration (bc), which is 0.0057 AE/cm for *E. procera* and 30.8 pg/cm for *L. humile*. The 75 %-discrimination threshold is dimensionless

use, like sensorimotor orientation, had not been considered. We know that *E. procera* depends on a high degree on pheromone-based communication (von Thienen et al. 2014) and we know from many field and laboratory observations that *E. procera* ants are very sensitive to visual stimuli despite their nocturnal activity pattern. Thus, it is reasonable to assume that they incorporate visual information into their trail-following behavior ranking

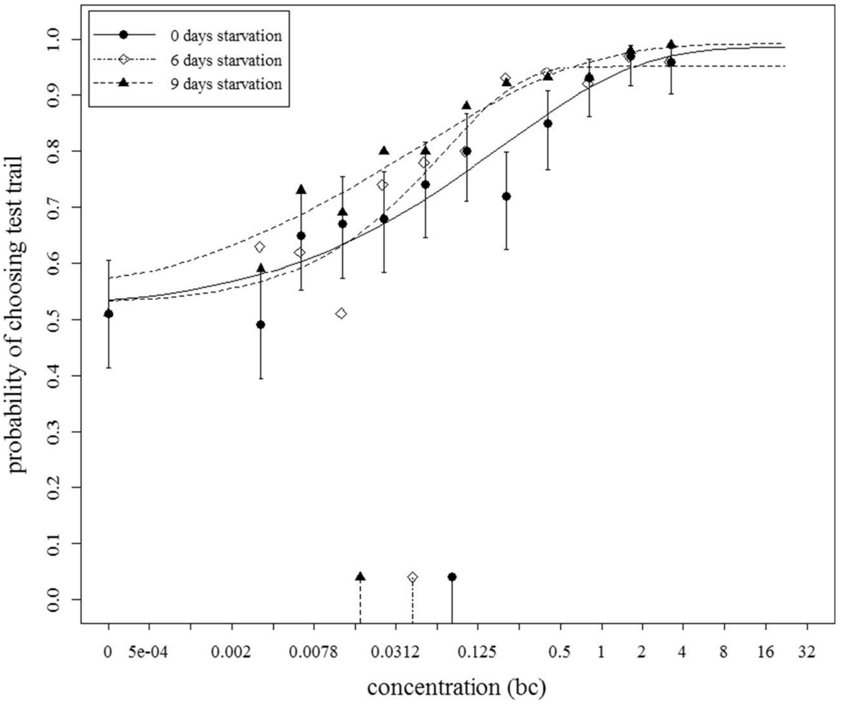
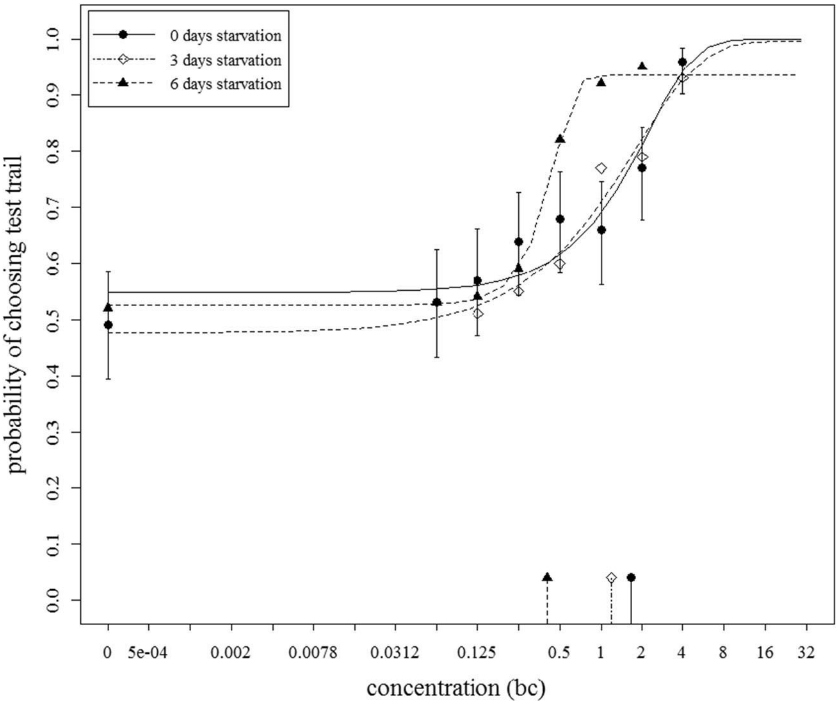


Fig. 4 Influence of motivation in detection experiments with *L. humile*. Results are presented for ants that had been without food for 0 (control), 6 and 9 days. The *symbols* (•,▲, ◊) show the probability (response) that ants take the test trail carrying the pheromone and the *bars* show the 95 %-confidence interval. For clarity reasons, confidence intervals are only given for the control, other CIs are of similar magnitude. The *x*-axis shows the pheromone concentration on the test trail with synthetic (*Z*)-9-hexadecenal measured in base concentrations (1 bc = 30.8 pg/cm) on a $\log_2$-scale. The reference trail carried no pheromone. The *symbols at the bottom* indicate the 75 %-detection thresholds, while *lines* show the fitted PFs. Number of measurements at each concentration = 10 (each measurement represents 10 binomial ant decisions)

Fig. 5 Influence of motivation in detection experiments with *E. procera*. Results are presented for ants that had been without food for 0 (control), 3 or 6 days. The *x*-axis shows the pheromone concentration on the test trail with extract from the rectum measured in base concentrations (1 bc = 0.0057 AE) on a $\log_2$-scale. The reference trail carried no pheromone. Number of measurements at each concentration *n* = 10 (each measurement represents 10 binomial ant decisions). For further explanations, please refer to Fig. 4



them between *L. niger* and *L. humile*. Further research is necessary to find a consistent definition and method to measure the degree of collectiveness in ant species. Parameters of the PF like lapse rate and detection/discrimination threshold might be important aspects in such a method.

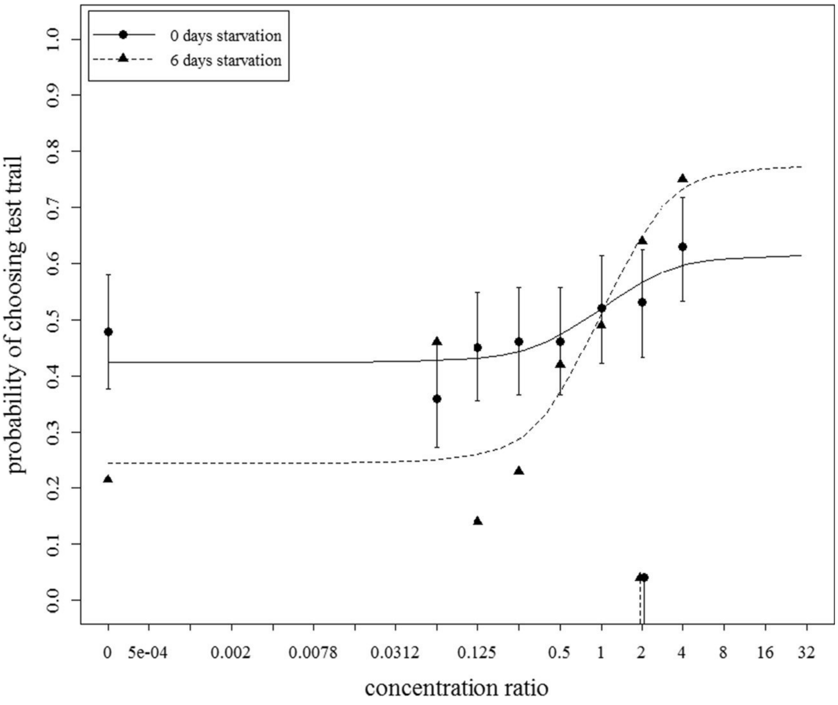


Fig. 6 Influence of motivation in discrimination experiments with *E. procera*. Results are presented for ants that had been without food for 0 (control) and 6 days. The *x*-axis shows the ratio of pheromone concentrations between test and reference trail on a $\log_2$-scale. The reference trail had a constant concentration of 1 base concentration of rectum extract (1 bc = 0.0057 AE/cm). The test trail had varying concentrations measured in proportions of bc. The *symbols at the bottom* indicate the 75 %-discrimination thresholds. Number of measurements at each concentration = 10 (except at concentration ratio 0 at 0 days: 9, and concentration ratio 0 at 6 days: 20), each measurement represents 10 binomial ant decisions. For further explanations, please refer to Fig. 4

Table 3 Comparison of hypothetical and actual results

Hypothesis				Testing method (see also statistics section)
1. The effects of memory are lower in a species that mainly depends on collective information (*L. humile*) compared to a species that uses private information as well (*L. niger*)		Yes		GLM with the covariable *pheromone concentration* and the factors *training* and *species*
	L. humile	*E. procera*	*L. niger*	
2. Memory changes the response to the pheromone	No	Yes	Yes	GLM with the covariable *pheromone concentration* and the factor *treatment*
3. Memory lowers the detection threshold	No	No	Yes	Comparison of the confidence intervals of the detection thresholds of *P+M* and *P-alone*.
4. (a) Contradictory information leads to a higher lapse rate (b) Congruent information leads to a lower lapse rate	No Yes	Yes Yes	Yes Yes	Comparison of the confidence intervals of the lapse rates of (a) *P+M* and *P-alone*, (b) *PvsM* and *P-alone*
5. Starvation changes the response to the pheromone	Yes	Yes	N/A [1]	GLM with the covariable *pheromone concentration* and the factor *hunger*
6. Starvation lowers the detection threshold	Yes	Yes	N/A [1]	Comparison of the confidence intervals of the detection thresholds
7. Starvation lowers the lapse rate	No	No	N/A [1]	Comparison of the confidence intervals of the lapse rates
8. Starvation changes the discrimination response	N/A [2]	Yes	N/A [2]	GLM with the covariable *pheromone concentration* and the factor *hunger*

Yes indicates that the results are consistent with the hypothesis, *No* if not

P+M pheromone plus memory, *PvsM* pheromone versus memory, *P-alone* pheromone alone

(1) Motivation was not tested in *L. niger*

(2) Discrimination experiments for motivation were done with *E. procera* only

In motivation experiments, the two species under investigation, *L. humile* and *E. procera*, showed similar results. The responses to the pheromone were significantly enhanced by starvation in both species. The detection thresholds were significantly lower after the longest starvation period. However, they were lower, but not significantly, at the medium starvation period. These results show that the effect of starvation increased with the starvation period in both species.

Looking separately at the results of each species, we find that the GLM-analysis of the memory experiments with *L. humile* showed no significant effect on the pheromone response after 24 h. In contrast, the analysis of the PF-parameters showed subtle details. The lapse rate was significantly lower in the *P+M* treatment compared to *P-alone*. In the opposite case, where both information sources were contradictory, the lapse rates did not differ significantly. We explain this asymmetry by collective information being much more important than private information to *L. humile* ants. In case of congruent information, visual memory helps reducing the uncertainty while alone, the effect of private information is too weak to increase the uncertainty. The detection threshold in *P+M* compared to *P-alone* was not significantly different, but in *PvsM* it was significantly lower compared to *P-alone*. The fact that we detected no differences in the responses by GLM-analysis is consistent with the results of Aron et al. (1993) showing only small effects of memory. However, they found that visual orientation decreased with rising pheromone concentration and visual orientation was used when no pheromone based information was available. We could not reproduce these effects. We assume that in our experiments, the memory decayed after 24 h and did not show similar strong effects like in Aron et al. (1993). In their experiments, the ants had been tested immediately after the training. Memory decaying effects are well known in different insect species, for instance honeybees (Hammer and Menzel 1995), butterflies (Rodrigues and Weiss 2012), and ants (Johnson 1991).

In motivation experiments, *L. humile* showed clear effects after 9 days of food deprivation. The GLM-analysis of the responses showed significantly higher values and the detection threshold was significantly lower. The PF after 6 days laid more or less between the control and the PF after 9 days, showing that motivation increased with the duration of the starvation period. The lapse rate after 6 and 9 days gave somewhat contradicting results. After 6 days, the lapse rate was significantly higher compared to the control, contrasting hypothesis 7, however, not different from the control after 9 days. Mailleux et al. (2011) had shown that a fraction of starved ants gather around the nest entrance. They proposed that the ants have a lower response threshold. In a similar way, we hypothesize that a fraction of the starved ants (for instance specialized scouts) have a higher lapse rate. They more or less ignore pheromone trails and explore the outside for new food sources if the colony is starving. It can be speculated that at a higher level of starvation, even these ants follow the pheromone trail to get food as fast as possible using all available workers, which would explain the low lapse rate after 9 days of starvation. In this way, the colony follows two different strategies to get food if they are starving. One is to follow a pheromone trail which leads to a recently discovered food source, while the other is to explore new food sources. Depending on the environment and the state of hunger, ants could be able to find an optimum between these two strategies by adapting the response thresholds and lapse rates of specialized workers. However, this hypothesis is speculative and needs to be investigated more deeply through experiment.

E. procera showed no memory effect after 24 h in the absence of pheromone (at 0 bc). The GLM-analysis of the responses showed no significant difference between *P+M* and *P-alone*. This shows that there is no or only a very weak orientation enhancing effect of route memory. *E. procera* is nocturnal so that visual orientation is of little use to the ants in the rain forest at night (Witte and Maschwitz 2008; von Beeren et al. 2014). This explains why route memory did not enhance their orientation. The detection threshold did not change significantly, which we again attribute to the ants being nocturnal. In strong contrast to these findings, the difference between the responses in *PvsM* and *P-alone* were significant. We attribute this mainly to an increased error rate since the lapse rates were different. This shows that the conditioned stimulus increased the error rate when information was contradictory. In the opposite case, the lapse rate was lower, showing that the uncertainty gets lower if two congruent information sources are available. We consider it as an important result of our experiments that, although a positive effect on the responses was not detectable by the GLM-analysis after 24 h, the memory effect still exists and alters the error rate of the ants.

The motivation experiments with *E. procera* showed clear differences from the control after 6 days without fresh food. The threshold of the fitted PF was lower compared to the control and motivation increased the ants' response to the trail pheromone. In contradiction to hypothesis 7, the lapse rate was increased. Similar to *L. humilis*, we explain this by an increased tendency to ignore established trails and explore new food sources by a fraction of the hungry ants. However, since we have only few measurements in the region of the upper asymptote, this result should be interpreted with caution. The responses after 3 days showed a lower detection threshold compared to the control but the difference was not significant. The lapse rates were similar. We assume that the ants had stored parts of the food in their crops and pieces of mushrooms in their nest and were not starving yet. The results of the discrimination experiment with *E. procera* showed a strong effect after 6 days without fresh food. Interestingly, the detection thresholds were nearly identical and the great differences in the PFs manifested in the differences between the lapse rates. This shows that only a small fraction of sated ants respond to pheromone differences while the majority of ants ignore them. However, those ants that respond to the pheromone differences do so in the same way as hungry ants with a nearly identical discrimination threshold. The discrimination threshold is regarded as a measure of the ants' ability to distinguish different pheromone concentrations which is very important for the ants to act collectively (von Thienen et al. 2014). It seems to be invariant to the motivational state of the ants. We see that the lapse rate is very high in discrimination experiments compared to that of detection experiments. A possible explanation is that a low error rate in the detection of trail pheromone is important for the ants regardless of hunger, since it enables them to stay on the pheromone trail (trail fidelity) or to detect a trail if they are lost or leaving the nest. This is especially important in the rainforest at night, where no visual cues are available. Thus, the lapse rate in detection experiments was low, regardless of satiation. On the other hand, a low lapse rate in the discrimination of trails of different pheromone concentration might be important for the ants to distinguish between food sources of different profitability. It is known that *L. niger* encodes food quality (sugar content and volume) in pheromone amounts (Beckers et al. 1992; Mailleux et al. 2000). Although empirical evidence is missing, it is reasonable to assume the same for *E. procera*. Our results imply that hungry ants have a higher tendency to follow the trail leading to the food source of higher quality than sated ants. Thus, hungry ants provide the colony with high quality food and reduce the state of hunger as fast as possible, while sated ants tend to exploit food supplies in the vicinity of the nest with little consideration for food quality.

As expected, *L. niger* showed the clearest effects of route memory of all three species. The ants' response to pheromone in combination with the conditioned stimulus was significantly higher than the response to pheromone alone. However, in the absence of pheromone at concentration 0 bc, the memory effect was not significant. As in *E. procera*, this shows again that, although memory alone has no significant effect after 24 h, it still has a strong effect in combination with pheromone. The detection threshold was 2.5 times lower, showing that the ants' sensitivity to the trail pheromone was increased by memory. Like in *E. procera*, the lapse rates were different between the treatments showing that the error rate decreases if two independent information sources provide congruent information. Evison et al. (2008) found a similar amplification effect on the pheromone response in *L. niger*. In the presence of memory, the longevity of pheromone trails, measured indirectly via the response to pheromone, was 20 h compared to a mean lifetime of 47 min found by Beckers et al. (1993) in experiments with no memory effect. As in our results, this shows that memory enhances the response to trail pheromones.

Like in *L. humile*, we found some evidence for decaying memory in *L. niger*. Aron et al. (1993) as well as Grüter et al. (2011), had shown that memory did not change the response to trail pheromone at different concentration levels for *L. niger*. In our experiments, we found that memory clearly increases the responses to trail pheromones at different concentration levels. We explain this discrepancy by a time-dependent decay in memory. In the experiments of Aron et al. (1993) and Grüter et al. (2011), the ants had been tested immediately after training. Thus, the ants' response was probably at its maximum and a higher concentration of pheromone did not increase the response. In terms of the PF, the response was at a level in the range of the upper asymptote, so a further increase in pheromone concentration did not lead to a stronger response. In our experiments, we measured a long term memory effect after 24 h and the memory was probably already decayed. Therefore, the concentration dependent nature of the ants' response was not masked by a strong memory effect in our experiments. In terms of the PF, the response was at a medium range between lower and upper asymptote in which the function shows a clear pheromone-dependent effect. It is important to note at this point that only by using psychophysical methods and investigating a series of suited pheromone concentrations, did the long-term memory effects become evident in subtle details. We want to point out that our evidence for decaying memory is based on the comparison of experiments using different colonies and bioassays. Thus, there might be other explanations for the discrepancies in the experimental outcome. Future research may clarify this point by measuring the PF immediately after training and after defined time intervals.

There are good reasons why psychophysical theory can be successfully applied to the collective trail-following behavior of ants. In many cases, behavior is a reaction to physical stimuli occurring in the environment. Stimuli are perceived by the sensory organs of the animal, processed by its' nervous system and finally, translated into behavior. Psychophysical theory was developed to understand this process and within its' context, important laws like Weber's law have been found and mathematical models have been developed to describe the relationship between stimulus, perception and behavior. By using psychophysical methods to investigate trail following in ants, an established model of perception was applied to explain important aspects of collective ant behavior (von Thienen et al. 2014; von Thienen et al. 2015). The results of the experiments presented in this study add further aspects to this approach. They show that the inner state of the ants (memory and motivation) modulates the process by which stimuli are translated into behavior. This modulation is shown in the shape and parameters of the PF, which reveal important biological information. Our findings may lead to the development of new or improved models of ant behavior, for instance to explain their collective choice between food sources of different quality depending on the level of starvation. Our results could also allow a better understanding of the ability of ants to find the shortest of different routes of varying complexity if visual learning and pheromones are combined. As discussed before, the results presented here may also lead to a consistent and quantitative definition of collectiveness in ant behavior by incorporating parameters of the PF into such a definition. Trail-pheromone following behavior can be considered as social learning (Franks and Sendova-Franks 2013). Thus, our approach may also be important for understanding what strategies animals, especially social insects, follow in their use of private and social learning (Laland 2004). While all three species studied use both learning types, they do so to a different extent due to their ecological requirements. Laland (2004) assumed that Hamilton's rule (Hamilton 1964) could apply to social learning and higher relatedness leads to increased social learning, because the benefits of information shared with relatives outweighs the costs to the individual. Our results show the opposite. *L. niger* shows a higher relatedness but a lower use of social learning, compared to *L. humile* (*L. niger* colonies are monogynous while *L. humile* colonies are polygynous (Seifert 2007)).

Psychophysics may be an appropriate tool for investigation whenever collective behavior is based on a communication process that transmits information about gradually different environmental states through a signal of varying intensity. This approach may even apply to other forms of collective behavior. For instance the swarming behavior of fish or birds has been explained by models in which distance to other individuals or the radius around an individual are key parameters (Reynolds 1987; Hildenbrandt et al. 2010; Hemelrijk and Hildenbrandt 2011). But how is distance or radius measured by an individual? So far, models have not incorporated a concept of perception of radius and distance, instead making assumptions that the individual has an exact knowledge of these parameters. However, we know from human examples that perception of distance and shape is not exact and follows psychophysical laws (Baird 1970). The same holds for the estimation of the size of cubes by chicken, which also follow a PF (Sarris 2006). Thus, the incorporation of a psychophysical theory of perception of distance and radius could also improve the swarm models of birds and fish by providing a more sophisticated explanation of the variable shape of swarms. Our findings may also be of great interest to computer scientists developing algorithms in the field of ant colony optimization (ACO). Such algorithms may be improved by incorporating parameters that modulate the response to the signal used by the algorithm. For instance, an algorithm that calculates transport routes or landing priorities at airports may use the concept of starvation to give target points or vehicles a higher priority improving efficiency.

Compliance with ethical standards

Ethical standards and conflict of interest The authors declare that the experiments comply with the current law of the country in which they had been performed and that they have no conflict of interest.

Appendix

Introduction into psychophysical methods used by von Thienen et al. (2014)

In psychophysics, a psychometric function (PF) describes the relationship between the probability of a positive response p to a stimulus and the stimulus strength x

$$p(x) = \gamma + (1{-}\lambda{-}\gamma)\cdot F(x).$$

x—stimulus strength, λ—guess rate, γ—lapse rate, $F(x)$—function describing the probability to detect a stimulus by the underlying sensory mechanism (Kingdom and Prins 2010 p. 74). For F(x) a probability distribution like the Weibull distribution is applied. For mathematical details, please refer to von Thienen et al. (2014).

von Thienen et al. (2014) used the PF to provide a mathematical description for the response of ants to trail pheromones of varying concentrations. They performed two types of psychophysical experiments: *Detection experiments* and *discrimination experiments*. The detection experiments measured the ability to detect a trail pheromone of varying intensity against a null stimulus or noise. Discrimination experiments measured the ants' ability to discriminate a trail pheromone of varying concentration from a reference trail pheromone of constant concentration. Both types are of specific biological importance for understanding the collective behavior of ants. Detection is the ants' ability to detect a pheromone trail and become attracted to it. It is important, for instance, to describe how ants at the nest entrance respond to a trail that leads out of the nest to a food source. Discrimination is the ability to differentiate pheromone concentrations at a trail bifurcation. This is especially important in situations where ants have to collectively find the better of two alternate solutions, for instance food of different quality. PFs are S-shaped curves providing a probability of response, theoretically ranging from 0.5 to 1 in detection experiments and from 0 to 1 in discrimination experiments. The stimuli were presented as trail pheromone of varying concentrations on one of the two branches of a y-shaped bridge. Parameters of the PF are the *lapse rate* (the distance between upper asymptote and 1) and the *75 %-detection/discrimination threshold*. von Thienen et al. (2014) interpreted the lapse rate biologically in two ways: as a measure for the errors that ants make and as a measure for the independence from pheromone guided behavior. It might be important for ants not to get stuck in suboptimal solutions and explore alternate solutions. The thresholds are standardized measures of the ants' ability to detect a stimulus or to discriminate between two stimuli, respectively. von Thienen et al. (2014) had shown that differences between the PFs of the three species (*L. humile*, *E. procera*, and *L. niger*) can be attributed to specific adaptations to their environment. *L. humile* ants coordinate the work of extremely large and interconnected colonies (Holway and Case 2000; Giraud et al. 2002). They depend on persistent as well as short-lived food supplies (Mallis 1942; Suarez et al. 1998). Thus, they depend highly on collective information and must be able to communicate even the smallest changes in their environment. This reflects in a low lapse rate and a low detection threshold. *L. niger*, on the other hand, has comparably small colony sizes and uses persistent as well as short-lived food sources (Seifert 2007). Thus, collective information is less important compared to *L. humile* while individual information plays an important part. This reflects in a higher lapse rate and a high detection threshold. *E. procera* is positioned between these two extremes. Their colonies are also much smaller than those of *L. humile*. Furthermore, the ants are nocturnal so that visual cues are rare (Witte and Maschwitz 2008; von Beeren et al. 2014). Consequently, they depend more on collective information and show a high trail fidelity, which reflects in the lowest lapse rate of all three species. Since their food sources are much more stable compared to the other two species, they do not have to share as much information and consequently have a comparatively high detection threshold.

References

Aron S, Deneubourg JL, Pasteels JM (1988) Visual cues and trail-following idiosyncracy in *Leptothorax unifasciatus*: an orientation process during foraging. Insectes Sociaux 35:355–66

Aron S, Beckers R, Deneubourg JL, Pasteels JM (1993) Memory and chemical communication in the orientation of two mass-recruiting ant species. Insect Soc 40:369–80

Baird JC (1970) Psychophysical analysis of visual space, 1st edn. Pergamon, Oxford, New York

Beckers R, Deneubourg J, Goss S (1992) Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). Insect Soc 39:59–72

Beckers R, Deneubourg JL, Goss S (1993) Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. J Insect Behav 6:751–9

Bestmann HJ, Kern F, Schafer D, Witschel MC (1992) 3,4-Dihydroisocoumarins, a new class of ant trail pheromones. Angew Chem Int Edit 6:795–6

Cavill GWK, Robertson PL, Davies NW (1979) An Argentine ant aggregation factor. Experientia (Basel) 35:989–90

Cavill GWK, Davies NW, McDonald JF (1980) Characterization of aggregation factors and associated compounds from the Argentine ant, *Iridomyrmex humilis*. J Chem Ecol 6:371–84

Czaczkes TJ, Grüter C, Jones SM, Ratnieks FLW (2011) Synergy between social and private information increases foraging efficiency in ants. Biol Lett 7:521–4

Czaczkes TJ, Gruter C, Ellis L, Wood E, Ratnieks FL (2013) Ant foraging on complex trails: route learning and the role of trail pheromones in *Lasius niger*. J Exp Biol 216:188–97

de Biseau JC, Pasteels JM (2000) Response thresholds to recruitment signals and the regulation of foraging intensity in the ant *Myrmica sabuleti* (Hymenoptera, Formicidae). Behav Processes 48:137–48

Dorai-Raj S (2009) Binomial confidence intervals for several parameterizations. In: R package version 10-6/r2

Evison SEF, Petchey OL, Beckerman AP, Ratnieks FLW (2008) Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. Behav Ecol Sociobiol 63:261–7

Franks NR, Sendova-Franks AB (2013) Learning and Decision Making in a Social Context. In: Menzel R, Benjamin PR (eds) Invertebrate Learning and Memory (Handbook of Behavioral Neuroscience). Academic Press/Elsevier, Amsterdam, pp 530–545

Giraud T, Pedersen JS, Keller L (2002) Evolution of supercolonies: the Argentine ants of southern Europe. Proc Natl Acad Sci U S A 99: 6075–9

Grüter C, Czaczkes TJ, Ratnieks FL (2011) Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav Ecol Sociobiol 65:141–8

Hamilton WD (1964) The genetical evolution of social behaviour, I, II. J Theor Biol 7:1–52

Hammer M, Menzel R (1995) Learning and memory in the honeybee. J neurosci : off j Soc Neurosci 15:1617–30

Hemelrijk CK, Hildenbrandt H (2011) Some causes of the variable shape of flocks of birds. PLoS One 6:e22479

Hildenbrandt H, Carere C, Hemelrijk CK (2010) Self-organized aerial displays of thousands of starlings: a model. Behav Ecol 21:1349–59

Hölldobler B (1995) The chemistry of social regulation: multicomponent signals in ant societies. Proc Natl Acad Sci U S A 92:19–22

Hölldobler B (1999) Multimodal signals in ant communication. J Comp Physiol 184:129–41

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge, Mass

Holway DA, Case TJ (2000) Mechanisms of dispersed central-place foraging in polydomous colonies of the Argentine ant. Anim Behav 59:433–41

Johnson RA (1991) Learning, memory, and foraging efficiency in two species of desert seed-harvester ants. Ecology 72:1408–19

Kingdom FAA, Prins N (2010) Psychophysics, 1st edn. Elsevier, London

Laland KN (2004) Social learning strategies. Learn Behav 32:4–14

Mailleux AC, Deneubourg JL, Detrain C (2000) How do ants assess food volume? Anim Behav 59:1061–9

Mailleux A-C, Buffin A, Detrain C, Deneubourg JL (2011) Recruitment in starved nests: the role of direct and indirect interactions between scouts and nestmates in the ant Lasius niger. Insect Soc 58:559–67

Mallis A (1942) Half a century with the successful Argentine ant. Sci Mon 55:536–45

R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reynolds CW (1987) Flocks, herds, and schools: a distributed behavioral model. Comput Graph 21:25–34

Robertson PL, Dudzinski ML, Orton CJ (1980) Excocrine gland involvement in trailing behaviour in the argentine ant (Formicidae: Dolichoderinae). Anim Behav 28:1255–73

Rodrigues D, Weiss MR (2012) Reward tracking and memory decay in the monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae). Ethology 118:1122–31

Sarris V (2006) Relational psychophysics in humans and animals. Psychology, New York

Seifert B (2007) Die Ameisen Mittel- und Nordeuropas. Lutra Verlags- und Vertriebsgesellschaft, Tauer

Suarez AV, Bolger DT, Case TJ (1998) Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79:2041–56

Van Vorhis Key SE, Baker TC (1982) Trail-following responses of the Argentine ant, *Iridomyrmex humilis* (Mayr) to a synthetic trail pheromone component and analogs. J Chem Ecol 8:3–14

von Beeren C, Lizon à l'Allemand S, Hashim R, Witte V (2014) Collective exploitation of a temporally unpredictable food source: mushroom harvesting by the ant *Euprenolepis procera*. Anim Behav 89:71–7

von Thienen W, Metzler D, Choe D-H, Witte V (2014) Pheromone communication in ants: a detailed analysis of concentration dependent decisions in three species. Behav Ecol Sociobiol 68:1611–27

von Thienen W, Metzler D, Witte V (2015) Modelling shortest path selection of the ant *Linepithema humile* using psychophysical theory and realistic parameter values. Journal of Theoretical Biology 372:168–178

Wetterer JK, Wild AL, Suarez AV, Roura-Pascual N, Espadaler X (2009) Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Myrmecol News 12:187–94

Wilson EB (1927) Probable inference, the law of succession, and statistical inference. J Am Stat Assoc 22:209–12

Wilson EO (1958) A chemical releaser of alarm and digging behavior in the ant *Pogonomyrmex badius* (Latreille). Psyche 65:41–51

Wilson EO, Pavan M (1959) Glandular sources and specificity of some chemical releasers of social behavior in dolichoderine ants. Psyche 66:70–6

Witte V, Maschwitz U (2008) Mushroom harvesting ants in the tropical rainforest. Naturwissenschaften 95:1049–54

General discussion and outlook

Overview

The results presented in this dissertation show that psychophysical theory is a well suited tool for investigating collective ant behavior. It enables us to look more comprehensively than before into some of the processes of collective decision making and behavior in ants and find new aspects of this behavior. Psychophysical theory is able to mathematically describe the relationship between pheromone concentration and behavioral response of ants to trail pheromones. For the first time, a theory of perception that was originally established by Weber and Fechner to understand sensory processes in humans, could be applied to the collective, self-organizing behavior of eusocial insects. By integrating psychophysical theory into the Deneubourg model, it is possible to considerably improve this model and find new biological important aspects of collective ant behavior. In this chapter, our main findings will be discussed in the light of the aim presented in the general introduction. Finally, we include an outlook on some approaches for further research.

Psychophysical theory as a framework to describe collective behavioral responses of ants to trail pheromones

To accomplish the first three tasks outlined in the general introduction, detailed measurements of ant responses had been done in discrimination as well as detection experiments over a broad range of pheromone concentrations for all three species (chapter 1). To our knowledge, this had never been done before in such detail. For instance, the works of Hangartner (1969), Van Vorhis Key (1982) and Choe et al. (2012) focused either on discrimination tasks or detection tasks. They measured only up to six pheromone concentrations, without knowing whether these concentrations matched natural conditions. Particularly low and high concentrations were not studied, which are presumably important in models of self-organization.

DCF and PF fitted well to the data of the measured dose-response relationship between pheromone concentration and ant response (chapter 1). This shows that both models are able to describe the collective response of ants to trail pheromones in the three species under investigation. However, the PF has some advantages over the DCF. The parameters of the DCF were originally not deduced experimentally but were set in such a way that the shortest path experiments could be explained satisfactorily. In strong contrast, the parameter values that had been deduced from our measurements presented in chapter 1 were quite different. In addition, the Deneubourg model was not based on a theory of perception and a biological interpretation of its parameters was not given (see general introduction). The PF overcomes these limitations. It fitted to the data at least as well the DCF with parameter values deduced from experiment. Under the additional assumption of pheromone modulation, the PF was closer to the experimental results of the shortest path experiments than the DCF. Since the PF is based on an established theory of perception, it opens the possibility of linking the collective behavior of ants to the neurological processes of stimulus perception and response in ants (see below). The parameters of the PF can be interpreted in a consistent biological manner and reveal deeper insights into the behavior of ants. The measurement of the dose-response relationship presented in chapter 1 included the responses to constant concentration ratios at different levels of pheromone concentration. Thus, it could be shown for the first time that Weber's law, a fundamental law of neurophysiology and psychophysics (see Box 2), is fulfilled by the collective responses of the ants in all three species. Considering these findings, the main conclusion of this dissertation is to modify the Deneubourg model by exchanging the Deneubourg choice function with the psychometric function.

In the following paragraph we will discuss the biological interpretation of the parameters of the PF and characteristic differences of the parameter values for the three species under investigation. The *discrimination threshold* is a measure for the ant's ability to discriminate between trails of different pheromone concentrations. Collective behavior of ants, such as the selection of high quality food sources (Beckers et al. 1993), greatly depends on information encoded in concentration ratios of the trail pheromones. Thus, the discrimination threshold can be interpreted as a measure for the ants' ability to act collectively. In addition, the ability to discriminate different pheromone concentrations is an important feature for ants to sense gradients of pheromone concentration left and right from their antennae. It allows them to stay on a pheromone trail and follow it (Hangartner 1967). In this way, the discrimination threshold can be taken as a measure for trail fidelity, which again, is important for collective behavior.

The *detection threshold* is the ability of ants to detect pheromones in their environment. Whenever a change in the ants' behavior depends on reaching a pheromone threshold, this parameter is important. For instance, it was proposed that division of labor within the ant colony can be explained by different response thresholds of specialized workers (Bonabeau et al. 1996). Mailleux et al. (2011) showed that ants of starving colonies gathering around the nest entrance show an increased tendency to leave the nest compared to ants deeper inside the nest. It was assumed that these ants have a lower response threshold to the recruitment signals (trail pheromone and/or tactile signals) and thus show a higher tendency to leave the nest and forage.

By relating the detection threshold to the amount of pheromone an ant carries, it is possible to define the *information capacity* as another biologically important parameter (chapter 1). It represents the total length of a trail an ant is able to mark with pheromone quantities that are detectable by other ants of the same species. It is inversely proportional to the detection threshold. Ants with a high information capacity can deposit more information units in their environment and thus change the behavior of the colony to a greater extent compared to ants with a lower information capacity. In this way, the information capacity defines the ability of individual ants to change the collective behavior of the colony. This parameter is independent from absolute pheromone concentrations which is a great advantage since, for most ant species, we are not able to measure absolute pheromone concentrations that make up the pheromone trails. This is due to the low pheromone amounts ants use and our lack of knowledge of exact chemical compositions of the pheromone trails. The information capacity overcomes these difficulties and gives us important new biological facts about the species under investigation that were not available so far.

The *lapse rate* defines the errors ants make in following a pheromone trail. Thus, it can be taken as a measure for the trail fidelity: the lower the lapse rate, the better ants follow and stay on a pheromone trail. On the other hand, a high lapse rate may help ants to keep flexible, explore new solutions and adapt to changing environments. If a certain percentage of ants ignore or leave the pheromone trails, there will always be a chance for them to discover unexplored terrain, discover new food sources, better homes and potential dangerous enemies.

The three species under investigation show considerable differences in these parameters, which can be explained by the different ecological needs of the three species (chapter 1). *L. humile* is highly collective orientated and thus depends to a great extent on collective information (Aron et al. 1993). This is consistent with high discrimination abilities (low discrimination threshold), a high information capacity and a low lapse rate. Due to these abilities, the ants are able to communicate slight changes in the available food supply and react to it. They are also able to maintain long lasting trails to stable food sources. This fits well to their ecological needs. They are dietary generalists and they use stable and persistent food sources, like honeydew, but also distributed and short lived food sources, like insects, seeds or carrion (see Mallis 1942; Suarez et al. 1998).

Compared to *L. humile*, *L. niger* depends less on collective information instead using optical orientation and visual memory to a great extent besides trail pheromones (Aron et al. 1993; Evison et al. 2008; Czaczkes et al. 2011; Grüter et al. 2011). Therefore, this species needs comparatively low discrimination abilities (high discrimination threshold) and a low information capacity although they feed on insects which represent a short lived and locally unstable food source (Seifert 2007). In addition, visual memory enables them to maintain long-lasting trails to trophobiosis sites, where they collect honeydew (Seifert 2007) despite their high lapse rate. On the other hand, a high lapse rate can be a great advantage, since the ants are not bound to the pheromone trails as sole medium of orientation, giving them a chance to find unpredictable food sources, like insects, and flexibly react to changes in their environment.

E. procera is positioned between the other two species in terms of collectivity. Compared to *L. humile*, it shows a similar discrimination threshold but a much lower information capacity. It is even five times lower than that of *L. niger*. The lapse rate, compared to *L. humile* is much higher but only half the lapse rate of *L. niger*. This can be explained by the specific ecological needs of this ant species. The ants are nocturnal and maintain stable trails to mushroom sites which are their main food supply and they follow these trails with high trail fidelity (Witte and Maschwitz 2008; von Beeren et al. 2014). The mushroom sites get exhausted after some days and are abandoned by the ants. However, after a few days the mushrooms regrow and the ants reactivate the trails, probably orienting on a long living component of the pheromone trail. Since they have no other means of orientation in the dark rain forest at night, the low discrimination threshold allows them to stay on their pheromone trails without optical aid. Although food sources are periodically changing, they are long lasting and many ants are active in maintaining stable trails leading towards them. Thus, the ants need only a very low information capacity.

Test of the models

Task 4 and 5 of this dissertation were to test the Deneubourg model as well as the new model, which is based on psychophysical theory. This was done by simulating the shortest path experiments by computer simulation to compare how accurate both models are able to reproduce the experimental results (chapter 2). In contrast to the original experiments and computer simulations of Goss et al. (1989) and Deneubourg et al. (1990), the parameter values of the choice functions were not set in such a way that gave the best fit to the shortest path experiments, but were deduced from actual measurements (see chapter 1). The simulations showed that neither the Deneubourg model nor the new model with the PF were able to satisfactorily explain the experimental results. The models were able to explain the general outcome i.e. the collective selection of the shortest path and symmetry breaking, but were not able to exactly reproduce the frequency with which the shortest path was selected. By incorporating the fact that the ants deposit more pheromone when returning to the nest (Aron et al. 1989) and making the hypothetical assumption that ants show a higher responsiveness in the beginning of the recruitment, the new model with the PF was able to fully reproduce the original experimental results of the shortest path experiments. This was not possible with the original Deneubourg model. These results show that although the Deneubourg model revealed some fundamental aspects of collective ant behavior by simulating ant behavior in a computer, real ants are far better in finding an optimal solution to the shortest path problem than virtual ants. Thus ants must use alternative or additional means to find solutions to the shortest path problem. Further experiments are needed to test if pheromone modulation and/or higher responsiveness are the source of the difference. Since the DCF and arbitrary parameter values had been used to explain other types of collective ant behavior (see “Summary”), these explanations should also be revised by further research in the light of the new findings presented here.

Collective vs. individual PF

Hölldobler and Wilson proposed that the collective behavior of ants is determined by a few and simple genetically determined social algorithms that individual ants follow (Hölldobler and Wilson 2009, pp. 53-58). Following this concept, we may ask how the algorithm that determines the response of individual ants to different pheromone concentrations is structured, and how it determines the collective behavior of the colony.

The experiments presented in chapter 1 measured the behavioral response of a collective of ants, not that of individual ants. It is remarkable that a theory, designed for the behavior of individuals, is able to describe the behavior of a collective consisting of several hundreds or thousands of individuals. The ant colony seems to respond to external signals like an individual, showing that it performs like an superorganism, as Hölldobler and Wilson (2009) proposed. This leads to the question of how far the PF of the collective is determined by the response of the individual ants to the pheromone, what the underlying neurophysiological processes are and how similar collective and individual PFs are. The individual workers of an ant colony are closely related sisters that share the same or only a few mothers (depending on whether they are polygynous or monogynous) and a few fathers. Thus, it is reasonable to assume that their genetically determined individual response to the trail pheromone is similar. In this case, the collective PF should be a good approximation of the average individual PF. However, this is only a conjecture and we do not know the variability of PF parameters between individual ants. We presently know of no experiments in which the individual behavioral dose-response relationship of individual ants was measured. It is questionable whether this is possible at all, since such an experiment needs repeated measurements at different concentration levels under constant conditions on individual ants. This is probably not possible since individual ants cannot be induced to respond to ca. 100 bifurcation choices without drastically influencing their behavior during the experiment. A possible approach to overcome these difficulties may be to directly measure the neurophysiological response of the olfactorial receptors like it has been done with silk moths (Kaissling 1970; Kaissling and Priesner 1970; Kaissling 2014), deduce a neurophysiological dose-response curve and fit a PF to these data. Presently we have no data of the underlying neurophysiological processes in ants and we do not know the relationship between neurophysiological responses and behavioral response. We can measure the stimulus strength as well as the behavioral response, but know nothing of what happens in between. However, there are data available from other animals. For instance, we know from experiments with monkeys that the PF, describing the behavioral response of individual monkeys to motion signals, highly correlates to the PF that describes cortical neuron response to the same motion signals (Britten et al. 1992). The authors concluded that “the psychophysical decisions [..] were based upon a relatively small number of neural signals.” Behavioral and neurophysiological experiments with the silkmoth *Bombyx mori* and the pheromone bombykol indicate a relationship between stimulus strength, neuron response and behavior. The dose-response relationship follows an S-shaped curve which the authors of the study explained by the probability distribution of pheromone molecules docking to the receptors at the antennal hairs (Kaissling and Priesner 1970). Thus, we have good reasons to assume that the specific shape of the PF might be the result of the stochastic process of pheromone molecules docking at the receptor cells of the ant's antennal hairs. However, our results show great differences between the three species we investigated, which indicates that additional, species specific factors influence the parameter values of the PF. These factors may be found in species specific differences of the kinetics and structure of the pheromone-receptor complex, the anatomy of the antennal hairs and the distribution of receptor cells on its surface and/or in the neurophysiological processing of the stimulus. Besides species specific differences in signal processing, our experiments with memory and motivation showed that the inner state of the ants modulates this process to a great extent (see chapter 3). Again, we do not know the factors that determine this modulation. It may be caused by changes in the pheromone-receptor complex, structure and exposition of the antennal hairs and/or in the neurophysiological processing. In the discussion of chapter 3 we have also indicated that there might be differences in the parameters of the PF between different worker groups. These differences may manifest at different levels of specialization i.e. caste, specialized worker or individual personality. All of these open questions show that we are still far from a full understanding of collective ant behavior.

Memory

Task 6 of this dissertation was to apply the new model to collective ant behavior by measuring the combined effect of trail pheromones and route memory (chapter 3). Memory can be viewed as information that is being acquired, stored and retrieved (Vaas 2000). According to this definition, memory is a process by which animals are able to utilize information that had been acquired and stored before. Information is extremely important for animals to adapt their behavior to a constantly changing environment (Dall et al. 2005). They use different kinds of information, like personal information generated by interactions of an animal with its environment and social information that is generated by the behavior of other animals (Dall et al. 2005). In this sense, route memory is information that individual ants acquire about the three dimensional structure of their environment which is stored for later retrieval. It can be viewed as source of private information. In contrast to private information, trail pheromones can be viewed as source of social or collective information (see for instance Czaczkes et al. 2011). Trail pheromones have also been interpreted as a means of social learning (Franks and Sendova-Franks 2013), in which the learned information is acquired, stored and retrieved by the members of the collective through an external medium.

Various studies showed that route memory acquired by visual learning changes the response of ants to trail pheromones (Aron et al. 1988; Aron et al. 1993; Czaczkes et al. 2011; Grüter et al. 2011; Czaczkes et al. 2013a). Ant species show considerable differences in their responses if visual learning and trail pheromones as source of information are both available. *L. humile*, a species that highly depends on collective information and less on private information provided by vision, shows a much higher response to trail pheromones than *Leptothorax unifasciatus or L. niger*, who use both information sources to a great extent (Aron et al. 1988; Aron et al. 1993). If route memory and trail pheromone are available to the ants as two independent information sources, the ants reduce the error rate in trail following and increase their trail following speed. This shows that two independent information sources allow the ants to increase their foraging efficiency (Grüter et al. 2011; Czaczkes et al. 2013a).

The results presented in chapter 3 show that route memory has clear effects in all three species. *L. niger* showed the strongest effects of memory while *L. humile* showed only small effects. This demonstrates that memory had a lower effect on the response to trail pheromones in a species that mainly depends on collective information compared to a species that uses private information as well. This confirms the findings of Aron et al. (1993). The strength of the memory effect in *E. procera* was intermediate compared to the other two species, but it is difficult to rank their degree of collectivity in relation to the other two species. The reason is that at present, we know of no consistent method to evaluate the degree of collectivity in ants. However, the results presented here give some hints as to which further research could lead to finding such a consistent method. First, the findings of (Aron et al. 1993) show that the extent to which social and private information are used are important indicators for collectivity. However, so far only the combined effect of visual memory and trail pheromones were investigated in this context. Other sources of information like sensorimotor orientation and tactile or acoustic communication have also to be considered as important factors although such a multifactorial approach will be very demanding. In chapter 1 the biological meaning of the parameters of the psychometric function have been described. Lapse rate, information capacity, detection and discrimination thresholds are considered as indicators for the collectivity of an ant species. They could be used to define the degree of collectivity. However, these parameters are not necessarily altered into the same direction if a species is more or less collective. For instance, like stated in chapter 1 and 3, we know from personal observations that *E. procera* is a highly collective orientated species. The low discrimination threshold and the low lapse rate clearly point into this direction. In contrast, they have only a very low information capacity, which is an indicator against collectivity. The specific strategies of this species in getting their main food supply through periodically growing mushroom sites was an explanation for this discrepancy (see chapter 1). The ants probably reactivate already existing trails and thus only a few ants are needed to deposit pheromone to reestablish a trail, which explains the low information capacity. This example shows that a definition for collectivity, based on a few parameters, might be difficult. However, the lapse rate is a clear indicator since it shows the extent to which ants ignore pheromone trails and are able to act independently from the main source of collective information. Further research including more ant species may clarify this issue (see also below “An outlook to a definition of collectivity”).

In all three species, memory had the effect of increasing the error that ants make in following the pheromone trails if information provided by trail pheromone and route memory were contradictory. In the opposite case, when both information sources provided the same information, the error rate sank. This confirms similar findings in *L. niger* (Czaczkes et al. 2011; Grüter et al. 2011; Czaczkes et al. 2013a). In contrast to former studies of the combined effect of memory and pheromone (Aron et al. 1988; Aron et al. 1993; Evison et al. 2008; Czaczkes et al. 2011; Grüter et al. 2011; Czaczkes et al. 2013a), we measured synergistic as well as antagonistic memory effects, which again revealed subtle details. For instance, in *E. procera* and *L. humile* the memory effect was low in a synergistic situation, in which the information provided by route memory and pheromone were the same. In the antagonistic situation, in which both information sources provided contradictory information, the memory effect was strong and mainly manifested in an increased error rate. This showed that even if a positive effect of memory is low or not detectable, memory is still present and influences the ants’ behavior. One of the main differences of our study from the above mentioned studies was the time scale in which the memory effect was measured. Except in some measurements of Evison et al. (2008), the measurements in the former studies were done immediately after the training. Some of these measurements showed only very low or not detectable memory effects (Aron et al. 1993; Grüter et al. 2011). In our study, the memory effects were measured 24 hours after the training and still showed clear effects in all cases. This shows that all three species maintain their route memory for at least 24 hours.

Motivation

Similar to route memory, an aim of this dissertation was to apply the new model to collective ant behavior by measuring the combined effect of trail pheromone and the ants' motivation to forage (chapter 3). Since food is the source of energy, proteins and micronutrients, starvation can be expected to influence ant behavior, especially their motivation to forage. For instance, it was shown in the ant species *Myrmica sabuleti* that starvation increases the motivation to forage as well as the communication among individuals by lowering the response threshold to recruitment signals (de Biseau and Pasteels 2000). If *L. niger* ants are experiencing a food shortage, more and more ants aggregate around the nest entrance, ready to speed up the process of food intake from returning ants by trophallaxis. In addition, the ants at the nest entrance show an increased tendency to leave the nest, probably due to a lower response threshold to recruitment signals (Mailleux et al. 2011).

L. humile and *E. procera* showed similar results if the combined effect of trail pheromone and starvation was measured. The responses to the pheromone were significantly enhanced by starvation and the detection thresholds were lower depending on the length of the starvation period. The lapse rate showed some unexpected results. It was expected that the lapse rate would be lower or at least would remain at a lower level in a starving colony compared to a sated colony. This expectation was reasonable because a low lapse rate increases the efficiency in the following of the trails leading towards existing food sources. In contrast to this expectation, the lapse rate after six days starvation was higher than the control while the lapse rate after nine days was lower compared to six days in *L. humile*. This can be explained by the hypothesis that different worker castes have different lapse rates and that these lapse rates are adapted to the level of starvation. For instance, if specialized ants like scouts have a high lapse rate after six days without food, they more or less ignore pheromone trails to be able to explore unknown terrain and find new food sources. If the degree of starvation of the colony becomes higher after nine days without food, even these ants may start following the pheromone trail to an existing food source to accelerate the food intake resulting in an overall lower lapse rate after nine days starvation compared to six days starvation. Thus, the colony may follow different foraging strategies: depending on the state of the colony and its environment. The ants find an optimum between the two strategies by adapting the lapse rates of specialized workers. A similar effect could be found in *E. procera*. Unexpectedly, their lapse rate increased with the length of the starvation period. Again, this can be explained by a fraction of specialized ants having a lower lapse rate to leave established trails and find new food sources for the starving colony.

In the discrimination experiments with *E. procera* there were great differences in the PFs of sated and starved colonies, which manifested in the differences between the lapse rates. In a sated colony, only a fraction of the ants seem to respond to pheromone differences. These ants respond in the same way as hungry ants because they have nearly identical discrimination thresholds. The discrimination threshold is an important measure of ants' ability to act collectively (von Thienen et al. 2014) and it seems to be invariant to the motivational state of the ants.

Outlook and proposals for further research

In this section, an outlook to possible further research approaches will be discussed based on the results presented so far.

Swarm behavior

The results presented in this dissertation may inspire further research in the field of swarm behavior of birds and fish, especially using some of the models that have successfully reproduced such behavior (Reynolds 1987; Hildenbrandt et al. 2010; Hemelrijk and Hildenbrandt 2011). These models base on a few simple assumptions about the behavior of the individual swarm members, mainly that they try to keep optimal distances to their closest neighbors. This simple local interaction is sufficient to reproduce real swarm behavior. However, the models are based on the simplifying assumption that the individual has perfect knowledge of distance and radius. We know from psychophysical theory that this is not the case (Baird 1970; Davies and Green 1994; Sarris 2006). By replacing this assumption with a more realistic, psychophysical based approach, we might get more realistic models of swarm behavior, especially in understanding its stochastic fluctuations.

For instance Hildenbrandt et al. (2010) developed a model of swarm behavior of starlings in which the birds try to keep an optimal distance to the starlings in their proximity. The model was able to reproduce the real swarm behavior very well. According to the model, a bird experiences four forces, one that directs him away from its neighbors, one that attracts him towards its neighbors, one that aligns it to the flight direction of its neighbors and one that keeps him in the vicinity of the roosting site. These forces depend on geometric parameters like distance and radius and they are constantly evaluated by the birds such that if one bird changes its position and heading, the neighboring birds react according to the interaction rules and adjust their position and heading. This results in a constantly changing three dimensional movement of the whole swarm. The model incorporates stochastic influences by adding a random force to the other four forces described above. It was shown by simulation that the variability in the swarm movement can be explained by local differences in the movement of the flock and depends on parameters like flock size and number of interacting neighbors. However, it was not investigated how far variability in the individual estimation of distance influences the variability of the swarm movements. By doing so, the model could be made more realistic, if the random force is replaced by the assumption that the estimation of distance follows a psychometric function.

Measuring the effect of neonikotinoids

In chapter 3 it was shown that the new approach described in this dissertation is able to reveal subtle details in the dose-response relationship of pheromone concentration and ant response. The same approach may be used in further research to investigate other factors that might influence collective ant behavior. For instance, we know from many examples that our ecosystems are threatened by various kinds of human activities like insecticides that are brought into the environment. We have strong evidence that insecticides like neonikotinoides, in addition to other possible factors, are responsible for the decline of honey bee populations in the US and Europe in the last decade (Grimm et al. 2012). It was demonstrated that sublethal doses of neonikotinoids change navigation, recruitment, and learning behavior of bees (Menzel 2014). In a similar way, we may ask if neonikotinoids alter the behavior of ant colonies. Due to the great importance of ants for the ecosystems worldwide, effects of neonikotinoids on ants may put great stress on our ecosystems. The measurement of the ant response to pheromones of varying concentrations and fitting a PF to the data, as described in this dissertation, may be used to measure the effect of neonikotinoids on ant behavior by exposing them to different doses of neonikotinoides.

The lapse rate — a mechanism to keep flexible

As it has been mentioned in chapter 1 and 2, the lapse rate is a measure of the ants' ability to act independently from pheromone trails. The lapse rate may help ant colonies to constantly explore the environment outside of the established pheromone trails and thus find new food sources, explore new nest sites or detect enemies. In a situation where a food source gets exhausted, it can be expected that ants with a high lapse rate are able to find and switch to a new food source faster than ants with a lower lapse rate. This hypothesis may be tested by computer simulations and experiments similar to the ones described by Czaczkes et al. (2013b). The simulations should be designed in such a way that the ants are offered a food source, establish a trail towards the food source and forage at that food source. Shortly before the food source gets exhausted, a new food source is offered and the time that it takes the ants to switch to the new food source is measured. By repeating this simulation for different lapse rates, the hypothesis can be tested. The lapse rates should be simulated in two different ways. One is to use the same lapse rate for all ants and change this lapse rate for the different simulation runs. In this case, all ants in a simulation run have the same lapse rate, which is equivalent to the collective lapse rate of the colony. The other is to assign different lapse rates to two fractions of the ants. This resembles the situation in which two different worker classes show different properties of the PF (see below) and the collective lapse rate resembles an average of the individual lapse rates. By modifying the size of the ant fractions, the collective lapse rate of the colony is modified and tested. It can be expected that both versions of the experiment lead to different behavior of the ant colony. As a secondary step, the hypothesis may be verified by experiment with real ants. The experiment should be done with ant species that show clearly distinct lapse rates like *L. humile* and *L. niger*. All information sources besides trail pheromones should be eliminated as much as possible by making the measurements in darkness or with dimmed red light, which the ants are not able to see.

Caste specific differences between PFs

In the discussion of the starvation experiments (chapter 3) it was hypothesized that the lapse rate shows significant differences between different worker castes and that the ants may be able to adjust the lapse rate to different degrees of starvation. This could be the case for other parameters of the PF as well. This would enable the ant colony to coordinate its workforce and fine tune it to the specific demands of the environment. This hypothesis could be tested on a species with a morphologically distinct worker class like species of the genera *Camponotus*.

An outlook to a definition of collectivity

In chapter 1 and 3 we discussed the interpretation of the PF-parameters in relation to the degree of collectivity of the three ant species we investigated. All social insects act collectively to a great extent, but besides collective information, ants also use private information like vision (Aron et al. 1988; Aron et al. 1993), polarized light (overview in Hölldobler and Wilson 1990, p. 366 ff), tactile information (Hölldobler and Wilson 1990, p. 258 ff), sensorimotor information (Macquart et al. 2007) and geomagnetic information (Anderson and Vander Meer 1993). In the work of Aron et al. (1993) the relative use of private information (route memory) and collective information (trail pheromone) was investigated in the ant species *L. niger* and *L. humile*. It was stated that *L. humile* is more collectively orientated compared to *L. niger* due to factors like colony size, food source longevity and emigration strategies. In other studies the relation between social (collective) and private information in social insects and other animals was discussed (Dall et al. 2005; Czaczkes et al. 2011; Grüter et al. 2011; Czaczkes et al. 2013a) as well as the relation between social and private learning (Laland 2004; Franks and Sendova-Franks 2013). Here we want to describe a possible approach to give an exact definition of to what degree an ant species is collectively organized with respect to its' use of trail pheromones as means to convey information to the collective. We propose to use the parameters of the PF since they can be interpreted as measures for different aspects of collective behavior (see chapter 1 and 3). The lapse rate defines how constrained the ants are to act according to the collective information or, putting it another way, how far they are able to act independently from the collective information and follow their private information and motivation. The information capacity defines the abilities of individual ants to change the collective behavior of the colony. The detection threshold defines the ability to detect pheromones or the sensitivity towards the collective information and the discrimination threshold defines the ability to act collectively. We propose to use following formula as an *index of trail pheromone oriented collectivity* of an ant species:

$$TCI = IC \cdot \frac{1}{\lambda_{dis} \cdot \lambda_{\det} \cdot t_{ds75}} . \tag{1}$$

With $IC = \frac{c}{tdt_{75}}$ where *c* is a proportional constant set to 1 (see chapter 1). We may also write

$$TCI = \frac{1}{\lambda_{dis} \cdot \lambda_{\det} \cdot t_{dt75} \cdot t_{ds75}} \tag{2}$$

TCI=trail orientated collectivity index
λ_{dis}= lapse rate discrimination
λ_{det} = lapse rate detection
t_{dt75}=75%-detection threshold
t_{ds75}=75%-discrimination threshold

All parameters are dimensionless except the detection threshold. Thus, the dimension is trail length (cm) per ant equivalent (AE). From the results in chapter 1 we may calculate the trail oriented collectivity index for the three species: *L. humile* shows the highest index with 3636 AE/cm, *L. niger* shows the lowest index with 44 AE/cm and *E. procera* lies in between with 227 AE/cm. This result is consistent with the statement of (Aron et al. 1993) that *L. humile* are more collectively orientated than *L. niger* and with our interpretation in chapter 1 that *E. procera* is positioned between these two species in terms of collectivity. However, future research is necessary to show if this definition is practical. As a first step, we propose to measure the index for other ant species which show collective behavior mediated by trail pheromones, for instance species of the genera *Formica*- such as *Formica fuscocinerea* and species of the genera *Lasius* such as *Lasius neglectus* or *Lasius fuliginosus*.

Better ACO-algorithms

In the field of informatics, the Deneubourg model inspired many solutions for optimization problems. These so called *Ant Colony Optimizations (ACO)* are based on the Deneubourg choice function (Dorigo et al. 1996; Dorigo and Stützle 2004). A famous example for a such a problem is the traveling salesman problem (TSP). This problem belongs to the class of *NP-complete problems* (NP = nondeterministic polynomial[1]) which play an important role in

---

[1] *Nondeterministic polynomial* means that an algorithm may be constructed which selects the different instances of the problem randomly (nondeterministic), constructs a solution for each instance and tests in polynomial time if the solution solves the problem. A NP-problem is *complete* if any other NP-problem can be reduced to it in polynomial time. *Polynomial time* means, that the time an algorithm needs to find a solution

decision theory. Algorithms that calculate an exact solution for NP-complete problems may take too long to be useful for practical purposes, even when the most powerful computers are being used. Therefore, algorithms like ACO have been developed to find a solution by approximating the exact solution in acceptable time. The TSP is an extensively studied example for such a problem. A travelling salesman has to visit different cities which are connected by roads of given length. The cities and their connections represent the instances of the problem. The salesman must find the shortest route that connects all cities that he wants to visit before coming home. The problem can be solved by a simple algorithm that sums up the distances between the cities for each possible city combination and tests if this sum represents the shortest sum found so far. If the salesman has to visit only a few cities (few problem instances), he can find a solution simply by mental calculation. However, if more and more cities are added to his tour (many problem instances), the possible combinations of cities grow faster than the number of instances and even if he has the fastest and most powerful computer at hand, the time needed to solve the problem will be unacceptable. For instance, if he has to visit 20 cities and his computer takes one nanosecond to calculate a single tour, it will take him nearly 2 years to find the shortest tour. An ACO algorithm follows a different approach which does not necessarily give an exact solution but usually gives a good approximation to it and is much faster. It lets a group of virtual ants explore the possible routes and find the best solution for the problem. This is done by letting each virtual ant walk randomly from city to city until it has visited each city once and returns home. After all ants have returned home, the shortest route found so far is calculated and marked with a virtual pheromone. In the next round, the same happens but this time, the virtual ants slightly prefer the routes marked with pheromone. This process is repeated many times and, after 100 to 1000 iterations, the algorithm usually converges to a solution that is close to the theoretical optimum in an acceptable time. The problem may be visualized as a Hamilton graph in which the shortest Hamilton circuit represents the solution and the ants randomly explore the state-space until the algorithm converges to an optimal solution (Dorigo and Stützle 2004, p. 40). One of the main differences to real ants is that no centralized instance exists which calculates and compares the lengths of the routes found so far.

---

for a problem is no more than a polynomial function of the problem size. Although the test for each instance can be performed in polynomial time, there are no algorithms known that are able to solve a NP-complete problems in polynomial time. The reason is that the number of possible solutions grow much faster than the number of problem instances. For more details, see Dorigo and Stützle (2004).

As stated in the discussion of chapter 2, we do not expect that ACO algorithms show a better time performance when using the PF since the PF is more complex than the DCF and therefore needs more computer power. However, certain new aspects that we found might lead to better ACO-algorithms or new solutions: The concept of the lapse rate might avoid situations in which ACO algorithms get into stagnation before reaching an optimum. This situation happens if all virtual ants follow the same path without coming close to the optimal solution. Usually this is avoided by adding a priori information to the algorithm about each node of the state-space or by performing local search algorithms that, for each ant, calculate optimal proposals for the step to take in the next iteration (Dorigo and Stützle 2004, p. 70, pp. 215-216). The concept of the lapse opens an alternate approach. By incorporating the lapse rate into ACO algorithms, there are always a set of ants which more or less ignore the pheromone trails and randomly explore solutions beyond established pheromone marked routes. This could be achieved by defining a set of virtual ants that weakly or not at all respond to the pheromone. Another improvement by the incorporation of the lapse rate may be that the algorithm finds closer approximations to the optimal solution. The reason is the asymmetry between short path and long path selection that had been described in chapter 2. This asymmetry is caused by the lapse rate and manifests in the tendency to reverse the selection of the long path towards the short path if it was selected in the initial phase of the simulation. In a similar way, it can be expected that good solutions are preferred over less good solutions.

The modulation of pheromones described in chapter 2, by which ants deposit more pheromone as they return to the nest, has already been incorporated in some ACO algorithms by the so called *Elitist Ant Systems* (Dorigo and Stützle 2004, p. 73). In this case, the best of all solutions found so far is marked with an additional pheromone amount. However, the idea behind the Elitist Ant System is different than the approach that real ants follow since the evaluation of a best solution needs a central instance which the ants do not have. Instead, they incorporate directional information into the system by dropping more pheromone while returning to the nest. Since classical problems solved by ACO, like the travelling salesman problem, do not incorporate information about direction, the concept of pheromone modulation will not improve these algorithms. However, if the problem is such that directional information is important, the concept of pheromone modulation may be useful. For example, if a salesman prefers to visit cities in the east before he visits cities in the west (a priori definition), the virtual ants may deposit more pheromone when heading east causing the algorithm to prefer cities in the east. Please note that the property "direction" may be replaced by any other property of the system which needs to be incorporated in the optimization process and which can be coded as virtual pheromone deposited at the nodes of the state-space.

We found that memory modulates the pheromone response (chapter 3). This concept is already being used by ACO algorithms by incorporating a priori information (Dorigo and Stützle 2004, p. 70). In addition, it could be used to develop learning ACO algorithms. As an example, we again take the travelling salesman. Here, he periodically takes the same tour. According to the actual traffic situation between the different cities, he may modify his tour due to traffic congestion caused by building operations that frequently occur at different segments of the tour. The information about the latest tour he has taken can be incorporated as a priori information for his next tours by slightly increasing the response of the virtual ants for the modified tour segments or by adding a second, long lasting virtual pheromone dose to the segment, increasing the ants' response. This would result in a tour that prefers certain tour segments depending how often they have been taken by the travelling salesman. Similar to memory, we found that starvation alters the response to the pheromone (chapter 3). This concept may be used to assign a time dependent priority to each node of the state-graph. As an example we again take the travelling salesman. The customers at the different cities may have given him certain time slots in which they want to be visited. This is stored as a priori information to the algorithm. In each iteration of the algorithm, the difference between actual time and the pre-defined time-slot is calculated and used to update the priority of the city. A similar concept has already been used by Bencheihk et al. (2011) to calculate the scheduling of aircraft landing at an airport by an ACO algorithm. In this algorithm, deviation from the scheduled time of arrival is being penalized.

Conclusion

For the first time we were able to explain and successfully apply aspects of collective ant behavior using an established model of perception, the psychophysical theory. In addition, it was the first time that psychophysical theory was successfully applied to collective behavior of animals. We have shown that the dose-response relationship between pheromone concentration and ant response can be well described by a psychometric function and follows Weber’s law in the three ant species we investigated. Thus, for the first time, an exact description of this dose-response relationship based on empirical measurements could be deduced. In addition, clear definitions for important biological parameters like detection- and discrimination-thresholds, information capacity and error rates could be given and measured. The results revealed considerable differences between the three species, which could be attributed to their specific ecological needs. The results and the deduction of realistic parameter values for the Deneubourg choice function and for the psychometric function gave us the possibility to test an established model of collective ant behavior. The results showed that the shortest path experiments with *L. humile* could not be explained by the established model, the Deneubourg model. Only by using the psychometric function and adding further assumptions like the modulation of pheromone deposition, could the shortest path experiments be explained satisfactorily. By using our methods to measure psychometric functions, we were able to show that memory and motivation modulate the responses to trail pheromones in the three ant species we investigated, changing detection thresholds and error rates. Again, the species showed considerable differences which could be explained by their ecological needs and by the extent up to which they use collective and private information. Our results showed that *L. humile* uses collective information to a much greater extent than *L. niger*, with *E. procera* positioned in between the other two species in terms of collectivity. Our results show that psychophysical methods may be a suitable tool to investigate collective animal behavior whenever this behavior is based on the transmission of information about gradually varying environmental states through a signal of varying intensity. The psychophysical approach may apply not only to ants but also to other animals showing collective behavior.

References

Akre KL, Johnsen S (2014) Psychophysics and the evolution of behavior. Trends in ecology & evolution 29:291-300

Anderson JB, Vander Meer RK (1993) Magnetic orientation in fire ant, Solenopsis invicta. . Naturwissenschaften 80:568-570

Aron S, Beckers R, Deneubourg JL, Pasteels JM (1993) Memory and chemical communication in the orientation of two mass-recruiting ant species. Insect Soc 40:369-380

Aron S, Deneubourg JL, Pasteels JM (1988) Visual Cues and Trail-Following Idiosyncracy in Leptothorax Unifasciatus: An Orientation Process During Foraging. Insectes Sociaux 35:355-366

Aron S, Pasteels JM, Deneubourg JL (1989) Trail-laying behaviour during exploratory recruitment in the argentine ant, Iridomyrmex humilis (Mayr). Biol Behav 14:207-217

Baird JC (1970) Psychophysical analysis of visual space, 1st edn. Pergamon Press, Oxford, New York,

Bandeira de Melo EB, Araújo AF (2011) Modelling foraging ants in a dynamic and confined environment. Biosystems 104(1):23-31

Beckers R, Deneubourg J, Goss S (1992) Trail laying behaviour during food recruitment in the ant Lasius niger (L.). Insect Soc 39:59-72

Beckers R, Deneubourg JL, Goss S (1993) Modulation of Trail Laying in the Ant Lasius niger (Hymenoptera: Formicidae) and Its Role in the Collective Selection of a Food Source. J Insect Behav 6:751-759

Bencheihk G, Boukacour J, EL Hilali Alaoui A (2011) Improved Ant Colony Algorithm to Solve the Aircraft Landing Problem. International Journal of Computer Theory and Engineering 3:224-233

Bonabeau E, Theraulaz G, Deneubourg JL (1996) Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. P Roy Soc Lond B Bio 263:1565-1569

Britten KH, Shadlen MN, Newsome WT, Movshon JA (1992) The analysis of visual motion: a comparison of neuronal and psychophysical performance. The Journal of neuroscience : the official journal of the Society for Neuroscience 12:4745-65

Camazine S, Deneubourg J-L, Franks NR, Sneyd J, Theraulaz G, Bonabeau E (2001a) Self-Organization in Biological Systems In. Princeton University Press

Camazine S, Deneubourg J-L, Franks NR, Sneyd J, Theraulaz G, Bonabeau E (2001b) Self-Organization in Biological Systems. Princeton University Press

Chittka L, Skorupski P, Raine NE (2009) Speed-accuracy tradeoffs in animal decision making. Trends in ecology & evolution 24:400-7

Choe D-H, Villafuerte DB, Tsutsui ND (2012) Trail Pheromone of the Argentine Ant, Linepithema humile (Mayr) (Hymenoptera: Formicidae). PLoS One 7(9)

Czaczkes TJ, Gruter C, Ellis L, Wood E, Ratnieks FL (2013a) Ant foraging on complex trails: route learning and the role of trail pheromones in Lasius niger. J Exp Biol 216:188-97

Czaczkes TJ, Grüter C, Jones SM, Ratnieks FLW (2011) Synergy between social and private information increases foraging efficiency in ants. Biology letters 7:521-4

Czaczkes TJ, Gruter C, Ratnieks FL (2013b) Negative feedback in ants: crowding results in less trail pheromone deposition. Journal of the Royal Society 10:20121009

Dall SRX, Giraldeau LA, Olsson O, McNamara JM, Stephens DW (2005) Information and its use by animals in evolutionary ecology. Trends in Ecology and Evolution 20:187-193

Davies MNO, Green PR (1994) Perception and motor control in birds : an ecological approach. Springer-Verlag, Berlin ; New York de Biseau JC, Pasteels JM (2000) Response thresholds to recruitment signals and the regulation of foraging intensity in the ant Myrmica sabuleti (Hymenoptera, Formicidae). Behavioural processes 48:137-48

Deneubourg JL, Aron S, Goss S, Pasteels JM (1990) The Self-Organizing Exploratory Pattern of the Argentine Ant. J Insect Behav 3:159-168

Detrain C, Deneubourg J-L (2006) Self-organized structures in a superorganism: do ants "behave" like molecules? Physics of Live reviews 3:162-187

Dorigo M, Maniezzo V, Colorni A (1996) Ant system: optimization by a colony of cooperating agents. IEEE transactions on systems, man, and cybernetics Part B, Cybernetics : a publication of the IEEE Systems, Man, and Cybernetics Society 26:29-41

Dorigo M, Stützle T (2004) Ant colony optimization. MIT Press, Cambridge, Mass.

Dussutour A, Beekman M, Nicolis SC, Meyer B (2009a) Noise improves collective decision-making by ants in dynamic environments. Proceedings Biological sciences / The Royal Society 276:4353-61

Dussutour A, Nicolis SC, Shephard G, Beekman M, Sumpter DJ (2009b) The role of multiple pheromones in food recruitment by ants. J Exp Biol 212:2337-48

Dyer AG, Spaethe J, Prack S (2008) Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. J Comp Physiol A 194:617-27

Evison SEF, Petchey OL, Beckerman AP, Ratnieks FLW (2008) Combined use of pheromone trails and visual landmarks by the common garden ant Lasius niger. Behav Ecol Sociobiol 63:261-267

Fechner G (1860) Elemente der Psychophysik. Breitkopf und Härtel

Franks NR, Sendova-Franks AB (2013) Learning and Decision Making in a Social Context. In: Menzel R, Benjamin PR (eds) Invertebrate Learning and Memory (Handbook of Behavioral Neuroscience). Academic Press/Elsevier, Amsterdam, pp 530-545

Gold JI, Shadlen MN (2007) The Neural Basis of Decision Making. Annual review of neuroscience 30:535-74

Goss S, Aron S, Deneubourg JL, Pasteels JM (1989) Self-organized Shortcuts in the Argentine Ant. Naturwissenschaften 76:579-581

Grimm M, Sedy K, Süßenbacher E, Riss A (2012) Existing Scientific Evidence of the Effects of Neonicotinoid Pesticides on Bees In: European Parliament PDAEASP (ed), Brussels

Grüter C, Czaczkes TJ, Ratnieks FL (2011) Decision making in ant foragers (Lasius niger) facing conflicting private and social information. Behav Ecol Sociobiol 65:141-148

Hangartner W (1967) Spezifität und Inaktivierung des Spurpheromons von Lasius fuliginosus Latr. und Orientierung der Arbeiterinnen im Duftfeld. Z Vgl Physiol 57:103-136

Hangartner W (1969) Orientierung von Lasius fuliginosus Latr. an einer Gabelung der Geruchspur. Insect Soc 16:55-60

Hemelrijk CK, Hildenbrandt H (2011) Some causes of the variable shape of flocks of birds. PLoS One 6:e22479

Hildenbrandt H, Carere C, Hemelrijk CK (2010) Self-organized aerial displays of thousands of starlings: a model. Behavioral Ecology 21:1349-1359

Hölldobler B (1995) The chemistry of social regulation: multicomponent signals in ant societies. Proceedings of the National Academy of Sciences of the United States of America 92:19-22

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge, Mass.

Hölldobler B, Wilson EO (2009) The superorganism : the beauty, elegance, and strangeness of insect societies, 1st edn. W.W. Norton, New York

Kacelnik A, Brito e Abreu F (1998) Risky choice and Weber's Law. J Theor Biol 194:289-98

Kaissling KE (1970) Mechanism of insect olfactory receptor stimulation. Neurosciences Research Program bulletin 8:526-30

Kaissling KE (2014) Pheromone Reception in Insects. The Example of Silk Moths. In: Mucignat-Caretta C (ed) Neurobiology of Chemical Communication. CRC Press, pp 99-134

Kaissling KE, Priesner E (1970) Die Riechschwelle des Seidenspinners. Naturwissenschaften 57:23-8

Kingdom FAA, Prins N (2010) Psychophysics, 1 edn. Elsevier, London

Klein A (2001) Measuring, estimating, and understanding the psychometric function: A commentary. Percept Psychophys 63(8):1421-1455

Laland KN (2004) Social learning strategies. Learning & behavior 32:4-14

Lanan MC, Dornhaus A, Jones EI, Waser A, Bronstein JL (2012) The trail less traveled: individual decision-making and its effect on group behavior. PLoS One 7:e47976

Macquart D, Latil G, Beugnon G (2007) Sensorimotor sequence learning in the ant Gigantiops destructor. Animal behaviour 75:1693-1701

Mailleux A-C, Buffin A, Detrain C, Deneubourg JL (2011) Recruitment in starved nests: the role of direct and indirect interactions between scouts and nestmates in the ant Lasius niger. Insectes Sociaux 58:559-567

Mallis A (1942) Half a century with the successful Argentine ant. Sci Mon 55:536-545

Menzel R (2014) Wie Pestizide (Neonicotinoide) die Navigation, die Tanz-Kommunikation und das Lernverhalten von Bienen verändern. In: Rundgespräche der Kommission für Ökologie »Soziale Insekten in einer sich wandelnden Welt«. Verlag Dr. Friedrich Pfeil, München

Perna A, Granovskiy B, Garnier S, Nicolis SC, Labedan M, Theraulaz G, Fourcassie V, Sumpter DJ (2012) Individual rules for trail pattern formation in Argentine ants (Linepithema humile). PLoS Comput Biol 8:e1002592

Ramsch K, Reid CR, Beekman M, Middendorf M (2012) A mathematical model of foraging in a dynamic environment by trail-laying Argentine ants. J Theor Biol 306:32-45

Reynolds CW (1987) Flocks, Herds, and Schools: A Distributed Behavioral Model. Computer Graphics 21:25-34

Sarris V (2006) Relational Psychophysics in Humans and Animals. Psychology Press, New York

Sasaki T, Granovskiy B, Mann RP, Sumpter DJT, Pratt SC (2013) Ant colonies outperform individuals when a sensory discrimination task is difficult but not when it is easy. Proceedings of the National Academy of Sciences of the United States of America 110:13769-73

Seifert B (2007) Die Ameisen Mittel- und Nordeuropas. Lutra Verlags- und Vertriebsgesellschaft, Tauer

Suarez AV, Bolger DT, Case TJ (1998) Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79:2041-2056

Vaas R (2000) Gedächnis. In: Lexikon der Neurowissenschaft. Spektrum Akademischer Verlag Heidelberg

Van Vorhis Key SE, Baker TC (1982) Trail-following responses of the Argentine ant, Iridomyrmex humilis (Mayr) to a synthetic trail pheromone component and analogs. J Chem Ecol 8:3-14

Vela-Pérez M, Fontelos MA, Velásquez JJL (2013) Ant foraging and geodesic paths in labyrinths: Analytical and computational results. J Theor Biol 320:100-112

Vittori K, Talbot G, Gautrais J, Fourcassie V, Araujo AF, Theraulaz G (2006) Path efficiency of ant foraging trails in an artificial network. J Theor Biol 239:507-15 von Beeren C, Lizon à l'Allemand S, Hashim R, Witte V (2014) Collective exploitation of a temporally unpredictable food source: mushroom harvesting by the ant Euprenolepis procera. Animal behaviour 89:71-77

von Thienen W, Metzler D, Choe D-H, Witte V (2014) Pheromone communication in ants: a detailed analysis of concentration dependent decisions in three species. Behav Ecol Sociobiol 68:1611-1627

von Thienen W, Metzler D, Witte V (2015) Modelling shortest path selection of the ant Linepithema humile using psychophysical theory and realistic parameter values. Journal of Theoretical Biology 372:168-178

von Thienen W, Metzler D, Witte V (2016) How memory and motivation modulate the responses to trail pheromones in three ant species. Behav Ecol Sociobiol 70:393-407

Weber EH (1834) De Pulsu, resorptione, auditu et tactu. Annotationes anatomicae et physiologicae. In: Koehler (ed), Leipzig

Wichmann FA, Hill NJ (2001) The psychometric function: I. Fitting, sampling, and goodness of fit. Percept & Psychophys 63(8):1293-1313

Wilson EO (1958) A chemical releaser of alarm and digging behavior in the ant Pogonomyrmex badius (Latreille). Psyche 65:41-51

Wilson EO (1962) Chemical communication among workers of the fire ant Solenopsis saevissima (Fr. Smith). Animal behaviour 10:134-164

Witte V, Maschwitz U (2008) Mushroom harvesting ants in the tropical rainforest. Naturwissenschaften 95:1049-1054

Curriculum Vitae

Personal Data

Name	Wolfhard von Thienen
Adress	Sachsengässchen 3, 86415 Mering
Telephone number	08233-31286
E-Mail	w@thienen.de
Birthdate	17/October/1952 in Lübeck/Germany
Status	married, 2 children
Nationality	German

Professional Experience

01/2000 – 11/2014	**IT-Management** Debis Systemhaus and T-Systems, München Consulting, head of department, delivery-management
03/1990 - 12/1999	**Software developer** HMT Informationssysteme GmbH, Grasbrunn Development of business software, project management, consulting, head of department
01/1989 - 02/1990	**Software developer** Markt & Technik Verlag AG Development of online-informations-systems, project management, head of department
06/1986 - 04/1988	**Software developer** Access Computer GmbH, Holzkirchen Development of business software
05/1975 - 09/1983	**Entrepreneur** Production of recycling products
03/1974 - 04/1975	**Case worker** Dresdner Bank AG, Frankfurt/M

Education

04/1987 – 03/1988	**Business Informatics** Digital Equipment GmbH, München
	Biology
10/1976 – 10/1978	Frankfurt/M
10/1983 – 03/1987	München
	Physics
10/1971 – 09/1973	Frankfurt/M
04/1962 – 06/1971	**Gymnasium** Georg-Büchner-Gymnasium, Bad Vilbel

Publications

von Thienen W, Metzler D, Choe D-H, Witte V (2014) Pheromone Communication in Ants: A Detailed Analysis of Concentration Dependent Decisions in Three Species. Behav Ecol Sociobiol 68:1611-1627

von Thienen W, Metzler D, Witte V (2015) Modelling shortest path selection of the ant Linepithema humile using psychophysical theory and realistic parameter values. Journal of Theoretical Biology

von Thienen W, Metzler D, Witte V (2015) How memory and motivation modulate the responses to trail pheromones in three ant species. Behav Ecol Sociobiol 70:393-407

von Thienen W (1996) LINUX Taschenreferenz, Markt&Technik, Haar, ISBN 3-8272-5141-9

von Thienen W (1993, 1999) Client Server, Technologie und Realisierung im Unternehmen, Vieweg, Braunschweig, 1st and 2nd edition, ISBN 3-528-05502-2

von Thienen W (1989, 1992) UNIX, Taschenreferenz, Markt&Technik, Haar, 1st and 2nd edition, ISBN 3-87791-325-3

Acknowledgements

I am very grateful to my supervisors Volker Witte and Dirk Metzler who accepted me as PhD student, discussed with me my scientific aims, the design of the experiments and the evaluation and interpretation of the results. Volker Witte gave me supervision for the biological aspects of my dissertation while Dirk Metzler supervised the simulations and modelling as well as the statistics. I feel very sad about the fact that Volker Witte passed away too early. With him, the scientific community lost a great biologist and myrmecologist, and we, the students, lost a great teacher.

I thank Prof. Rolf Ulrich and Dr. Karin Bausenhart from the University of Tübingen as specialists in psychophysics for discussing my psychophysical approach to the collective ant behavior and for the quality check of this approach.

I thank the students, who helped me performing the experiments during their research practicals under my supervision, Kai von Szadkowski, Sandra Franz and Nora Kipferler

Many thanks to Ekaterina Morozova for grammar- and spell-checking my manuscripts as native speaker.

Finally I wish to thank Tomer Czaczkes as well as my fellow-PhD-students, who discussed with me my approaches and results, Christoph von Beeren, Sophia Lizon à l’Allemand, Sebastian Pohl, Deborah Schweinfest, Angelika Pohl and Magdalena Mair.

Appendix

Supplemental material chapter 1

Electronic supplementary material

Article title

Pheromone Communication in Ants: A Detailed Analysis of Concentration Dependent Decisions in Three Species

Journal

Behavioral Ecology and Sociobiology

Year 2014

Authors

Wolfhard von Thienen, Dirk Metzler, Dong-Hwan Choe, Volker Witte

e-mail: w@thienen.de

Online Appendix A: Equations

Weber's law

Equation 1 – Weber's law

$$k = \frac{EU}{R}, \tag{1}$$

EU – "ebenmerklicher Unterschied" (just detectable difference), R – reference stimulus, k – constant.

Psychometric function

We fitted the psychometric function using the Weibull distribution as the probability distribution. The general form of the Weibull distribution is:

Equation 2 – Weibull distribution

$$p = 1 - e^{\left(\frac{x}{s}\right)^{b}}, \tag{2}$$

s – Scale parameter, b – Form parameter.

Equation 3 – Psychometric function

The general form of the psychometric function is given by

$$p = \gamma + (1 - \lambda - \gamma) \cdot F(x), \tag{3a}$$

With *F(x)* "describing the probability of correct stimulus detection or discrimination by the underlying sensory mechanism" (Kingdom and Prins 2010 p. 74). For F(x) we apply the Weibull distribution from above in a modified form (see comment below):

Detection:

$$p = \gamma + (1 - \lambda - \gamma) \cdot \left[1 - e^{\ln(1-\gamma)\cdot\left(\frac{c}{t}\right)^{b}}\right], \tag{3b}$$

Discrimination:

$$p = \lambda + 2 \cdot (\gamma - \lambda) \cdot \left[1 - e^{\ln(1-\gamma)\cdot\left(\frac{c}{t}\right)^{b}}\right], \tag{3c}$$

t – Pheromone concentration at threshold, *c* – Pheromone concentration of test trail, λ – lapse rate, γ – guess rate, *b* – slope.

In the fitting procedure, the parameters λ, *b* and γ were fitted and the parameter *a* was set to *a*=0.5 (detection) and a=γ *(discrimination)*. In detection experiments, the parameter *t* was also fitted and in discrimination experiments it was set to *t*=1 *bc*.

In discrimination experiments the ants have no information about which trail is the test trail and which is the reference trail. Since the PF is asymmetrical, both cases yield slightly different results for the exponential term. Following common practice in psychophysics, we take the average $(e^{\ln(1-a)\cdot\left(\frac{c}{t}\right)^b} + 1 - e^{\ln(1-a)\cdot\left(\frac{t}{c}\right)^b})\cdot 0.5$ of both cases as the exponential term when we fit the data.

For the concentration dependency experiment, we use equation 3c with *t* set to the concentration of the reference trail and *c* set to $c{=}0.5\cdot t$. We chose the Weibull function because in pre-evaluations, it gave a good fit compared to other functions (for instance exponential) and it is reported to give a good fit for the psychometric function (Klein 2001, p. 1430). We have formulated it in a way that threshold and slope are independent and the threshold does not change the shape of the function, it only results in a parallel shift along the x-axis.

Equation 4 – 75%-threshold

The sensory thresholds are defined as the stimulus strength at which the response probability is

$$p = \gamma + 0.5\cdot(1-\lambda-\gamma) \approx 0.75\,. \tag{4}$$

This represents half the distance between guess rate and upper asymptote of the PF. In discrimination experiments, the 75%-discrimination threshold is evaluated by the inverse of equation 3c by setting p according to equation 4. In detection experiments, the parameter *t* is free and included in the fitting process since we do not know the absolute concentrations, and it gives the 75%-detection threshold. In discrimination experiments, we take the ratio between the 75%- and the 50%-discrimination thresholds as a measure for the *contrast* that the test subjects are able to recognize. Since the 50%-discrimination threshold is situated at 1, this is equivalent to the 75%-discrimination threshold.

Under low threshold conditions, the psychometric function can be modified by adding a small constant amount of pheromone *k_pf* to the pheromone concentrations with the effect that at low concentrations the Weibull-term becomes asymptotic towards 0.5.

Equation 5– Information capacity

$$IC = \frac{c}{tdt_{75}} \tag{6}$$

IC – Information capacity, measured in units length (cm) of a trail an ant can mark with one gland, tdt_{75} –75%-detection threshold, c – proportionality constant, set to 1.

Equation 6– Deneubourg choice function

$$p = \frac{(k + c)^b}{(k + c)^b + (k + c\_ref)^b}, \tag{5}$$

c_ref – pheromone concentration of reference trail, c – pheromone concentration of test trail,
b – exponent, k – constant.

Online Appendix B: Detailed results

Table B1: Detailed results of fitting the psychometric function

Experiment	Species	N	GOF	AIC	z-GLM	Dsp	Function parameters					
					p-GLM		b	λ	γ	a	t_{75}	IC
Detection	*E.*	138	0.96	563	2.7	1.8	*0.72*	*0.03*	*0.48*	0.5	*0.62 bc*	*146*
	procera				8e-3		1.05	0.05	0.52		0.89 bc	197
							1.63	*0.07*	*0.56*		*1.2 bc*	*282*
Detection	*L. humile*	100	0.25	388	6.4	1.8	*0.42*	*0.09*	*0.35*	0.5	*0.04 bc*	*6452*
					1.5e-10		0.6	0.11	0.43		0.05 bc	13450
	Pavan's gland						*1.08*	*0.13*	*0.52*		*0.11 bc*	*17857*
Detection	*L. humile*	60	0.64	147		1.2	*0.39*	*0.02*	*0.3*	0.5	*0.62 pg/cm*	*4228*
	(Z)-9						*0.68*	0.06	0.44		1.64 pg/cm	11185
							2.34	*0.1*	*0.56*		*3.96 pg/cm*	*27005*
Detection	*L. niger*	80	0.39	294	4.4	1.2	*0.6*	*0.15*	*0.4*	0.5	*0.08 bc*	*708*
					1e-5		1.21	0.18	0.49		0.16 bc	1094
							3.86	*0.20*	*0.55*		*0.25 bc*	*2065*
Discrimin.	*E.*	237	0.55	873	1.8	2.0	*0.82*	*0.09*	*0.50*	0.5	*1.85*	
Low	*procera*				5e-71		1.0	0.11	0.52		2.14	
							1.23	*0.14*	*0.53*		*2.52*	
Discrimin.	*E.*	110	0.89	362	9.5	1.4	*0.54*	*0.0*	*0.50*	0.5	*2.77*	
High	*procera*				2e-21		0.63	0.03	0.51		3.33	
							0.75	*0.05*	*0.52*		*4.07*	
Discrimin.	*L. humile*	80	0.86	221	8.9	0.8	*0.75*	*0.0*	*0.45*	0.5	*2.16*	
					4e-19		0.87	0.02	0.47		2.48	
							1.01	*0.05*	*0.48*		*2.85*	
Discrimin.	*L. niger*	210	0.57	821	11	1.6	*0.4*	*0.15*	*0.52*	0.5	*2.59*	
					1e-29		0.56	0.21	0.54		3.82	
							0.79	*0.25*	*0.55*		*6.49*	
												k_pf
Conc.Dep.	*E.*	306	0.15	1514	-5	2.7	*0.65*	0		0.5		*0.12*
	procera				4e-7		0.8					0.34
							1.0					*0.85*
Conc.Dep	*L. humile*	150	0.34	660	0.97	2.0	*0.41*	0		0.5		*0.0*
					0.3		0.52					0.02
							0.64					*0.06*
Conc .Dep.	*L. niger*	141	0.08	646	-1	1.8	*0.4*	0		0.5		*0.0*
					0.3		0.5					0.02
							0.63					*0.09*

Notes: Values for fitted parameters are given in the order lower confidence limit, fitted value, and upper confidence limit from top to bottom with confidence limit in italics. Parameters with no confidence limits are fixed. Confidence limits were calculated at 95%-level by parametric bootstrapping (n = 1,000).

N	total number of measurements,
GOF	goodness of fit,
AIC	AIC (Akaike information criterion),
z-GLM, p-GLM	z- and p-value of GLM for factor concentration,
Dsp	Dispersion,
b	slope,
λ	lapse rate,
γ	guess rate,
a	decision probability at threshold,
t75	75%-threshold (detection resp. discrimination),
IC	information capacity,
k_pf	constant added to concentrations, measured in *bc*.

Table B2: **Detailed results of fitting the Deneubourg choice function**

Experiment	Species	N	GOF	AIC	Dsp	Function parameters	
						b	k
Detection	*E. procera*	138	0.97	560	1.78	*1.16*	*0.50*
						1.57	0.91
						2.54	*2.05*
Detection	*L. humile* Pavan's gland	100	0.41	384	1.84	*0.44*	*0.01*
						0.52	0.02
						0.64	*0.04*
Detection	*L. humile* (Z)-9-hex.	60	0.13	153.3	1.26	*0.44*	*0.01*
						0.48	0.02
						0.73	*0.06*
Detection	*L. niger*	80	0.28	293	1.29	*0.40*	*0.03*
						0.5	0.06
						0.65	*0.13*
Discrimin. Low	*E. procera*	237	0.11	879	2.01	*0.96*	*0.09*
						1.08	0.15
						1.23	*0.23*
Discrimin. High	*E. procera*	110	0.96	359	1.41	*0.83*	*0.00*
						0.92	0.03
						1.02	*0.06*
Discrimin.	*L. humile*	80	0.22	226	0.93	*0.96*	*0.00*
						1.06	0.02
						1.19	*0.06*
Discrimin.	*L. niger*	210	0.45	821	1.61	*0.49*	*0.06*
						0.56	0.13
						0.65	*0.23*
Conc.Dep.	*E. procera*	306	0.15	1514	2.67	*0.73*	*0.0*
						1.03	0.32
						1.26	*0.77*
Conc.Dep.	*L. humile*	150	0.34	660	2.04	*0.55*	*0.0*
						0.68	0.02
						0.83	*0.06*
Conc.Dep.	*L. niger*	141	0.08	646	1.84	*0.52*	*0.0*
						0.66	0.02
						0.82	*0.1*

Note: See table above for parameter descriptions.

b	exponent,
k	constant.

Table B3: Pheromone concentrations used in the experiments

Experiment	Species	Lowest concentration	Highest concentration	Number of concentrations measured
Detection	*E. procera*	0 bc	4 bc	11
Detection	*L. humile* Pavan's gland	0 bc	2 bc	10
Detection	*L. humile* (Z)-9-hexadecenal.	0 pg/cm	200 pg/cm	5
Detection	*L. niger*	0 bc	2 bc	8
Discrimination Low	*E. procera*	0 bc	16 bc	12
Discrimination High	*E. procera*	0 bc	8 bc	10
Discrimination	*L. humile* Pavan's gland	0 bc	8 bc	8
Discrimination	*L. niger*	0 bc	32 bc	13
Concentration dependency	*E. procera*	0 bc	32 bc	11
Concentration dependency	*L. humile* Pavan's gland	0 bc	32 bc	15
Concentration dependency	*L. niger*	0 bc	8 bc	12

Note: The ranges of pheromone concentration (i.e. highest and lowest values) on the test trail are shown.

Table B4: Effects of three factors in generalized linear models and mixed effects models

Experiment	Species	glm full	glm date	glm colony	glm conc	glmer full	glmer date	glmer colony	glmer conc
Detection	*E. procera*	552	551	-	645	558	556	-	654
Detection	*L. humile* Pavan's gland	411	-	417	496	420	-	421	503
Detection	*L. niger*	303	-	314	348	311	-	314	356
Discrimination low	*E. procera*	1151	1168	-	1627	1161	1173	-	1643
Discrimination high	*E. procera*	451	457	-	748	458	460	-	757
Discrimination	*L. humile* Pavan's gland	247	272	272	449	267	284	284	468
Discrimination	*L. niger*	947	973	974	1118	961	959	978	1135
Concentration dependency	*E. procera*	1536	1535	1536	1553	1558	1562	1556	1564
Concentration dependency	*L. humile* Pavan's gland	669	669	669	668	673	671	671	672
Concentration dependency	*L. niger*	645	642	643	650	653	652	651	656

Notes: The effect of the factors pheromone concentration, colony and date are evaluated by dropping the factors from a full model and comparing the AIC (Akaike information criterion). Resulting AICs are shown for generalized linear models (glm) and generalized linear mixed models (glmer). No results are given (-) if the relevant factor has only one level. A lower value of the AIC compared to the full model indicates an effect of the factor. Compared to the factor “concentration”, the factors “date” and "colony” show only small effects.

full	AIC (Akaike information criterion) of full model,
date	factor date is dropped
colony	factor colony is dropped
conc	factor pheromone concentration is dropped

Online Appendix C: Supplementary Figures

Figures C1a–c	detection experiments fitted with the psychometric function,
Figures C2a–c	discrimination experiments fitted with the psychometric function,
Figures C3a–b	concentration dependency experiments fitted with the psychometric function,
Figures C4a–c	symmetry tests,
Figures C5a–b	detection experiments fitted with the Deneubourg choice function,
Figures C6a–c	discrimination experiments fitted with Deneubourg choice function,
Figures C7a–c	concentration dependency experiments fitted with Deneubourg choice function.

Fig. C1

Detection experiment (a) *Linepithema humile* (Pavan's gland), (b) *Lasius. niger*, (c) *L. humile* (pygidial gland) fitted with the psychometric function. The response to different pheromone concentrations is shown. Circles show the probability that ants take the test trail (response) and bars show the 95%-confidence interval. The x-axis shows the pheromone concentration of the test trail measured in base concentrations on a $\log_2$-scale; the reference trail carried no pheromone. The solid line shows the fitted psychometric function and dashed lines show its 95%-prediction interval. The symbol (•) at the bottom indicates the 75%-detection threshold.

GOF=Goodness of fit, bc=base concentration, b=slope, t_{dt75}=75%-detection threshold, λ=lapse rate, N=total sample size, n=number of measurements per concentration (each measurement represents 10 binomial ant decisions).

L. humile (Pavan's gland): GOF=0.25, bc=0.0014 AE/cm, b=0.6 (range 0.42–1.08), t_{dt75} =0.05 bc (range 0.04–1.11 bc), λ=0.11 (range 0.09–0.13), N=100, n=10 (each).

L. niger: GOF=0.39, bc=0.0057 AE/cm, b=1.21 (0.6–3.86), t_{dt75}=0.16bc (range 0.08–0.25 bc), λ=0.18 (range 0.15–0.2), N=80, n=10 (each). The measurement at 0.125 bc was regarded as an outlier and not included in the fitting process.

L. humile (pygidial gland): bc=0.0057 AE/cm, N=70, n=10 (each). Kruskal-Wallis test shows no significant difference between measurements (p=0.372, Kruskal-Wallis, N=70).

Fig. C1

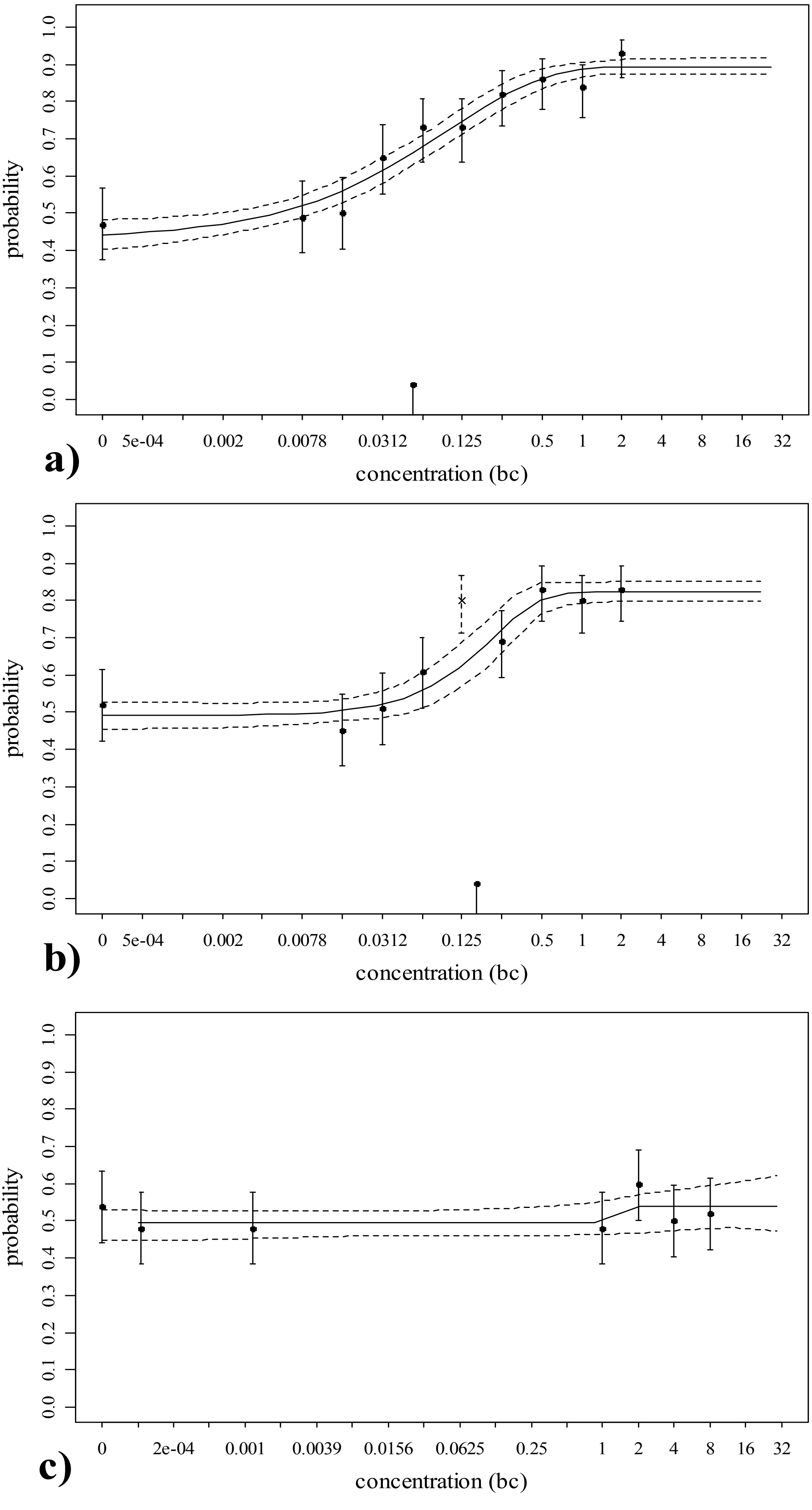

probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
a)
concentration (bc)
probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
b)
concentration (bc)
probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
2e-04
0.001
0.0039
0.0156
0.0625
0.25
1
2
4
8
16
32
c)
concentration (bc)

Fig. C2

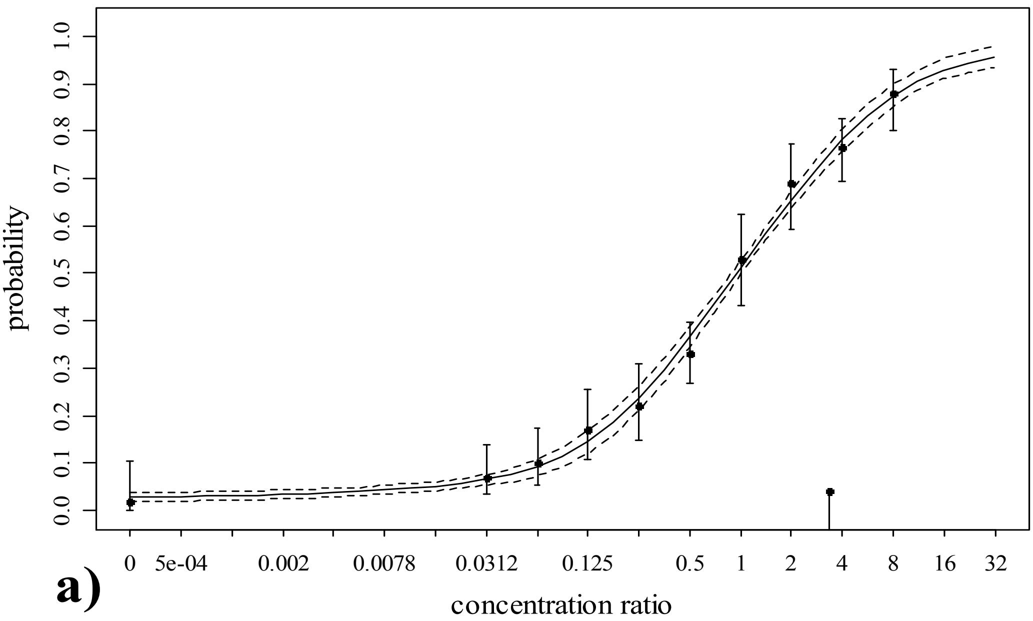
Discrimination experiment fitted with the psychometric function showing the response to different pheromone concentration ratios. (a) *Euprenolepis procera*, high concentration measurement series. (b) L*inepithema humil*e, (c) *Lasius niger*. The x-axis shows the concentration ratio between test trail and reference trail on a $\log_2$-scale. The test trail had varying concentrations measured in base concentrations. The reference trail had a constant concentration of 1 *bc*. Circles show the probability that ants take the test trail (response) and bars show the 95%-confidence interval. The solid line shows the fitted psychometric function and dashed lines show its 95%-prediction interval. Symbol (•) at bottom indicate the 75%-discrimination threshold.

GOF=Goodness of fit, bc=base concentration, b=slope, t_{ds75}=75%-discrimination threshold, λ=lapse rate, N=total sample size, n=number of measurements per concentration (each measurement represents 10 binomial ant decisions).

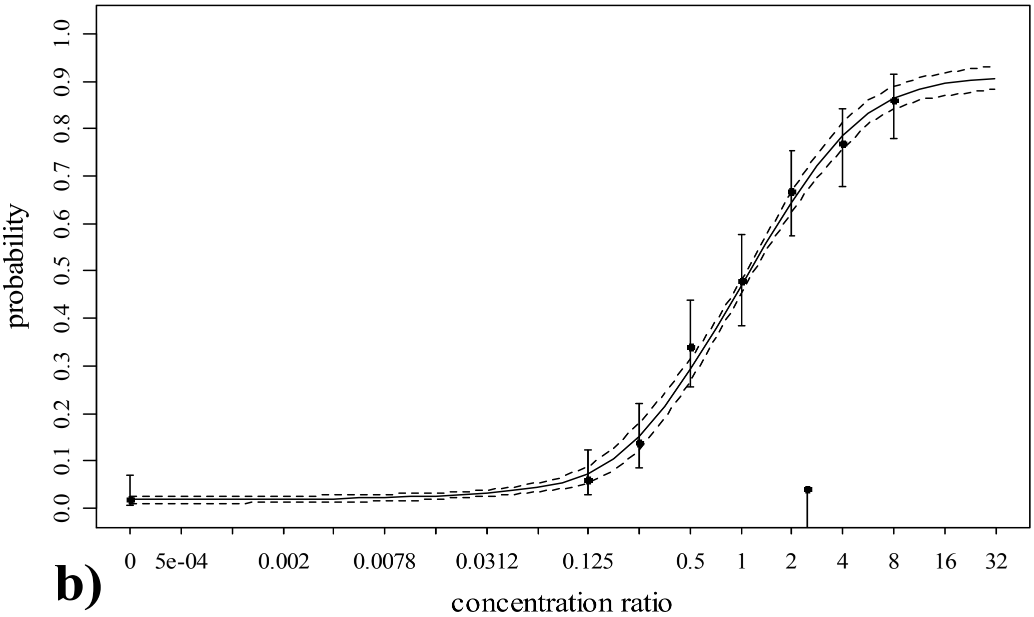
E. procera, high concentration: GOF=0.89, bc=0.01425 AE/cm, b (slope)=0.63 (range 0.54–0.75), t_{ds75} =3.33 (range 2.77–4.07), λ=0.03 (range *0.0–0.05*), N=110, n=5/10/10/10/10/20/10/10/15/10 (from left to right).

L. humile: GOF=0.86, bc=0.0014 AE/cm, b=0.87 (range 0.75–1.01), t_{ds75}=2.48 (range2.16–2.85), λ=0.02 (range 0–0.05), N=80, n=10 (each).

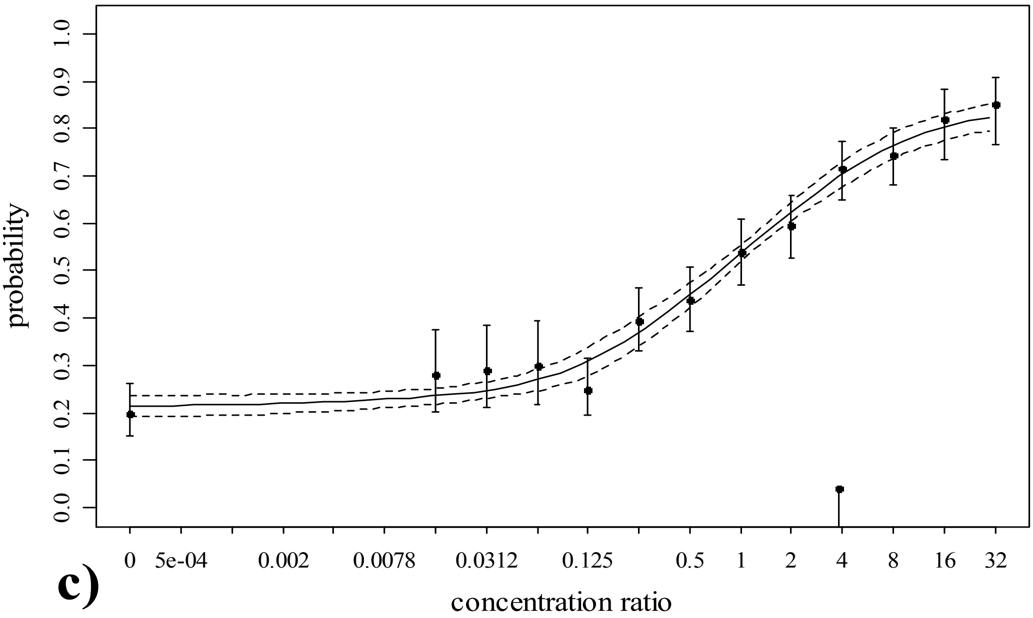
L. niger: GOF=0.57, bc=0.0057 AE/cm, b=0.56 (range 0.4–0.79), t_{ds75}=3.82 (range 2.59–6.49), λ=0.21 (range 0.15–0.25), N=210, n=20/10/10/10/20/20/20/20/20/20/20/10/10 (from left to right).


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration ratio

a)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration ratio

b)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration ratio

c)

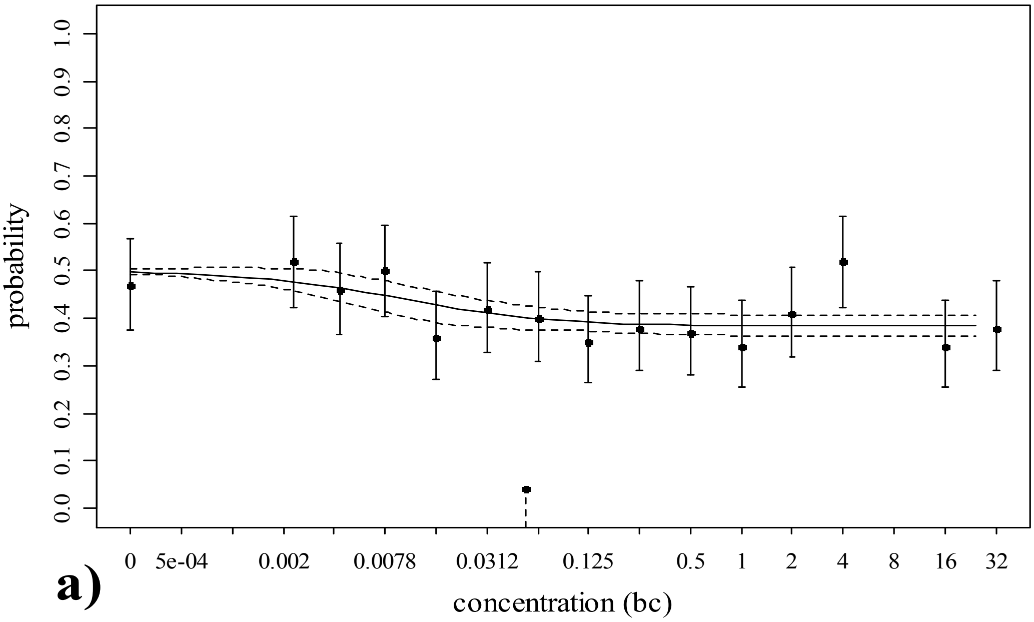
Fig. C3

Concentration dependency of fixed concentration ratio. (a) *Linepithema humile*, (b) *Lasius niger*. The response to the same concentrations ratio of 0.5 between test and reference trail at different absolute concentrations are shown. The x-axis shows the pheromone concentration of the test trail measured in base concentrations on a $\log_2$-scale. The concentration of the reference trail was twice the concentration of the test trail. Circles show the probability that ants take the test trail (response) and bars show the 95%-confidence interval. The solid line shows the fitted psychometric function with additional parameter k_pf and the corresponding 95%-prediction interval as dashed lines. The asterisk (*) marks a measurement series with both trails carrying no pheromone. The dotted vertical line shows the 75%-detection threshold measured in detection experiments.

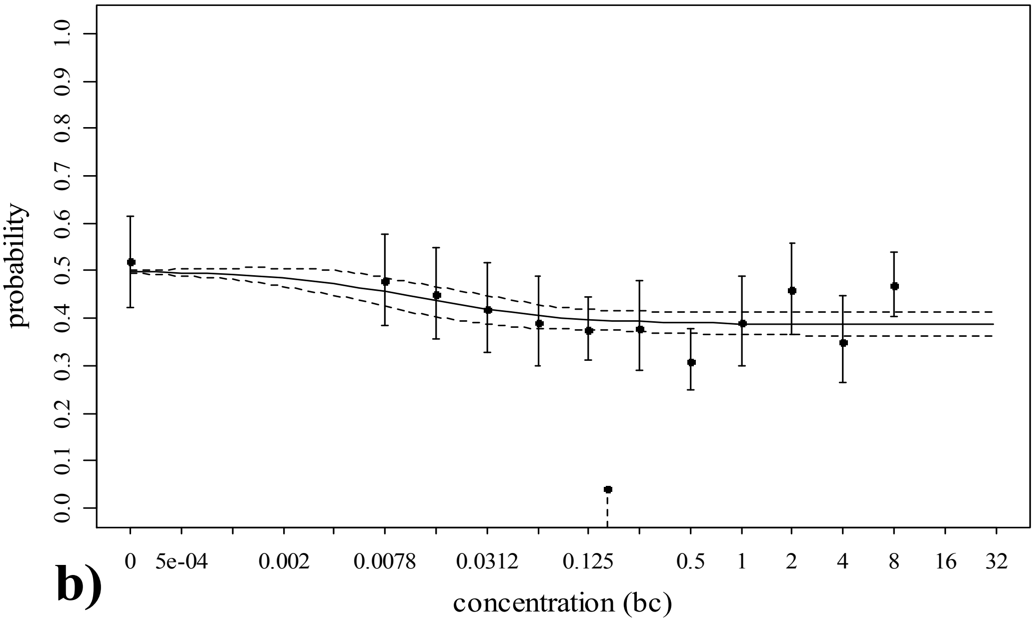
GOF=Goodness of fit, bc=base concentration, b=slope, k_pf=constant, N=total sample size, n=number of measurements per concentration (each measurement represents 10 binomial ant decisions).

L. humile: GOF=0.34, bc=0.0014 AE/cm, b=0.52 (*range 0.41–0.64*), k_pf=0.02 (range 0.0–0.06), N=150, n=10 (each).

L. niger: GOF=0.08, bc=0.0057 AE/cm, b=0.5 (range 0.4–0.63), k_pf=0.02 (range 0.0–0.09), N=151, n=10/10/10/10/10/20/10/20/10/10/10/21 (from left to right).


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration (bc)

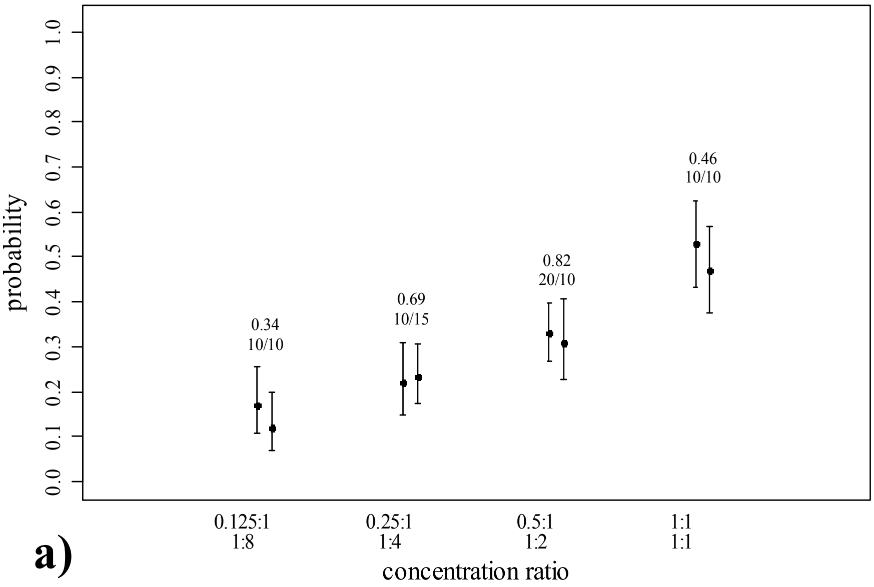
a)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration (bc)

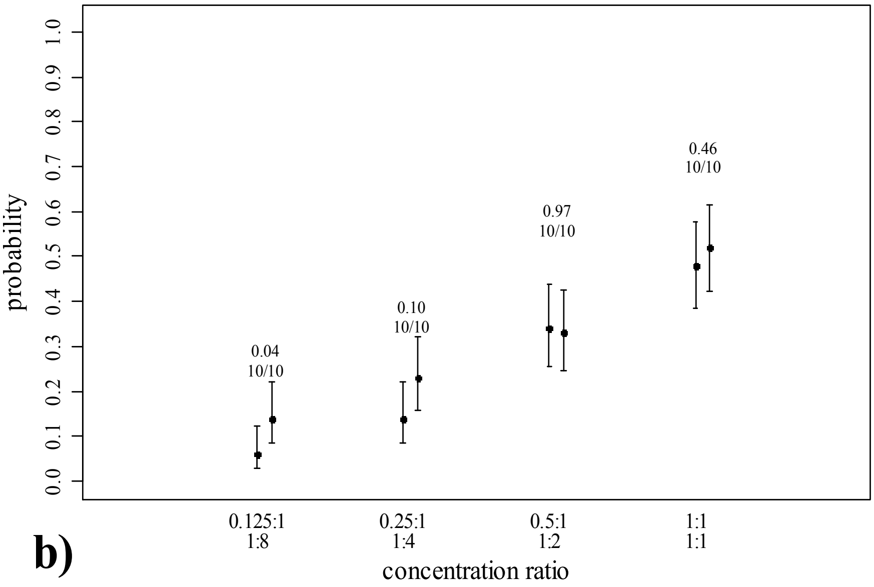
b)

Fig. C4

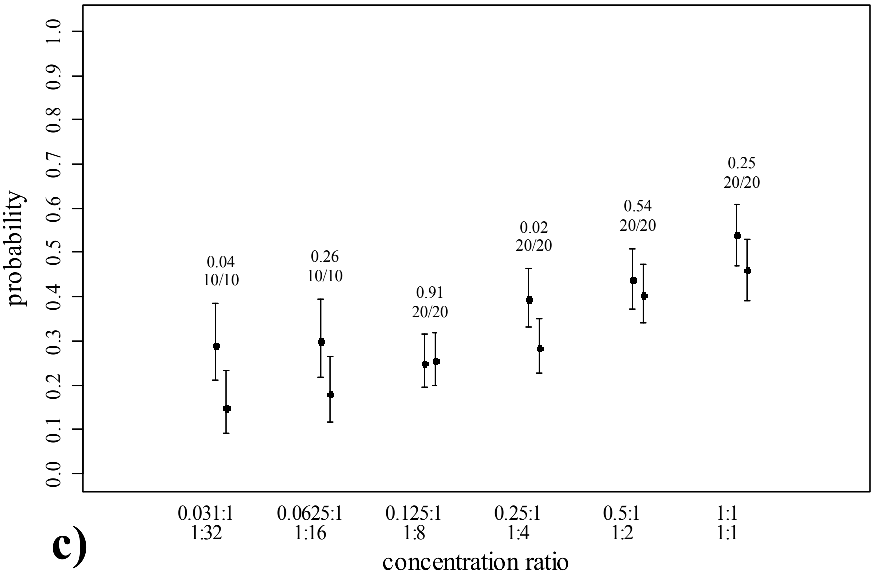
Symmetry of concentration ratios. (a) *Euprenolepis procera* (high concentration measurement series), (b) *Linepithema humile*, (c) *Lasius niger*. Shown is the probability that ants take the test trail (response) at different concentration ratios of the discrimination experiments. Each pair shows an equal concentration ratio but differs in the absolute concentrations. The left part of each pair shows the concentration ratio below 1, and the right shows the same concentration ratio above 1. The absolute concentrations differ for *E. procera* and *L. humile* by the factors of 8, 4, 2, 1 (from left to right), and for *L. niger* by the factors of 32, 16, 8, 4, 2, 1. Number above bars give p-value and sample size of two-sample Wilcoxon-test.


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.34
10/10
0.69
10/15
0.82
20/10
0.46
10/10
0.125:1
1:8
0.25:1
1:4
0.5:1
1:2
1:1
1:1
concentration ratio

a)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.04
10/10
0.10
10/10
0.97
10/10
0.46
10/10
0.125:1
1:8
0.25:1
1:4
0.5:1
1:2
1:1
1:1
concentration ratio

b)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.04
10/10
0.26
10/10
0.91
20/20
0.02
20/20
0.54
20/20
0.25
20/20
0.031:1
1:32
0.0625:1
1:16
0.125:1
1:8
0.25:1
1:4
0.5:1
1:2
1:1
1:1
concentration ratio

c)

Fig. C5

Detection experiment fitted with Deneubourg choice function for (a) *Linepithema humile*, (b) *Lasius niger*. The response to different pheromone concentrations is shown. The x-axis shows the pheromone concentration of the test trail measured in base concentrations on a $\log_2$-scale; the reference trail carried no pheromone. Circles show the probability that ants took the test trail (response) and bars show the 95%-confidence interval. The solid line shows the fitted Deneubourg choice function and dashed lines show its 95%-prediction interval. The measurement at 0.125 bc in (b) was regarded as an outlier and not included in the fitting process.

GOF=Goodness of fit, bc=base concentration, b=exponent, k=constant, N=total sample size, n=number of measurements per concentration (each measurement represents 10 binomial ant decisions).

L. humile: GOF=0.41, bc=0.0014 AE/cm, b=0.52 (range 0.44–0.64), k=0.02 (range 0.01–0.04), N=100, n =10 (each).

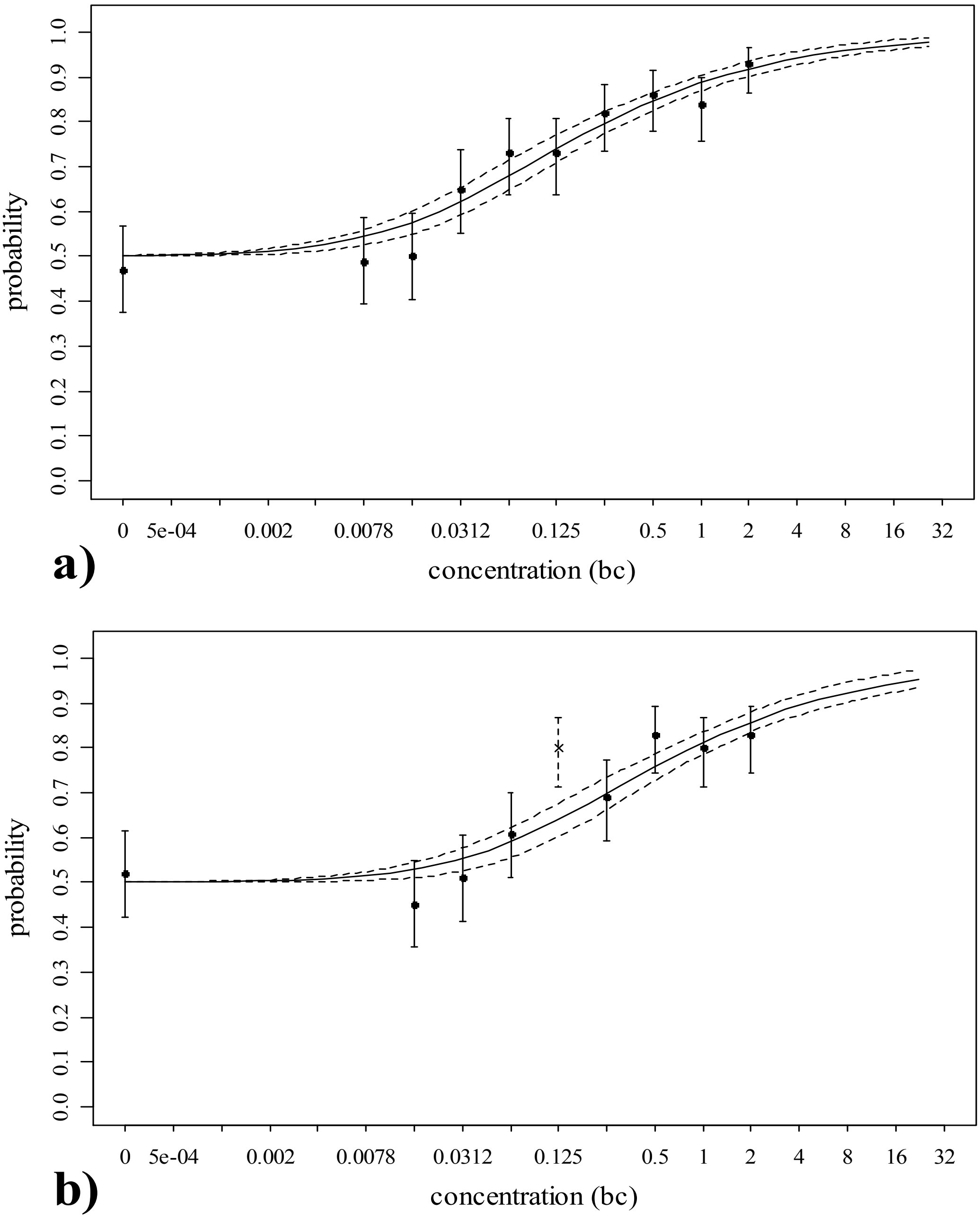


Fig. C6

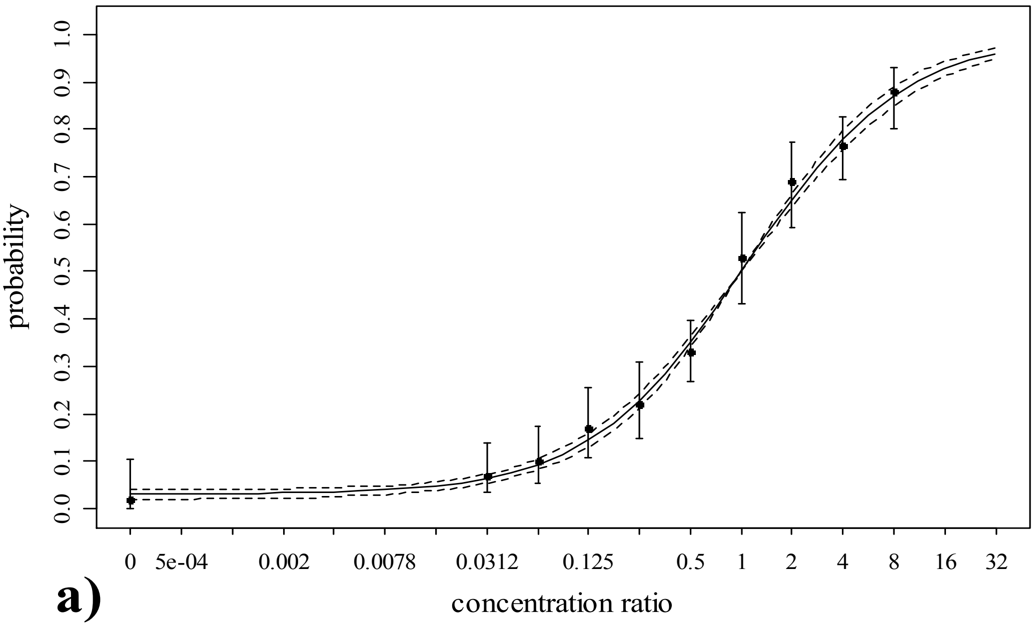
Discrimination experiments fitted with the Deneubourg choice function. The response to different pheromone concentration ratios is shown. (a) *Euprenolepis procera*, high concentration measurement series, (b) *Linepithema humile*, (c) *Lasius niger*. The x-axis shows the pheromone concentration of the test trail measured in base concentrations on a $\log_2$-scale; the reference trail carried no pheromone. Circles show the probability that ants take the test trail (response) and bars show the 95%-confidence interval. The solid line shows the fitted Deneubourg choice function and dashed lines show its 95%-prediction interval.

GOF=Goodness of fit, bc=base concentration, b=exponent, k=constant, N=total sample size, n=number of measurements per concentration (each measurement represents 10 binomial ant decisions).

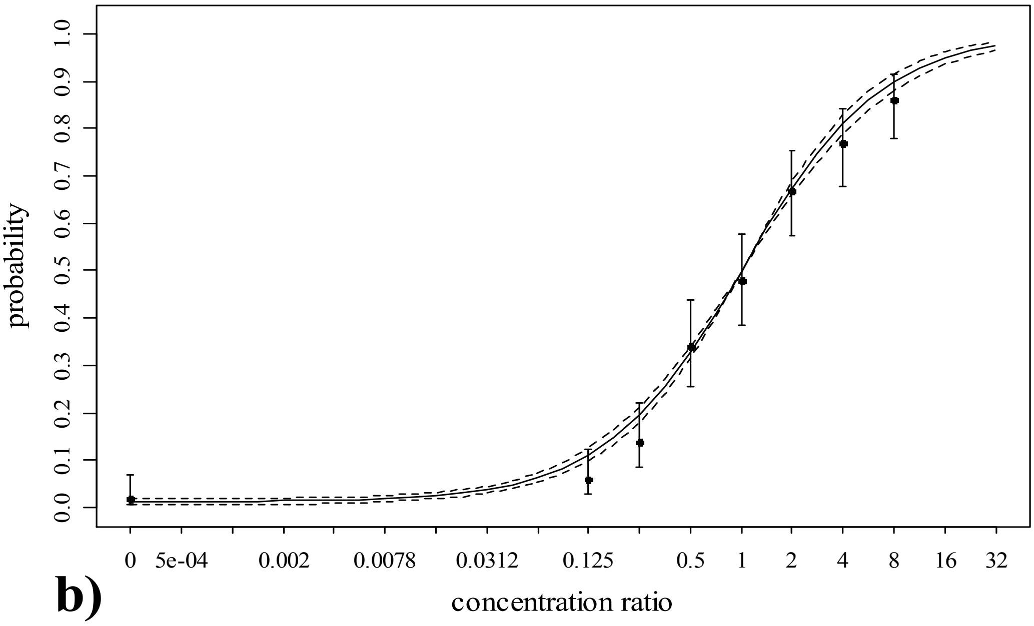
E. procera, high concentration: GOF=0.96, bc=0.0057 AE/cm, b=0.92 (range 0.83–1.02), k=0.03 (range 0.0–0.06), N=110, n=5/10/10/10/10/20/10/10/15/10 (from left to right).

L. humile: GOF=0.22, bc=0.0014 AE/cm, b=1.06 (range 0.96–1.19*)*, k=0.02 (range 0.0–0.06), N=80, n=10 (each).

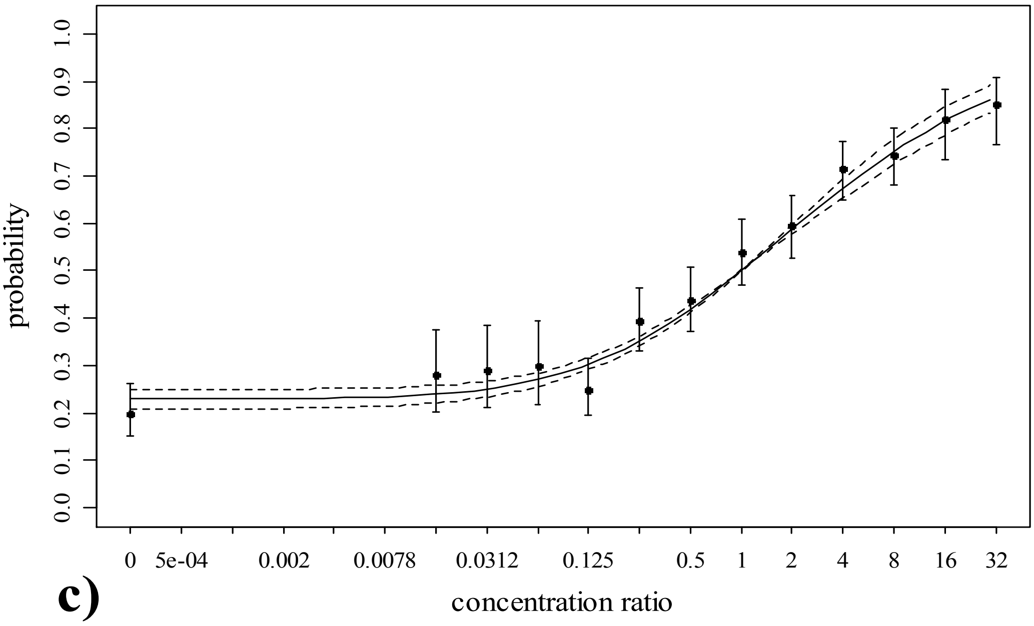
L.niger: GOF=0.45, bc=0.0057 AE/cm, b=0.56 (range 0.49–0.65), k=0.13 (range 0.06–0.23), N=210, n=20/10/10/10/20/20/20/20/20/20/20/10/10 (from left to right).


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration ratio

a)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration ratio

b)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration ratio

c)

Fig. C7

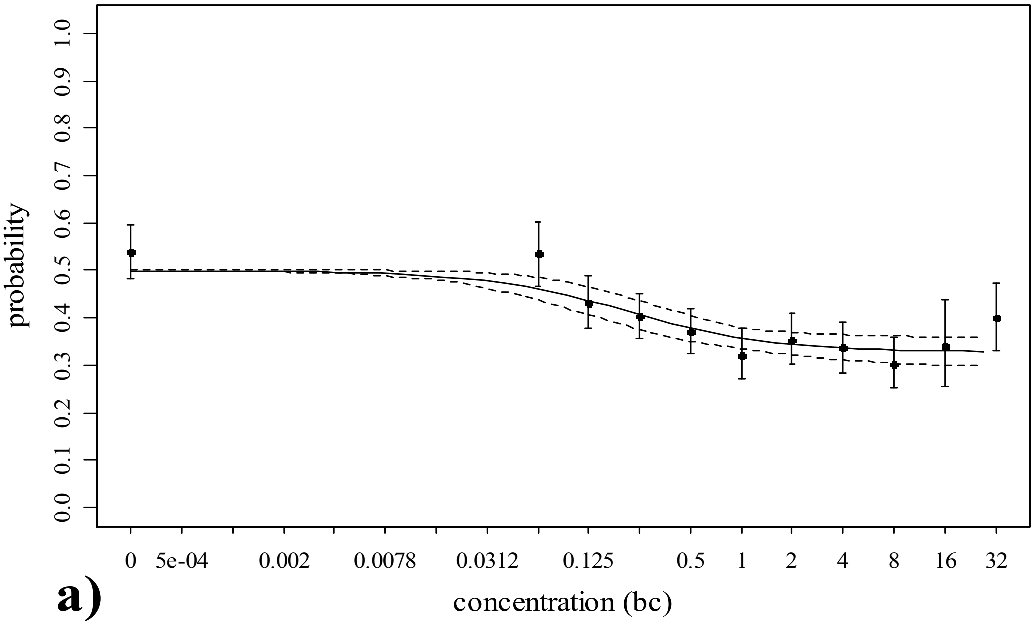
Concentration dependency of fixed concentration ratios fitted with the Deneubourg choice function. (a) *Euprenolepis procera*, (b) *Linepithema humile*, (c) *Lasius niger*. The response to the same concentrations ratio of 0.5 between test and reference trail at different absolute concentrations is shown. The x-axis shows the pheromone concentration of the test trail measured in base concentrations on a $\log_2$-scale. The concentration of the reference trail was twice the concentration of the test trail. Circles show the probability that ants take the test trail (response) and bars show the 95%-confidence interval. The solid line shows the fitted Deneubourg choice function and the corresponding 95%-prediction interval as dashed lines. The asterisk (*) marks a measurement series with both trails carrying no pheromone.

GOF=Goodness of fit, bc=base concentration, b=exponent, k=constant, N=total sample size, n=number of measurements per concentration (each measurement represents 10 binomial ant decisions).

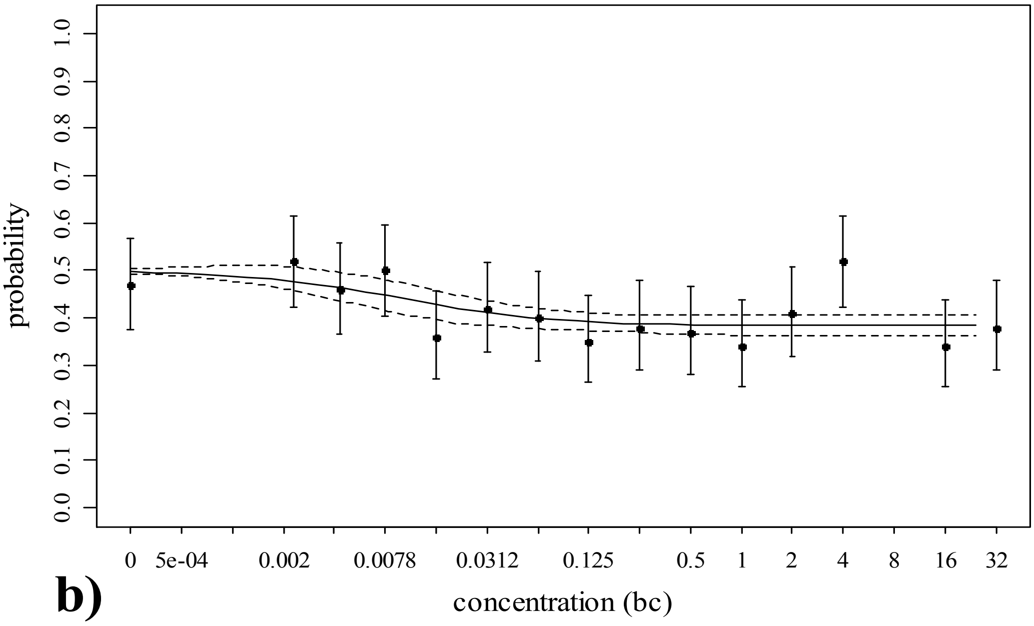
E. procera: GOF=0.15, bc=0.0057 AE/cm, b=1.03 (range 0.73–1.26), k=0.32 (range *0.0-0.77*), N=306, n=30/20/30/40/40/30/30/30/28/10/18 (from left to right).

L. humile: GOF=0.34, bc=0.0014 AE/cm, b=0.68 (range *0.55–0.83*), k_pf=0.02 (range *0.0–0.06*), N=150, n=10 (each).

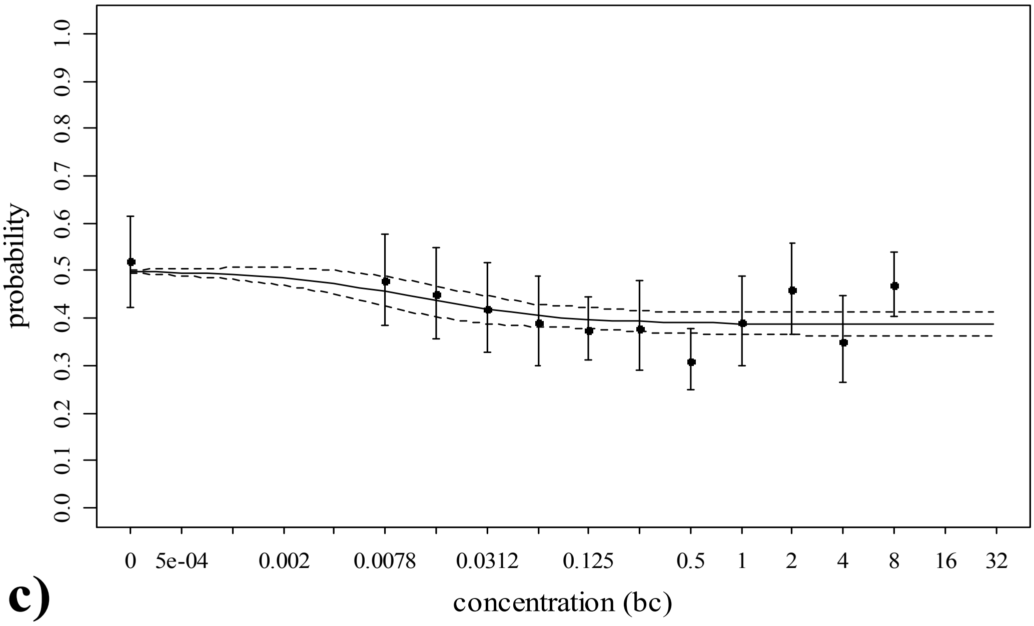
L. niger: GOF=0.08, bc=0.0057 AE/cm, b=0.66 (range 0.52–0.82), k_pf=0.02 (range 0.0–0.1)), N=151, n=10/10/10/10/10/20/10/20/10/10/10/21 (from left to right).


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration (bc)

a)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration (bc)

b)


probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5e-04
0.002
0.0078
0.0312
0.125
0.5
1
2
4
8
16
32
concentration (bc)

c)

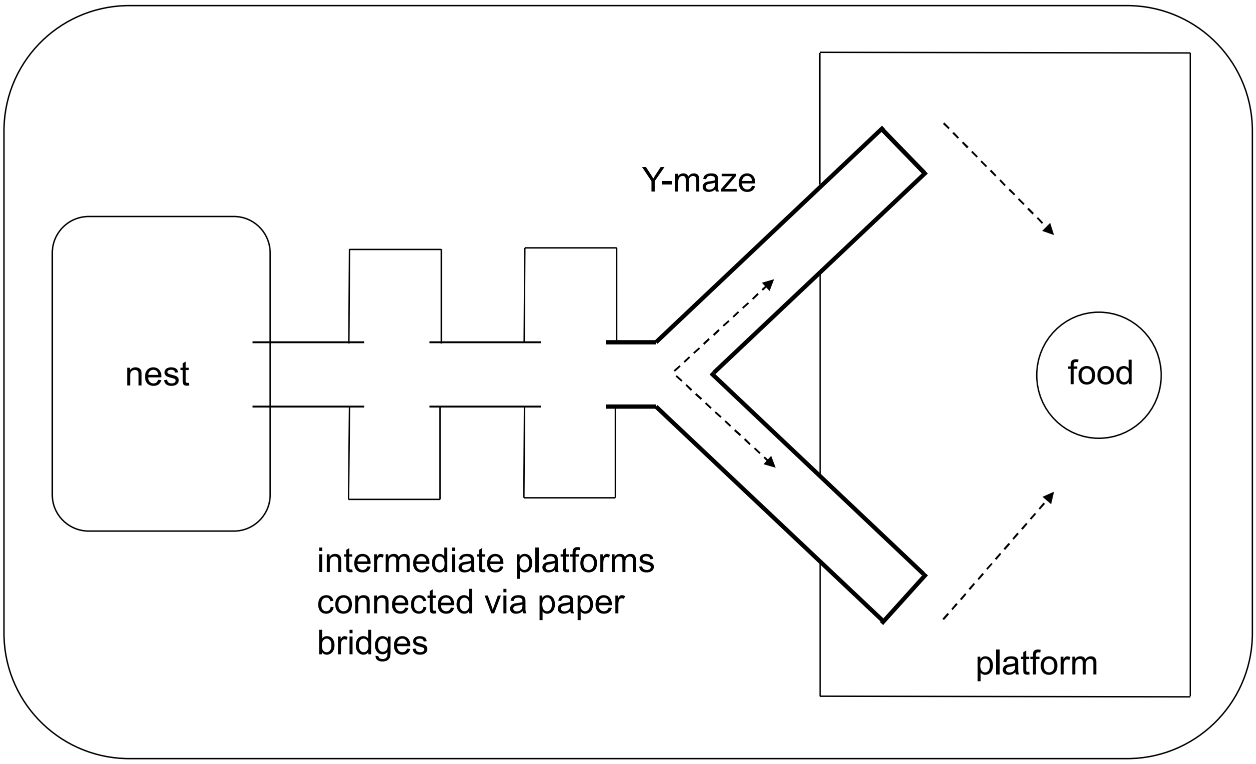
Online Appendix D: Libraries of R being used

Library	Used for	Version
base	Basic functions	2.14.0
stats	Statistical functions	2.14.0
MASS	Statistical functions	7.3-16
coin	Chi-square-Test	1.0-21
lme4	Mixed-effects model	1.0-5

Online Appendix E: Experimental setup

Fig. E1

Experimental setup (schematic). The connection between nest and food was established via paper bridges. The ants had to decide which trail to follow at the bifurcation of the Y-maze. The two branches of the Y-maze had been prepared with pheromone solutions of different concentrations while the straight part of the Y-maze was prepared with both pheromone solutions. The paper bridges were 1 cm wide. The bifurcation was positioned 2 cm distant from the left edge of the Y-maze and the distance between bifurcation and right edge of an arm was 5 cm. Thus, the total length of one complete arm from the left edge to the right edge of the Y-maze was 7 cm. The angle between both Y-arms was 100°.



Online Appendix F: Estimation of realistic concentration ranges and base concentrations

Realistic concentration ranges

Physiologically realistic concentration ranges of pheromones were estimated prior to our experiments. The experimental setup was similar to that described in the main article (see Fig. E1) but instead of the Y-bridges, we offered ants the choice between two different trails to follow (see Fig. F1a). One was a trail that had been established by the ants for 30-45 minutes on a paper bridge connecting their nest to a food source (referenced here as "natural trail"). The other trail was a paper bridge (width=1 cm, length=5 cm) marked with gland extract of known pheromone concentration leading away from the natural trail but also towards the food source (referenced here as "artificial trail"). We offered a series of such artificial trails marked with different pheromone concentrations and attempted to measure at which pheromone concentration half of the ants would follow the artificial trail. We assumed that if half of the ants followed the artificial trail (decision probability=0.5), its pheromone concentration to be equal to the pheromone concentration of the natural trail. Since all artificial bridge pheromone concentrations were known, we were able to estimate the pheromone concentration of a naturally constructed trail by graphical linear interpolation between the concentrations that were giving the closest results below and above the decision probability p=0.5. For each pheromone concentration, 10 artificial trails were tested by counting 10 ant decisions each. To minimize possible memory effects, in half of the tests for each concentration the artificial trail was placed on the left side of the natural trail and in the other half on the right side. For results see Table F1 below.

Table F1: Estimation of realistic trail concentrations and base concentrations

Species	Natural trail concentration (AE/ml)	Preparation of pheromone solution (glands/ml=AE/ml)	Base concentration (bc) (AE/cm)
E. procera	9.77 (range 7–14)	10	0.0057
L. niger	10.56 (range 8–13)	10	0.0057
L. humile	1.5 (range 0.8–2.7)	2.5	0.0014

We performed an control experiment for our assumption that ants take both trails with equal chance if the pheromone concentration on both trails is equal: As before, we lead ants away from the natural trail by paper bridges that had been cut out of the natural trail immediately before the test at a different position ca. 40 cm towards the food source (see Fig. 1b). Although at a different position, both trails can be expected to have the same or at least similar pheromone concentrations. Each bridge was tested with 20 ants, then it was exchanged by a new bridge to avoid possible differences in pheromone concentrations caused by ants marking the trails during the test. For *E. procera* 6 bridges (120 ants) were used and for *L.*

humile and *L. niger* 4 bridges (80 ants). 95%-Confidence intervals and p-values were calculated with a binomial proportion test (R-function binom.test()). Results see table F2. The null hypothesis that the ants took both trails with equal chance could not be rejected.

Table F2: Control experiment for realistic trail estimation

Species	Proportion of ants taking the trail leading away from the natural trail	n	p (proportion test)
E. procera	45% (range 34%–56%)	120	0.43
L. niger	54% (range 41%–64%)	80	0.74
L. humile	47% (range 38%-56%)	80	0.52

Base concentrations

Based on our estimations for realistic concentration ranges, we attempted to simplify the pheromone preparation and standardize the pheromone concentrations for our bioassay (see "Experimental setup" in the main article). To achieve this, we always prepared solutions with concentrations of 10 glands/ml for *E. procera* and *L.niger* and 5 glands/2 ml for *L. humile*. In our bioassay, 4 µl of pheromone solution was applied to 7 cm of artificial trail on a Y-bridge. Thus, the concentrations expressed in units of AE/cm are 0.0057·AE/cm for *E. procera* and *L. niger* and 0.0014·AE/cm for *L. humile*. These concentrations were taken as *base concentration* (*bc*) in all experiments using glands as pheromone source.

Example for the Estimation of base concentration

Dissolving 10 glands in 1 ml DCM gives a *pheromone concentration* of

$$cPh = 10\frac{AE}{ml}\cdot$$

Applying 4 µl of this solution to a trail of length 7 cm gives a *base concentration* of

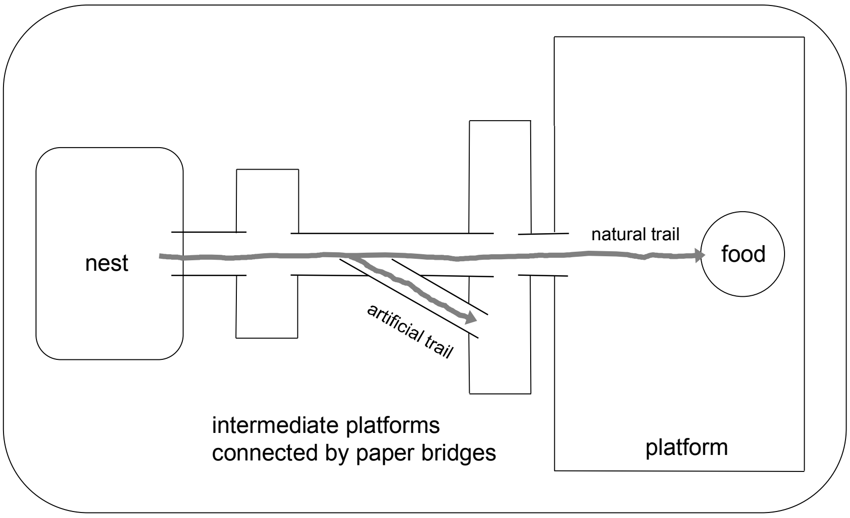
$$bc = \frac{10AE}{ml}\cdot\frac{4\mu l}{7cm} = 5.7\cdot 10^{-3}\frac{AE}{cm}\cdot$$

Fig. F1

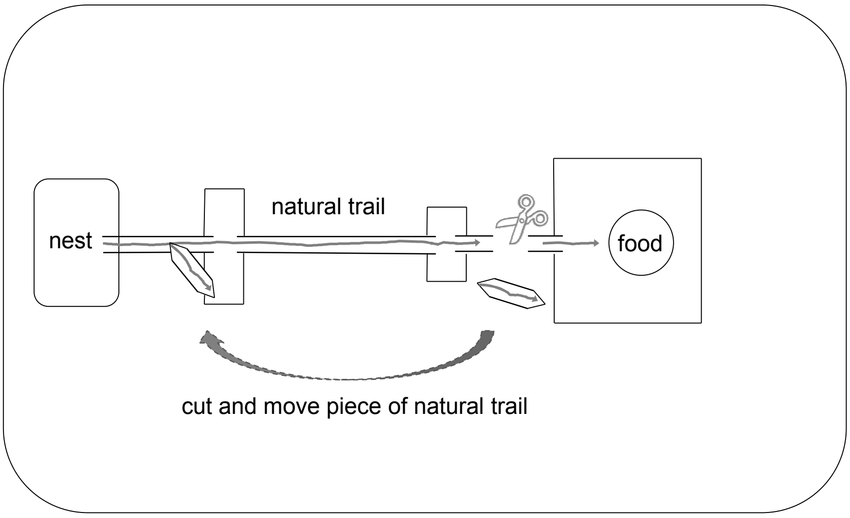
Experimental setup for estimating realistic concentration regions (schematic).

a) The connection between nest and food was established via paper bridges (width=1 cm). The ants had the choice between two trails, one natural and the other artificial. The artificial trails we tested (width=1 cm, length=5 cm) were marked with artificial pheromone of different concentrations. We assumed that the number of ants taking both trails was equal, if the pheromone concentration on both trails was equal. By using artificial trails with different pheromone concentrations and counting the ants, we were able to estimate the concentration at which equal number of ants took both trails. Thus we were able to estimate the concentration of the natural trail.

b) As control, we cut a piece out of the paper bridge of the natural trail (width=1 cm, length=3 cm) at a different position and again offered the ants the choice of two trails by placing the outcutted piece beside the natural trail. If the assumption we made before was correct, the number of ants taking the trails should be equal since the pheromone concentrations can be expected to be equal.



a)



b)

Online Appendix G: Model comparison

The hypothesis that the psychometric function fits the data (D) better than the Deneubourg choice function was tested. First the Akaide information criterion (AIC) of both models was calculated. A lower AIC indicates a better fit. We further estimated the significance of this result by calculating the log-likelihood ratio LLr(D) between the fitted psychometric function and the fitted Deneubourg choice function. Then we performed a parametric booststrap by generating 1,000 sets of simulated data (S) using the Deneubourg choice function and its' fitted parameters. In the same way as before, we fitted both functions to the simulated data and calculated the log-likelihood ratio. Thus, we gained a set of 1,000 simulated log-likelihood ratios LLr(S). The null-hypothesis that the Deneubourg choice function fits the experimental data as well or better than the psychometric function was rejected if LLr(D) was smaller than the lower 5%-confidence bound of LLr(S). The p-value was evaluated by calculating the number of LLr(S)<LLr(D) divided by the number of simulations. For results, see Table G1 below.

Table G1: Comparing the psychometric function to the Deneubourg choice function

Experiment	Species	LLr(D)	$LLr_{0.05}$	ΔAIC	par	N	p
Detection	*E. procera*	-0.41	-2.53	-3.2	2/4	1,000	0.62
Detection	*L. humile* Pavan's gland	-0.23	-3.02	-3.6	2/4	1,000	0.72
Detection	*L. niger*	-1.69	-3.03	-0.7	2/4	1,000	0.18
Discrimination Low	*E. procera*	-3.91	-1.8	5.8	2/3	1,000	0.007
Discrimination High	*E. procera*	0.24	-2.11	-2.4	2/3	1,000	0.83
Discrimination	*L. humile* Pavan's gland	-3.18	-1.77	4.3	2/3	1,000	0.01
Discrimination	*L. niger*	-1.17	-1.62	0.3	2/3	1,000	0.006

LLr(D)	log likelihood-ratio between Deneubourg choice function and psychometric function,
$LLr_{0.05}$	lower 5% confidence bound of simulated log likelihood-ratios,
ΔAIC	Difference between AIC for the Deneubourg choice function and AIC for the psychometric function,
par	number of free parameters for calculating the AIC (Deneubourg choice function/psychometric function),
N	number of simulations,
p	p-value.

Supplemental material chapter 2

Supplemental content

Article: Modeling shortest path selection of the ant Linepithema humile using psychophysical theory and realistic parameter values

Wolfhard von Thienen,[1*] Dirk Metzler,[1] Volker Witte[1]
1. Fakultät für Biologie, Department Biologie II, Ludwig-Maximilians Universität München
Großhaderner Str. 2, D-82152 Planegg, Germany
e-mail: w@thienen.de, metzler@bio.lmu.de, witte@bio.lmu.de
Telephone: 049-8233-31286
* Corresponding author

S1. Tests of additional assumptions

The results of the simulations of different path lengths with the PF were of slightly better quality than the original simulations of Goss et al. (1989), showing an 8% higher *spsr80*. However, there was still a discrepancy between experiment and model since the model does not reach the high *spsr80* that occurred in the experiments (experiment: 93%, model PF 72% and model DCF 57%, see Fig. 5). Thus, real ants exclusively decided for the short path, while the virtual ants did not. Therefore, we tested additional and speculative assumptions, like simulating a combination of two pheromones with different diffusion rates, path memory, negative and positive feedback between pheromone concentration and pheromone deposition, and higher pheromone deposition on the return path in the initial phase. These additional assumptions did not clearly enhance the decision rate for the short path. We know from a study on Pharao's ants that ants involved in foraging show a significantly higher response to pheromone trails (Jackson and Châline, 2006). We speculated that this could also be the case for *L. humile* and extended the model by adding the assumption that ants involved in the beginning of foraging have an increased pheromone response. We did so by increasing the slope four times in the first 100 iterations of the simulations. By doing so, we were able to nearly exactly reproduce the experimental results. In 89% of the simulations, the short path was taken by most of the ants, which was significant ($p<0.0001$, one sided binomial test, $n=1000$). The *spsr80* was 89%, which was close to the original experimental results of Goss et al. (1989) (93%). For the DCF, we tested the same assumption by increasing the exponent. This showed the opposite effect and there was no significant preference of the short path. In 51% of the simulations, the short path was taken by most of the ants, which was not significant ($p=0.38$, one sided binomial test, $n=1000$). The *spsr80* was 51% and the *lpsr80* 49%. To test if the cause for this difference between DCF and PF was the same as described in the main article (see 4.4), we repeated the simulations and forced the first ant to select either the long or the short path. The results show that the ability to reverse the initial selection of the long path was enforced by the higher responsiveness in the initial phase. In case of the PF, 79% of the simulations with an initial long path selection were reversed towards the short path, while in case of the DCF, this did not happen at all (0%) (see Table ST1).

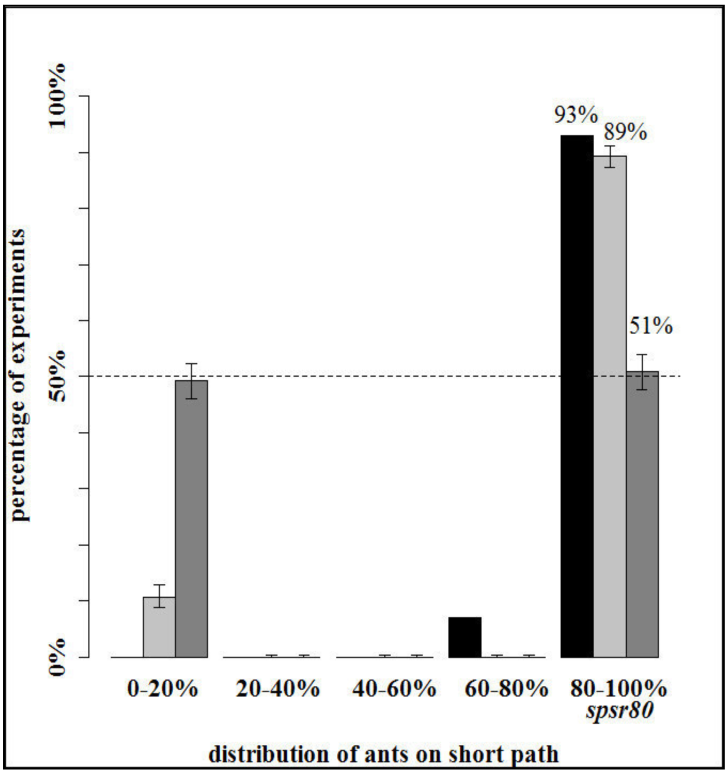


FIG S1.—Monte Carlo simulations as in Fig 5 of the main article. The assumption was made that ants deposit four times more pheromone on their way back from food to nest and that in the initial phase (first 100 iterations) the slope (PF) or exponent (DCF) of the function (detection and discrimination) was increased four times. The x-axis shows the distribution of ants that chose the short path (n=500). The y-axis shows the number of experiments (n=14) or simulations (n=1000) in percent. Black=original experimental results modified from Goss et al. (1989), light grey=simulation of the PF, dark grey=simulation of the DCF. Error bars give 95%-binomial confidence intervals of the simulations. Parameters for the DCF: b=1.19, k=0.02 (discrimination) and b=0.52, k=0.02 (detection). Parameters for the PF: γ=0.48, λ=0, b=1.02 (discrimination) and γ=0.43, λ=0.13, b=1.1, tdt_{75}=0.04 (detection).

Table ST1

Results of simulations with 1st ant selection fixed

1st ant	Function	*spsr80*
long path	PF	79 %
	DCF	0 %
short path	PF	96 %
	DCF	100 %

Notes: The simulations of Fig. S1 had been repeated with the first ant forced to select the long path for 1000 simulations. This was repeated for the short path for another 1000 simulations.

S2. Parameter space

S2.1 Simulations

Simulations were performed as described in the main article. The only difference was that we systematically varied the discrimination parameters of the functions and repeated 100 simulations for each parameter combination and calculated the *80%-short path selection rate* (*spsr80)* (see 3.2 in the main article). This rate was 93% in the original experiments of Goss et al. (1989). Table ST2 shows the values defining the simulated parameter space. Simulations were done with different path lengths as described in the main article. Detection parameters for the PF were kept constant and set to their fitted values according to Table A2 in the main article. The exponent of the DCF was set to its fitted value according to table A1 in the main article. The parameters *k* of the DCF for discrimination and detection were set equal.

Table ST2

Parameter space

Function	Parameter	Low	High	Steps	Increment per step
DCF	*k*	0.000575	35.875	80	multiply by 1.15
DCF and PF	*b*	0.125	3.1	120	add 0.025
PF	λ	0.005	0.4	80	add 0.005
PF and DCF	*phpa*	0.04	4.8	120	add 0.04

Notes:
DCF = Deneubourg choice function
PF = psychometric function
phpa = pheromone units per ant (amount of pheromone an ant releases on the trail at each side)
k = constant
b = exponent (DCF), slope (PF)
λ = lapse rate

S2.2 *Results*

Results are presented in the figures below. The *spsr80* in these figures is encoded as a heat map ranging from 0% to 100% .

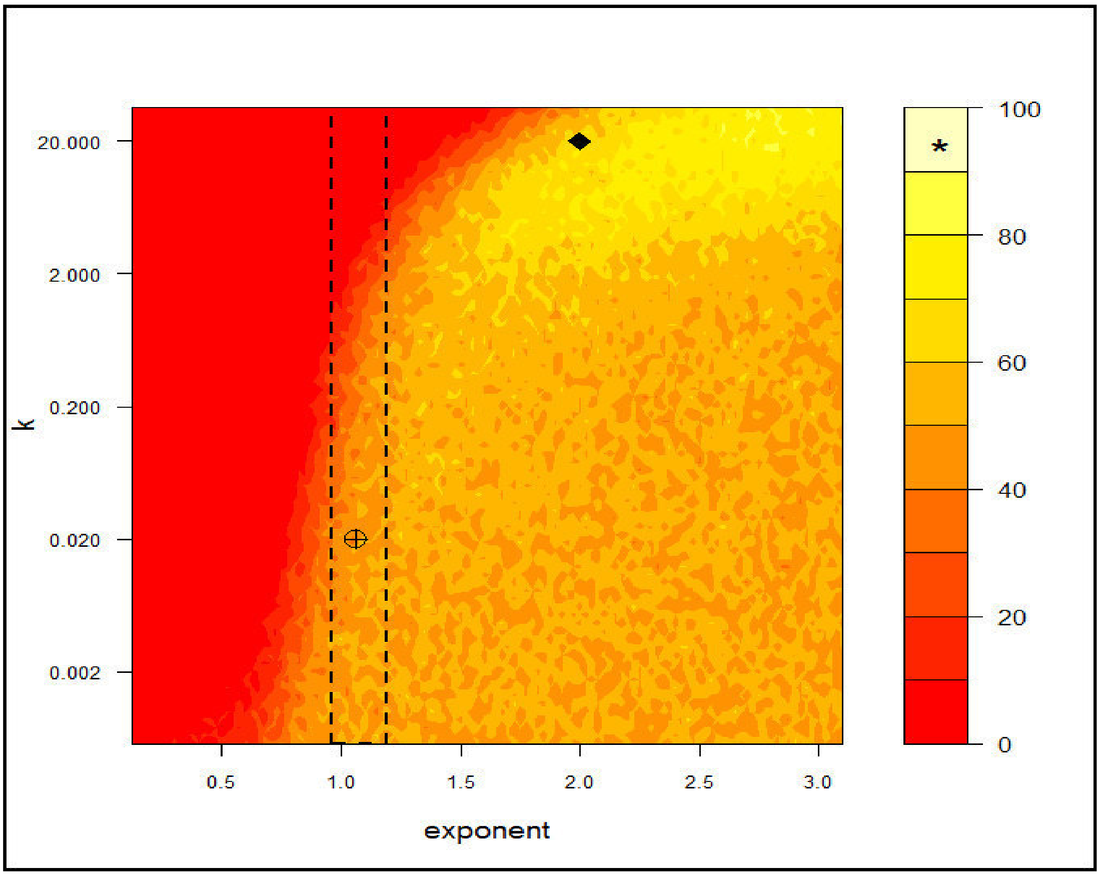
By applying the fitted parameters, the simulations with both functions were not able to reproduce the experimental results (see Fig. S2A and B). The confidence regions of the DCF and the PF were positioned in regions with a *80%-short path selection rate (spsr80)* well below the experimental results of Goss et al. (1989).

Assuming that ants modulated pheromone deposition and released four times more pheromone on their return path than in the opposite direction, the simulations with the PF gave values for the *spsr80* between 80% and 90% over a wide and contiguous region of the parameter space including some small regions with values >90% (see Fig. S3B). However, only a small fraction of the confidence region of the PF covered values >80%. The same simulations with the DCF showed that the short path selection rate exceeded 80% only for smaller and more scattered regions. Only parts of the confidence region covered values between 60% and 70% (see Fig. S3A). This suggests that both functions are only able to give an acceptable explanation of the shortest path experiments by taking into consideration that ants release more pheromone on their return path towards the nest than in the opposite direction. Simulations with the PF were closer to the experimental results than simulations with the DCF, but still, both functions could not fully reproduce the exact experimental results if realistic parameter values were applied.

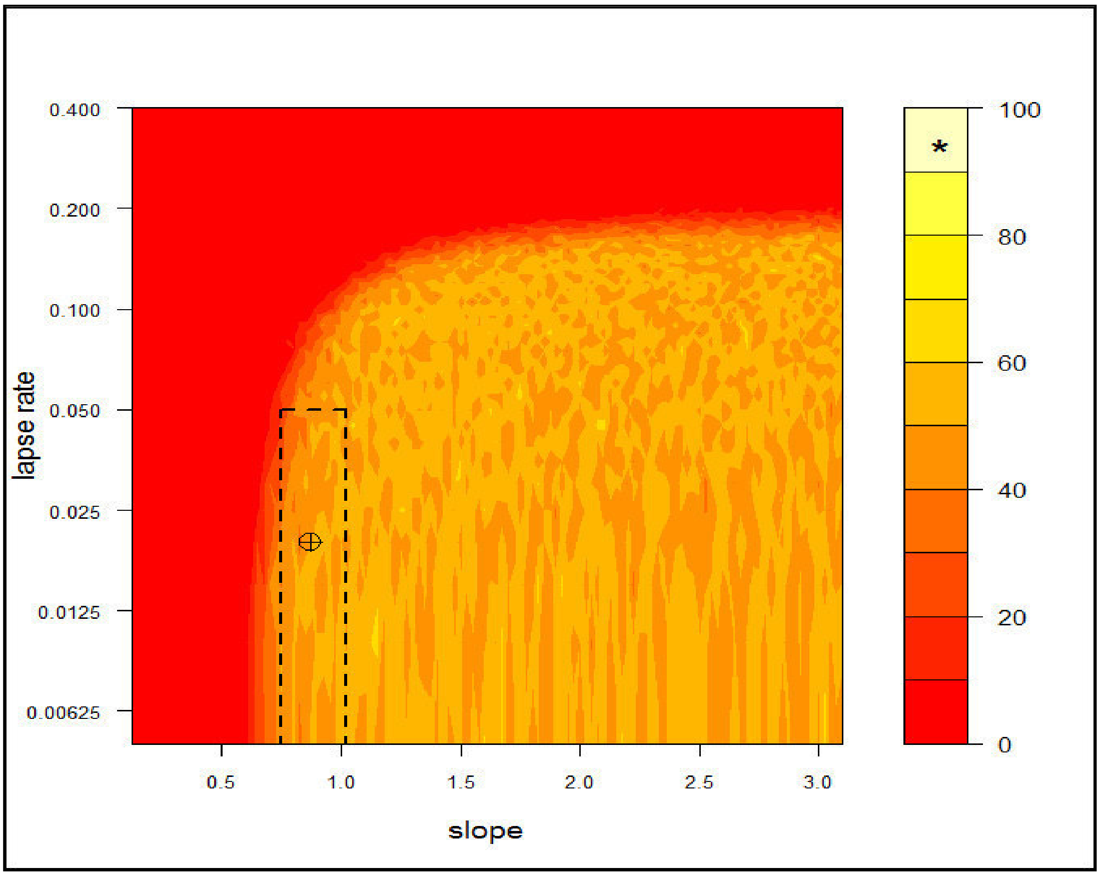
We made further simulations with the additional assumption that in the initial phase the exponent/slope is increased four times (see paragraph S1 above). The simulations with the PF yielded results between 90% and 100% over a vast and contiguous region of the parameter space. This region included most of the confidence region of the PF parameters (see Fig. S4B). Thus, the results were consistent with the experimental results of Goss et al. (1989). The same simulations with the DCF showed that only a small region of the parameter space reached values around 80% and the whole confidence region lay outside of this region (see Fig. S4A).

Finally, we tested if the simulations are sensible to the scale in which pheromone amounts are measured (see paragraph 4.7 in the main article). This scale is proportional to the amount of pheromone a single ant releases at the beginning and end of the trail each time it passes these points (*pheromone unit per ant – phpa*). It was arbitrary set to 0.5 pheromone units in our simulations, giving one pheromone unit per passagelike in the simulations of Goss et al. (1989). The simulations with the PF (see Fig. S5) displayed a pattern with horizontal layers indicating that the results remained relative stable over the given pheromone range.

The same simulations with the DCF and the parameter combination *phpa* and exponent showed horizontal patterns only for exponents <1.2, indicating that the results were invariant to the pheromone scale only for exponents <1.2 (see Fig. S6B). Since the confidence region of the exponent lay in this region, the simulations were more or less invariant to the pheromone scale giving values between 45% and 55% for the *spsr80*. The simulations with the parameter combination *phpa* and *k* showed a high dependency on the pheromone scale (see Fig. S6A). The results showed a clear pattern with layers parallel to a line representing the proportionality between *k* and *phpa*. This showed that the results of the simulations were invariant to the pheromone scale as long as *k* and *phpa* were kept proportional.

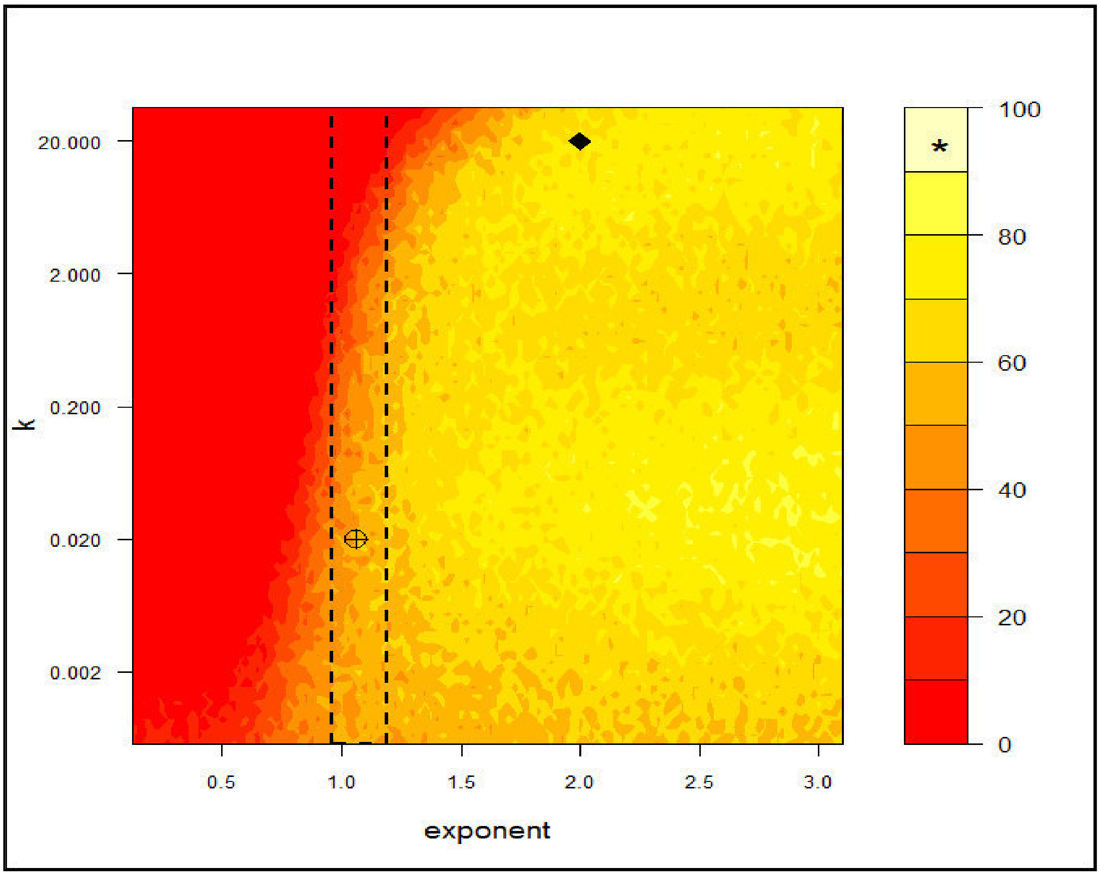

k
20.000
2.000
0.200
0.020
0.002
0.5
1.0
1.5
2.0
2.5
3.0
exponent
100
*
80
60
40
20
0

A

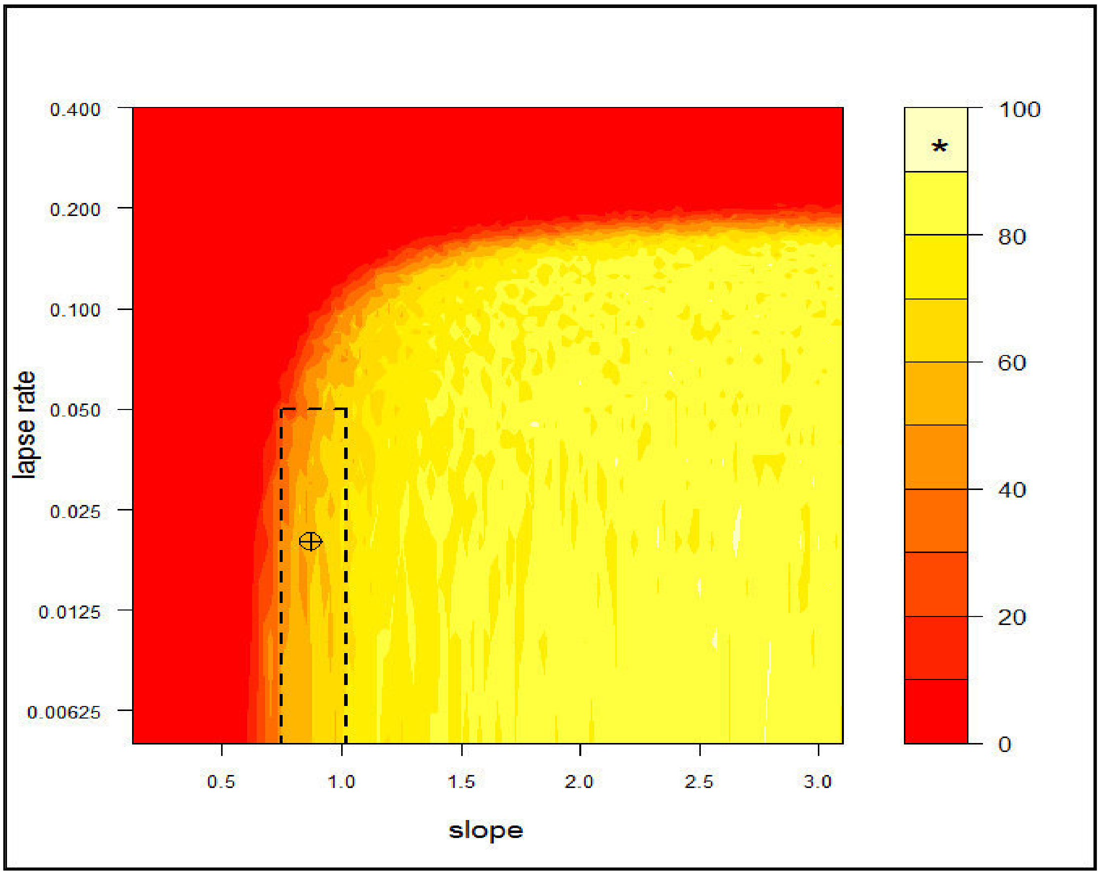

lapse rate
0.400
0.200
0.100
0.050
0.025
0.0125
0.00625
0.5
1.0
1.5
2.0
2.5
3.0
slope
100
*
80
60
40
20
0

B

FIG. S2.─Simulations covering the parameter space of the DCF (A) and the PF (B). 9600 parameter combinations were simulated, each with 100 simulations. Colors indicate the *80%-short path selection rate (spsr80)* of each parameter combination. The star symbol in the heat map indicates the result of the original experiments of Goss et al. (1989), which was 93%. The circled plus symbol represents the position of the fitted parameters, dashed lines show their confidence region (see table A1 and A2 in the main article). The parameter *k* of the DCF was free, thus it covers the whole y-axis. The black diamond in (A) represents the position of the parameter setting of Goss et al. (1989) (*k*=20, *b*=2). The y-axes are scaled logarithmic.

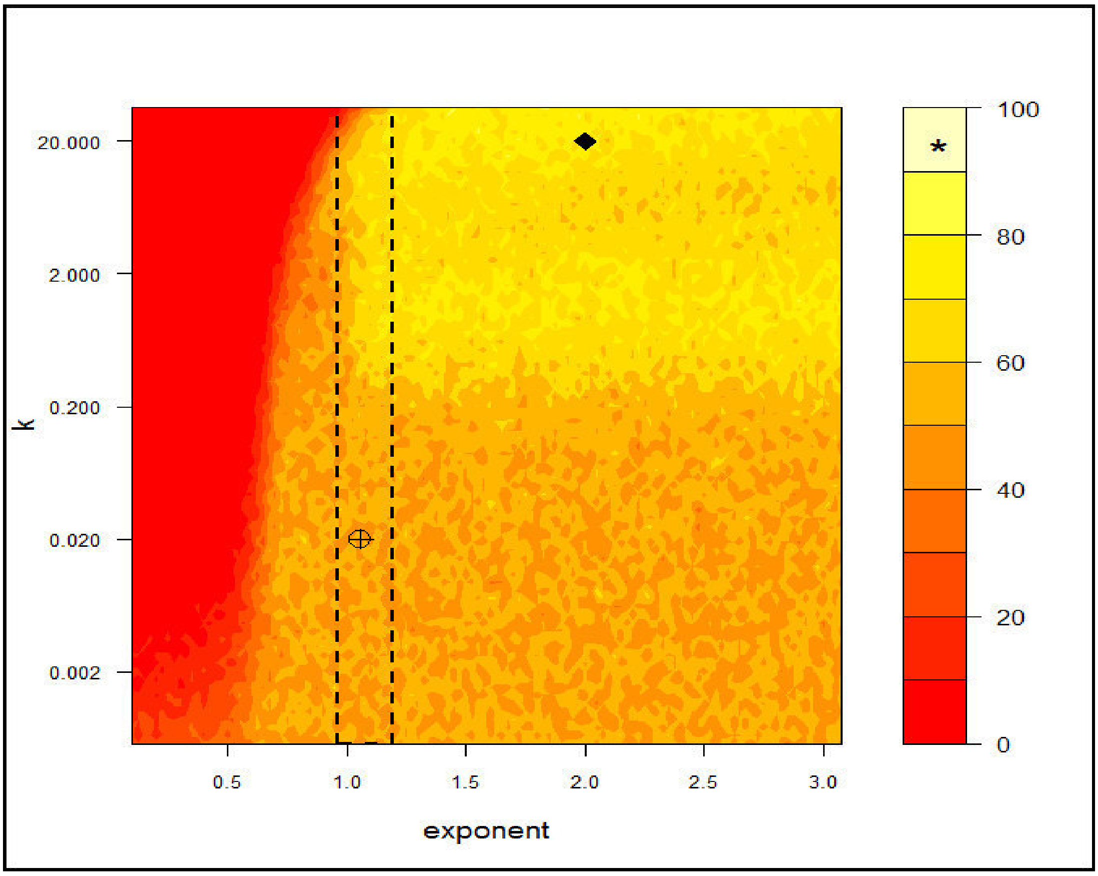

k
20.000
2.000
0.200
0.020
0.002
0.5
1.0
1.5
2.0
2.5
3.0
exponent
100
*
80
60
40
20
0

A

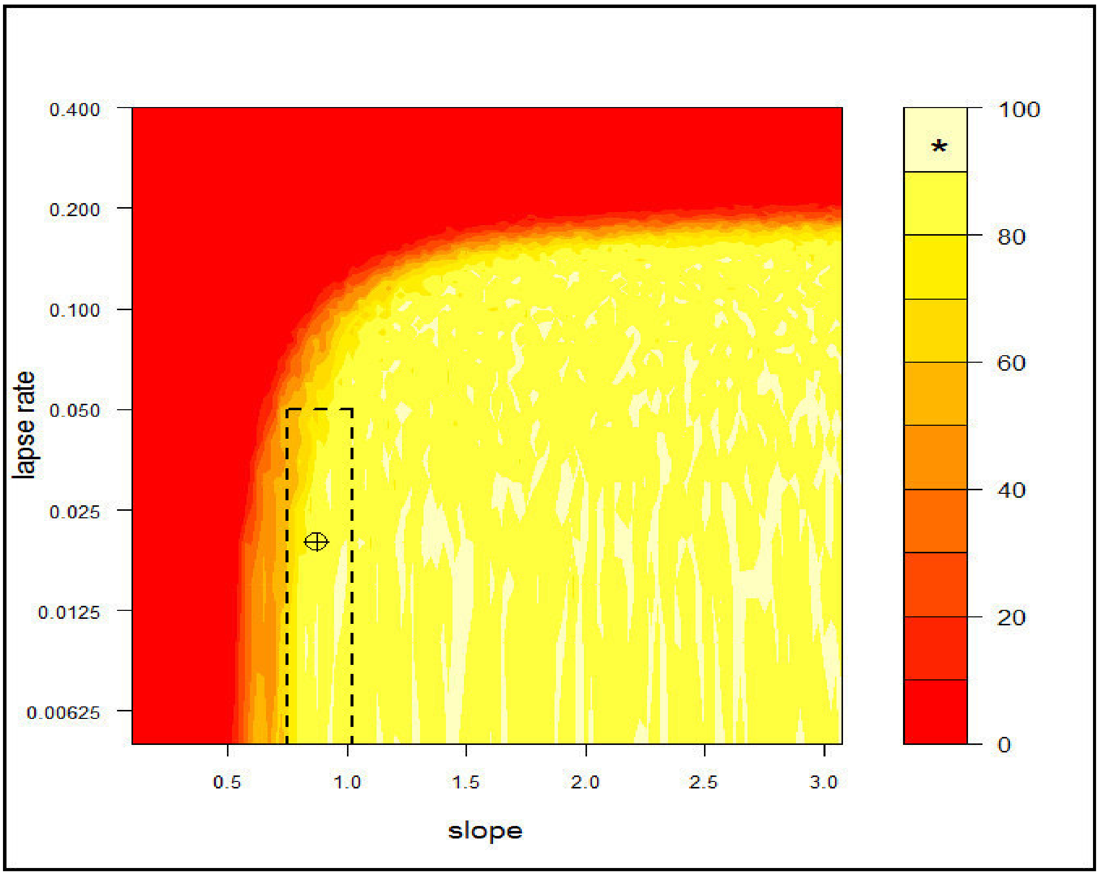

lapse rate
0.400
0.200
0.100
0.050
0.025
0.0125
0.00625
0.5
1.0
1.5
2.0
2.5
3.0
slope
100
*
80
60
40
20
0

B

FIG. S3.─Simulations covering the parameter space of the DCF (A) and the PF (B). Ants deposited four times more pheromone returning from food to nest than in the opposite direction. 9600 parameter combinations were simulated, each with 100 simulations. Colors indicate the *80%-short path selection rate (spsr80)* of each parameter combination. The star symbol in the heat map indicates the result of the original experiments of Goss et al. (1989), which was 93%. The circled plus symbol represents the position of the fitted parameters, dashed lines show their confidence limits (see table A1 and A2 in the main article). The parameter k of the DCF was free, thus it covers the whole y-axis. The black diamond in (A) represents the position of the parameter setting of Goss et al. (1989) (k=20, b=2). The y-axes are scaled logarithmic.

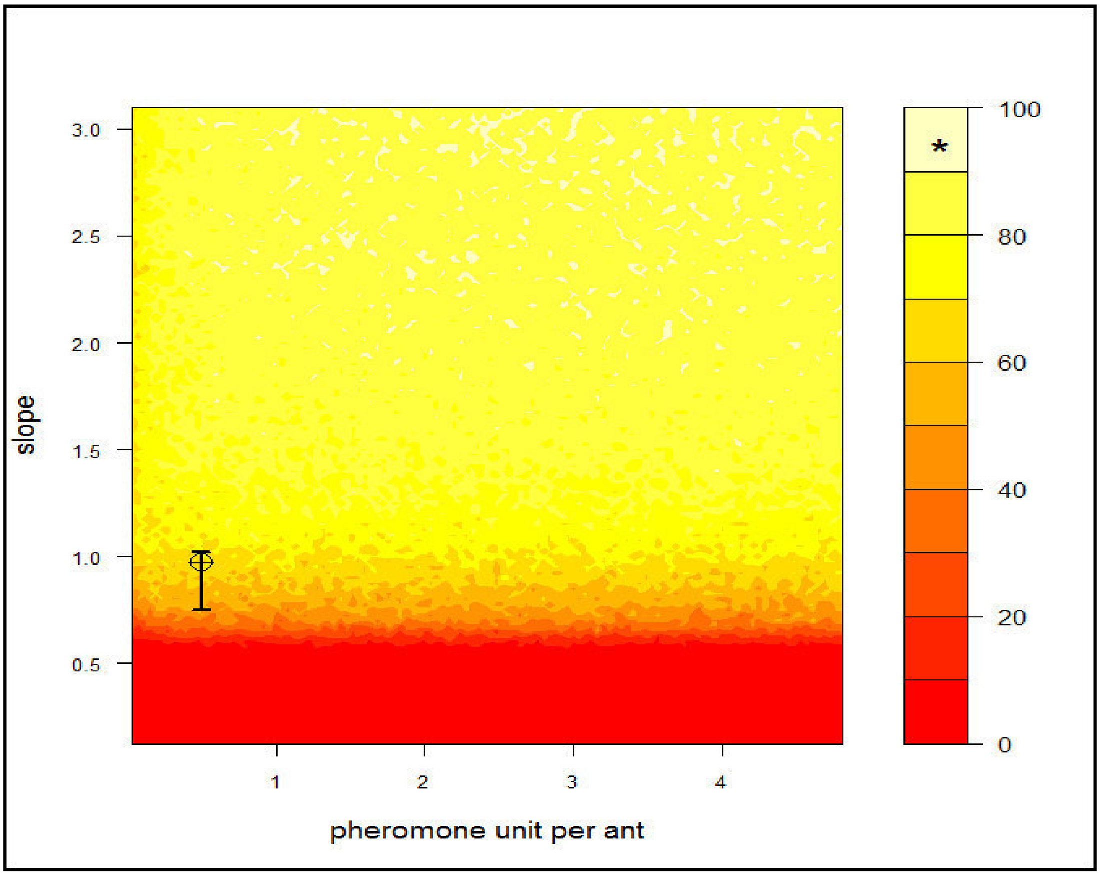

k
20.000
2.000
0.200
0.020
0.002
0.5
1.0
1.5
2.0
2.5
3.0
exponent
100
80
60
40
20
0
*

A

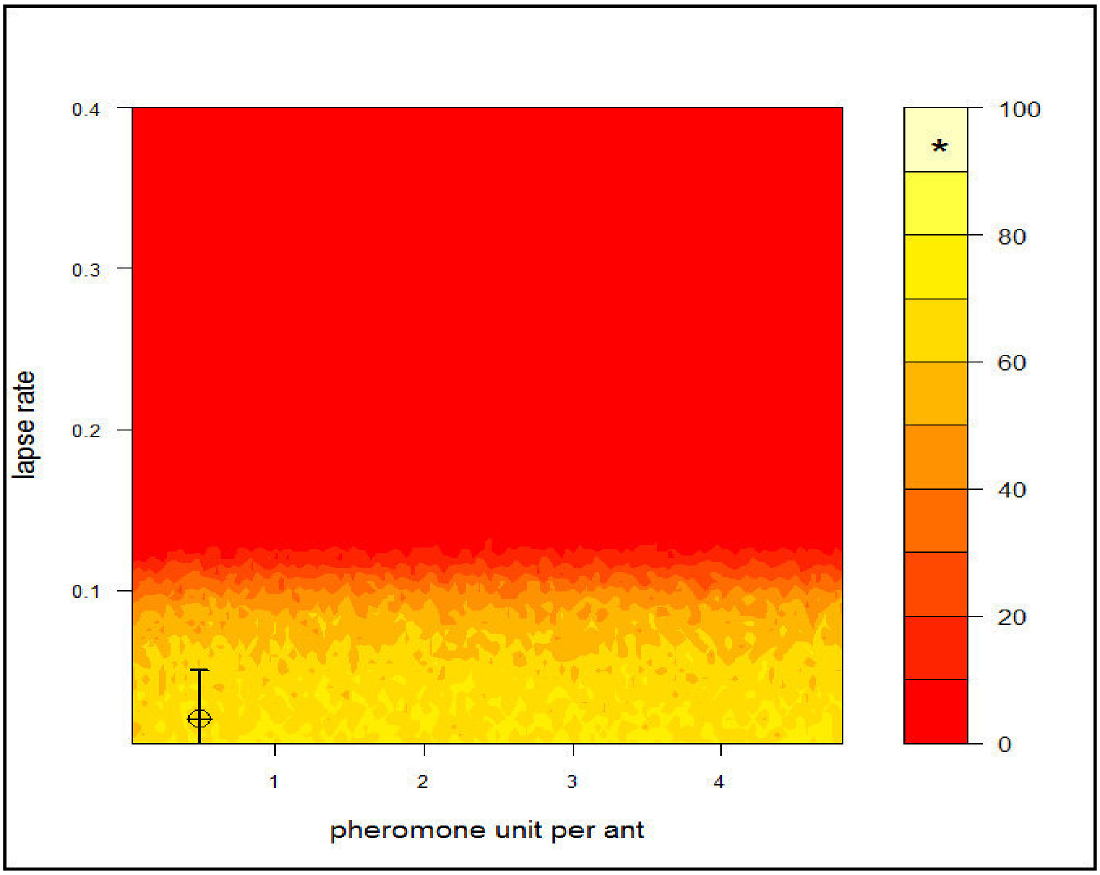

lapse rate
0.400
0.200
0.100
0.050
0.025
0.0125
0.00625
0.5
1.0
1.5
2.0
2.5
3.0
slope
100
80
60
40
20
0
*

B

FIG. S4.─Simulations covering the parameter space of the DCF (A) and the PF (B). Ants deposited four times more pheromone returning from food to nest than in the opposite direction and the additional assumption was made that the exponent/slope was increased by factor four in the initial phase of the simulations (first 100 iterations). 9600 parameter combinations were simulated, each with 100 simulations. Colors indicate the *80%-short path selection rate (spsr80)* of each parameter combination. The star symbol in the heat map indicates the result of the original experiments of Goss et al. (1989), which was 93%. The circled plus symbol represents the position of the fitted parameters, dashed lines show their confidence limits (see table A1 and A2 in the main article). The parameter *k* of the DCF was free, thus it covers the whole y-axis. The black diamond in (A) represents the position of the parameter setting of Goss et al. (1989) (*k*=20, *b*=2). The y-axes are scaled logarithmic.

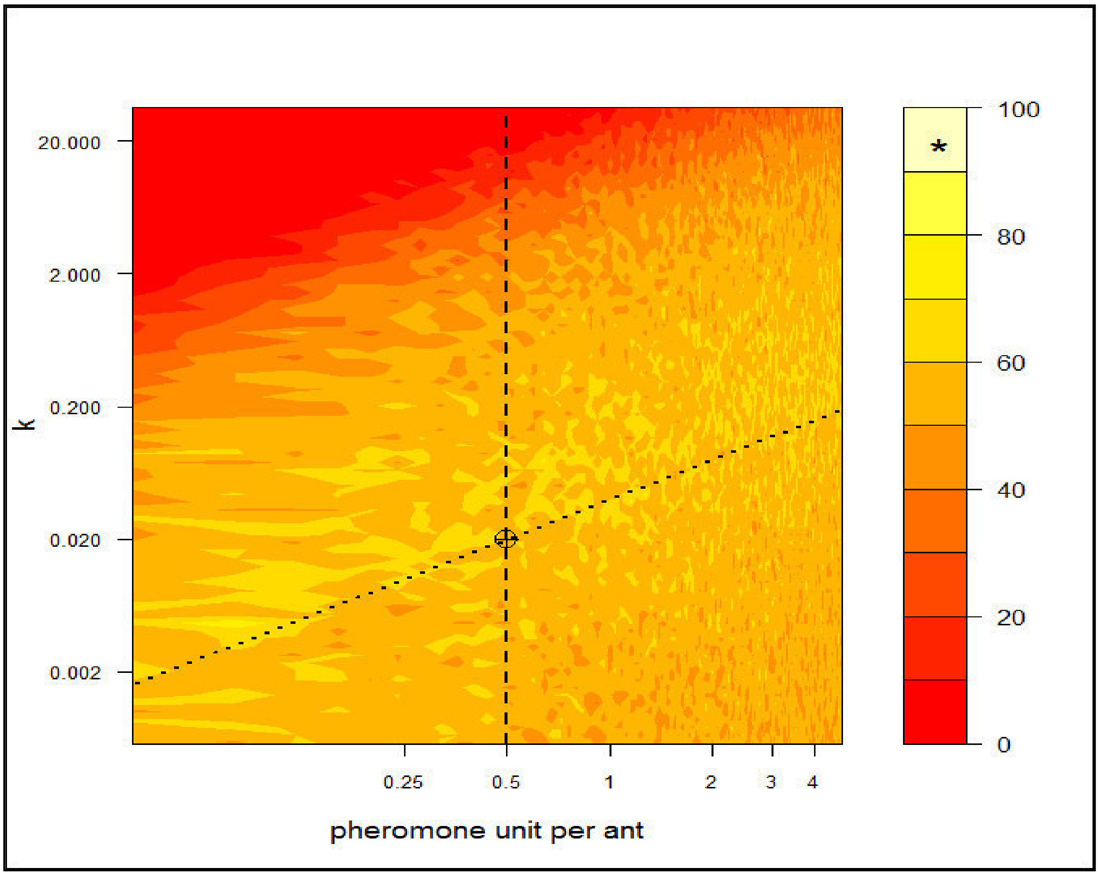

3.0
2.5
2.0
1.5
1.0
0.5
slope
1
2
3
4
pheromone unit per ant
100
*
80
60
40
20
0

A

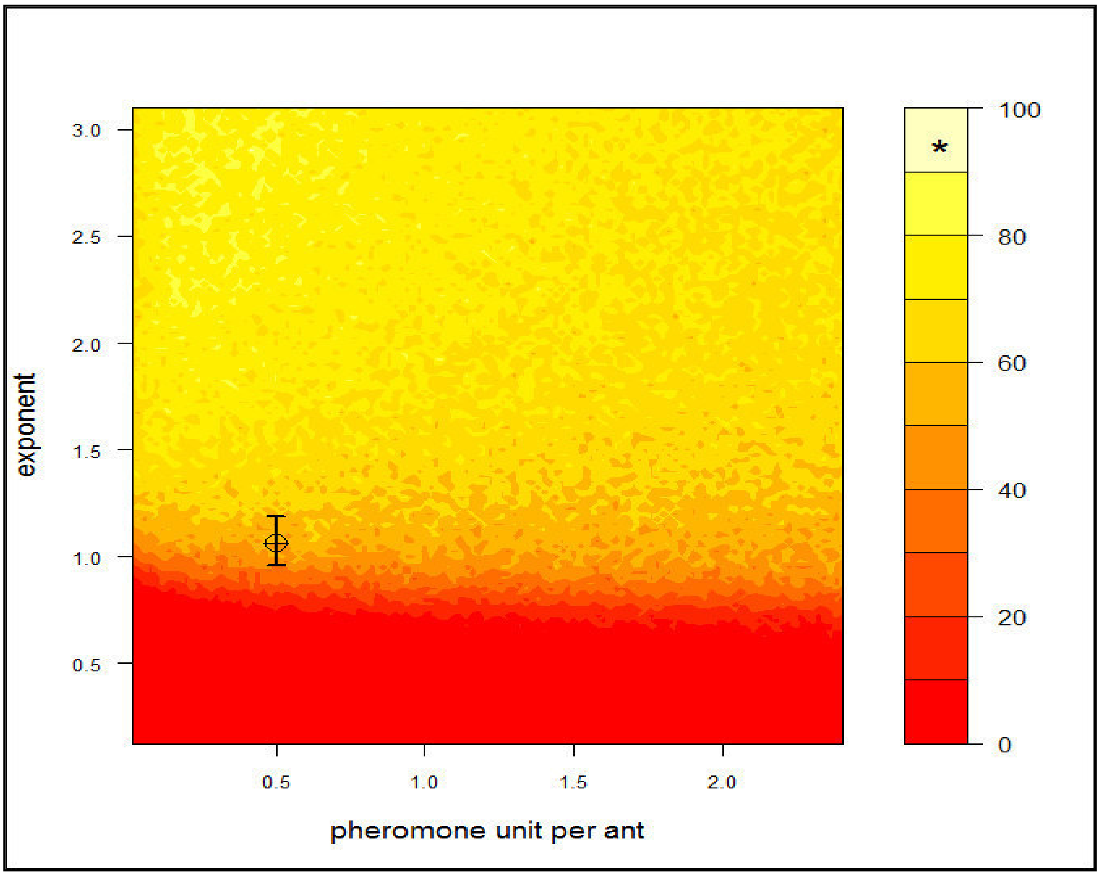

0.4
0.3
0.2
0.1
lapse rate
1
2
3
4
pheromone unit per ant
100
*
80
60
40
20
0

B

FIG. S5.—Simulations with varying *pheromone units per ant (phpa)*, covering the parameter space of the PF. In (A), *slope* and *phpa* were varied. In (B), lapse rate and *phpa* were varied. 14400 (A) and 9600 (B) parameter combinations were simulated, each with 100 simulations. Colors indicate the *80%-short path selection rate (spsr80)* of each parameter combination. The star symbol in the heat map indicates the result of the original experiments of Goss et al. (1989), which was 93%. The circled plus symbol represents the position of the fitted parameter and the *phpa* that was used in our simulations of the main article (0.5), bars show the confidence limits of the fitted parameter according to table A2 in the main article. Ants deposited four times more pheromone heading from nest to food than in the opposite direction. Note that horizontal layers indicate that the simulations were invariant to changes of the pheromone scale.


20.000
2.000
0.200
0.020
0.002
k
0.25
0.5
1
2
3
4
pheromone unit per ant
100
*
80
60
40
20
0

A


3.0
2.5
2.0
1.5
1.0
0.5
exponent
0.5
1.0
1.5
2.0
pheromone unit per ant
100
*
80
60
40
20
0

B

FIG. S6.─ Simulations with varying *pheromone units per ant (phpa)*, covering the parameter space of the DCF. In (A), *k* and *phpa* were varied. In (B), *exponent* and *phpa* were varied. 9600 (A) and 14400 (B) parameter combinations were simulated, each with 100 simulations. Colors indicate the *80%-short path selection rate (spsr80)* of each parameter combination. The star symbol in the heat map indicates the result of the original experiments of Goss et al. (1989), which was 93%. The circled plus symbol represents the position of the fitted parameter and *phpa* that was used in our simulations of the main article (0.5). The vertical line in (A) shows the confidence limits of *k*. Since *k* was a free parameter, this line covers the whole y-axis. Bars in (B) represent the confidence limits of the exponent according to table A2 in the main article. Ants deposit four times more pheromone heading from nest to food than in the opposite direction. Along the dotted line in (A), the ratio between *k* and *phpa* is constant. Layers, parallel to this line, indicate that the *spsr80* is constant, as long as *k* and *phpa* are kept proportional. orizontal layers in (B) indicate that the simulations were invariant to changes of the pheromone scale. The axes in (A) and (B) are scaled logarithmic.

S3. Calculation of pheromone deposition in directions to and from the food

Table ST3

Streak length and streak distance *L. humile*, modified from (Aron et al., 1989)

	toward nest	towards food
Minimum streak length (cm)	0.19 – 0.03 = 0.16	0.10 – 0.01 = 0.09
Maximum streak length (cm)	0.19 + 0.03 = 0.22	0.10 + 0.01 = 0.11
Minimum distance between streaks	0.31 – 0.09 = 0.22	0.57 – 0.07 = 0.50
Maximum distance between streaks	0.31 + 0.09 = 0.40	0.57 + 0.07 = 0.64

The total length of pheromone streaks *Pl* on a trail of length *Tl* can be calculated from these data using following formula:

$$Pl = sl \cdot \frac{Tl}{sl + d} \tag{9}$$

Pl = total length of pheromone streaks
Tl = Trail length
sl = average streak length
d = average distance between streaks

This gives following results:

Maximum Pl towards nest	Minimum Pl towards food	Ratio Maximum
50.0 cm	12.3 cm	4
Minimum Pl towards nest	**Maximum Pl towards nest**	**Ratio Minimum**
28.6 cm	18.0 cm	1.6

Literature cited (supplement chapter 2)

Aron, S., Pasteels, J. M., Deneubourg, J. L., 1989. Trail-laying behaviour during exploratory recruitment in the argentine ant, Iridomyrmex humilis (Mayr). Biol Behav 14, 207-217.

Goss, S., Aron, S., Deneubourg, J. L., Pasteels, J. M., 1989. Self-organized Shortcuts in the Argentine Ant. Naturwissenschaften 76, 579-581, doi: doi:10.1007/BF00462870

Jackson, D. E., Châline, N., 2006. Modulation of pheromone trail strength with food quality in Pharao's ant, Monomorium pharaonis. Anim Behav 74, 463-470.