
Development of foraging strategies in bees

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Table of contents

Summary.....	6
Resume.....	7
Declaration.....	9
Preface.....	10
Acknowledgments.....	11
Chapter I. General Introduction.....	13
1 - Bees: a model for optimal foraging.....	14
2 - Technological challenge.....	17
3 - Modelling approach.....	20
4 - Thesis prospectus.....	21
Chapter II. Understanding traplining as a foraging strategy.....	30
Abstract.....	31
1 - Introduction.....	32
2 - Spatial constraints.....	33
2.1 - Flower constancy.....	33
2.2 - Nearest-neighbour movements.....	33
3 - Temporal constraints.....	35
3.1 - History of rewards.....	35
3.2 - Exploration to exploitation ratio.....	36
4 - Social interactions.....	39
4.1 - Competition and resource partitioning.....	39
4.2 - Social learning.....	40
5 - Conclusion.....	41
Chapter III. A model of resource partitioning between foraging bees based on learning.....	49
Abstract.....	50
1 - Introduction.....	51
2 - Results.....	54

2.1 - Overview of models.....	54
2.2 - Simulations with one forager.....	56
2.3 - Simulations with two foragers.....	59
3 - Discussion.....	65
4 - Methods.....	69
4.1 - Description of models.....	69
4.2 - Environments.....	71
4.3 - Movements.....	72
4.4 - Learning.....	73
4.5 - Competitive interactions.....	75
4.6 - Data Analyses.....	75
Supporting Information.....	88

Chapter IV. Partial partitioning of foraging resources

by honey bees at small spatial scale.....	120
Abstract.....	121
1 - Introduction.....	122
2 - Methods.....	124
2.1 - Foraging arena.....	124
2.2 - Tagging and training of bees.....	126
2.3 - Artificial flowers.....	127
2.4 - Experimental protocol.....	128
2.5 - Data analysis.....	128
2.6 - Comparison to model simulations.....	131
3 - Results.....	133
3.1 - Route similarity.....	133
3.2 - Partitioning.....	133
3.3 - Time spent on flowers.....	134
4 - Discussion.....	139

Chapter V. Foraging strategies of bees in a competitive

intra-patch situation.....	147
Abstract.....	148
1 - Introduction.....	149

2 - Methods.....	150
2.1 - Natural experiment.....	150
2.2 - Controlled experiments.....	153
2.3 - Modelling.....	158
2.4 - Comparisons between observations and simulations.....	162
3 - Results.....	162
3.1 - Natural experiment.....	162
3.2 - Controlled experiment: One bee.....	167
3.3 - Controlled experiment: Five bees.....	172
4 - Discussion.....	176
4.1 - An absence of traplines.....	177
4.2 - Bees improved their foraging efficiency.....	178
Chapter VI. General Discussion.....	184
1 - Summary of results.....	185
2 - Traplines as “paths of least resistance”.....	186
3 - Principle of parsimony and cognitive load.....	188
4 - Models, a tool of answer “What If...” questions.....	189
5 - Conclusion.....	192

Summary

Effective foraging for food is such a necessity for most animals it is reasonable to expect natural selection to favour individuals that optimise their nutrient intake and minimise energy expenditure. One way to achieve these goals is through the use of specific behaviours, called “foraging strategies”. Pollinators such as bees present a very interesting case of foraging optimisation. Since the nectar offered by plants is a renewable resource bees have a strong incentive to learn and memorise the positions of the flowers they have discovered. Many studies have investigated the foraging behaviour of bees, leading to the identification of two foraging strategies: the use of stable, repeated routes between subsets of flowers (“traplines”) and the development of areas of exclusion of other bees in competitive situations (“resource partitioning”). The use of these two strategies by bees has been demonstrated multiple times in different situations, but we still know very little about how such strategies develop. These two strategies have mostly been described through cognitively complex mechanisms. However, while they have been observed and characterised in controlled environments, these strategies were seldom seen in more natural environments, suggesting our current explanations of these phenomena are incomplete. This gap in knowledge leads me to question what are the behavioural rules individual bees follow to establish these strategies? My thesis focused on attempting to gain some insight on how these foraging strategies form by complementing experiments with a modelling approach. I built an agent-based model of multiple bees foraging in a wide variety of environments. With it I tried to explain the establishment of these strategies through the use of simple positive and negative reinforcement rules as bees found flowers with or without rewards, respectively. Exploration of the model showed that both traplining and partitioning strategies could emerge in simple competitive situations with two bees foraging on 10 feeding sites. I then conducted three experiments to challenge the assumptions of the model. My results suggest that the foraging strategies of bees could emerge from simple foraging rules, but more importantly that their development in natural conditions could be mostly driven from the spatial and temporal constraints of the environment which are altering the availability in resources. Bees were able to improve their foraging efficiency in most experimental conditions, but how they did so was not limited to the establishment of traplines or resource partitioning. By explaining their formation mostly through these constraints, we are able to present these foraging strategies not as cognitively intensive processes, but rather paths of least resistance to environmental constraints.

Keywords: foraging behaviour, traplines, partitioning, agent-based model

Résumé

Être efficace lors de la recherche de nourriture est tant important, qu'il est raisonnable de s'attendre à ce que la sélection naturelle favorise les individus qui optimisent leurs apports nutritionnels et minimisent leurs dépenses énergétiques. Cette optimisation peut prendre la forme de comportements spécifiques, que l'on nomme "stratégies de recherche de nourriture". Les pollinisateurs, tels que les abeilles (au sens large), présentent un cas très intéressant d'optimisation de recherche de nourriture. Le nectar offert par les fleurs se renouvelant au fil du temps, les abeilles sont encouragées à apprendre et mémoriser les positions des fleurs qu'elles découvrent. Plusieurs études sur leur comportement lors du butinage ont mené à l'identification de deux stratégies: l'utilisation de routes stables et répétées entre plusieurs fleurs (nommées "traplines"), ainsi que le développement de zones d'exclusion d'autres abeilles dans des situations compétitives (nommée "partitioning"). Ces stratégies ont été démontrées dans diverses situations, mais nous ne savons encore que peu sur comment elles se développent. Ces stratégies ont toujours été décrites au travers de mécanismes cognitifs complexes. Cependant, bien qu'elles aient été observées en milieu contrôlé, ces stratégies n'ont pas souvent été observées en milieu naturel, suggérant une connaissance incomplète de ces phénomènes. Cette lacune m'amène à me demander quelles sont les règles comportementales suivies par les abeilles pour développer ces stratégies? Ma thèse a eu pour but d'apporter des réponses sur comment ces stratégies se forment, en combinant des approches expérimentale et de modélisation. J'ai développé un modèle individu-centré de plusieurs abeilles butinant sur plusieurs environnements. Avec ce modèle j'ai tenté d'expliquer l'établissement de ces stratégies via l'utilisation de simples règles de renforcement positif et négatif lorsque les abeilles trouvaient des fleurs avec ou sans nectar, respectivement. L'exploration de ce modèle a démontré que les "traplines" et "partitioning" pouvaient émerger en situations compétitives simples avec deux abeilles butinant sur 10 ressources. J'ai réalisé trois manipulations afin de confronter les prédictions du modèle. Les résultats suggèrent que ces stratégies de recherche de nourriture pourraient émerger à partir de simples règles d'apprentissage, mais également que leur émergence en conditions naturelles pourraient être majoritairement dû aux contraintes spatiales et temporelles de leur environnement; qui affecte la disponibilité des ressources. Les abeilles ont été capables d'améliorer l'efficacité de leur recherche de nourriture dans la majorité des situations expérimentales. Cependant, cette amélioration ne s'est pas limitée à l'utilisation des stratégies de "trapline" et "partitioning". En expliquant leur formation au travers de ces contraintes environnementales, j'ai pu présenter ces stratégies de recherche de nourriture

non pas comme le résultat de mécanismes cognitifs complexes, mais comme des chemins de moindre résistance aux contraintes environnementales.

Mots-clés: recherche de nourriture, trappes, partitioning, modèle individu-centré

Declaration

On the 20/10/21,

I certify that the thesis “Development of foraging strategies in honey bees” presented here is only submitted to the Université Toulouse III - Paul Sabatier and Macquarie University, as agreed upon in the cotutelle agreement dated 03/10/2018, and has not been submitted to any other institution.

All the work presented in this document is my own, with all contributions, collaborations and assistances appropriately acknowledged. All information taken from the literature has also been properly acknowledge and the original work cited.

No approval by the Macquarie University Ethics Review Committee was required as this work was conducted on invertebrates.

Thibault DUBOIS

Preface

The different chapters of this thesis were written in the form of papers in preparation for submission (Chapters II, IV and V), or already published papers (Chapter III), with adjustments for the thesis format.

Publication included in this thesis:

Dubois, T.*, Pasquaretta, C.* , Barron, A. B., Gautrais, J., & Lihoreau, M. (2021). A model of resource partitioning between foraging bees based on learning. *PLoS computational biology*, 17(7), e1009260.

[*Co-first authors]

- Included as Chapter III.

TD, CP and ML designed the study, TD and JG developed the model, TD and CP analysed the data, TD, CP and ML wrote the first draft of the manuscript, all authors contributed to revisions.

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Chapter I.

General Introduction

1 - Bees: a model for optimal foraging

Social bees, and in particular honey bees, have featured in mythology, literature and art for millennia, as far back as ancient Egypt where their caste and social system was richly represented in hieroglyphs. The works of pioneers in the study of animal cognition, such as Charles H. Turner and Karl von Frisch, a century ago, sparked a new interest in the cognitive abilities of bees (von Frisch, 1915; Dona & Chittka, 2020). Notably, it has been demonstrated that bees are capable of olfactory, visual and tactile learning (honey bees: Schubert et al., 2002), path integration (Collett & Collett, 2000; Wehner & Srinivasan, 2003), social learning (Leadbeater & Chittka, 2007), concept learning (honey bees: Avarguès-Weber & Giurfa, 2013), numerosity (honey bees: Chittka & Geiger, 1995; Dacke & Srinivasan, 2008) and metacognition (honey bees: Perry & Barron, 2013), among other cognitive abilities. Honey bees and bumblebees have been notoriously favoured in studies of insect foraging behaviour, since they are easily maintained, manipulated (i.e. individually tagged), docile, numerous, they adapt easily to new foraging tasks presented to them, and forage constantly (Goulson & Osborne, 2010). Thus, these species have often been used to study foraging optimisation problems. Throughout this thesis, references to honey bees and bumblebees are made as a simplification for studies on *Apis mellifera* and various species of *Bombus sp.*, respectively.

Under the hypothesis that a better foraging success would consequently increase one's survival and reproduction, we have expected animals to be selected for optimal foraging. Hypotheses as to how to assess foraging performance have been improved by the establishment of the Optimal Foraging Theory ("OFT"; Emlen, 1966). This theoretical framework tries to predict the foraging behaviour of animals by assuming that they should exploit optimally their environment (Pyke et al., 1977; Pyke, 1984), based on the assumptions that (i) a more efficient foraging behaviour affects the fitness of the next generation; (ii) the foraging behaviour is inheritable; (iii) the effect of the foraging behaviour

on the fitness (i.e. the “currency”) is known; (iv) there is no genetic reason preventing the evolution of the foraging behaviour; (v) the evolution of the foraging behaviour is constrained by the biology of the animal; and (vi) the foraging behaviour evolves faster than the rate at which the relevant conditions change (Pyke, 1984).

That bees are effective and efficient foragers is not in doubt, but how they achieve this is an open question. Bees naturally forage in large areas on resources that are dispersed, ephemeral and unpredictably varying, making the foraging task very difficult. How are bees able to forage efficiently in such environments? What is the extent of their cognitive abilities enabling their effective foraging?

Bees exploit patches of flowers, from which they extract pollen and nectar. Foragers of a colony usually specialise in the exploitation of either resource (honey bees: Page et al., 2006), and are usually distinguishable during foraging by the presence of accumulated pollen in their pollen basket. While there is no reason to suspect a difference in how bees forage for these two resources, manipulating pollen and designing experimental protocols around the exploitation of pollen is generally more difficult than nectar, which is easily replaced in experimental setups by sucrose solution. As such, studies on foraging strategies and resource optimisation have mostly focused on nectar foraging. Unless stated otherwise, the “foraging behaviour” mentioned throughout this work will specifically refer to nectar foraging.

The foraging behaviour of bees consists in visiting a series of flower patches distributed in their environment, and within each visit a certain number of flowers for nectar. Within a patch of flowers, studies have observed rules generally followed by bees and structuring how they exploit such resources. Namely, bees were reported moving between flowers by moving to the nearest unvisited flower (i.e. “nearest-neighbour movements”, bumblebees: Ohashi et al., 2007; Saleh & Chittka, 2007), often retaining their heading for

multiple successive movements (i.e. “directionality of movements”, Levin et al., 1971; Pyke & Cartar, 1992; Cresswell et al., 1995), and making decisions as to when they should leave a patch to visit another one (Pyke, 1982a). These foraging behaviours based on hard-wired rules are cognitively simple solutions for maximising foraging efficiency, in accordance with optimal foraging theory.

However, when navigating between patches, bees typically cannot follow these rules. Indeed bees usually cannot rely on visual cues as these patches are too distant from each other. As a consequence, they need to use a completely different set of behaviours and cues to navigate within patches (i.e. “intra-patch foraging”) and between patches (i.e. “inter-patch foraging”). One behaviour in particular has drawn interest when studying the foraging behaviour of bees over large spatial scales. Bees have been observed establishing routes between the same patches of flowers, and revisiting them in a similar order every time they would do a foraging bout. These stable routes have been called traplines (Thomson, 1996; Ohashi et al., 2007; Lihoreau et al., 2012a), and have since been studied for their role as a foraging strategy. In recent studies, bees have been observed establishing traplines in a way that reduces the overall distance travelled, i.e. by using the shortest path starting from their nest, passing by all the flowers and back to their nest (bumblebees: Ohashi et al., 2007; Lihoreau et al., 2012a; Lihoreau et al., 2012b; honey bees: Buatois & Lihoreau, 2016). Finding the shortest path between a set of points is analogous to the Traveling Salesman Problem, a NP-complete mathematical problem for which the solution cannot be known unless all options are tested. This analogy highlights the complexity of the foraging task of bees, and puts the focus on an aspect of foraging optimisation often debated: the cognitive load of the task. The notion of cognitive load presented throughout this thesis is used to depict the theoretical amount of information transferred to execute a behavioural response to a stimulus. Since measuring the load of information between neurons is difficult, we chose to use the information as it is presented in the behavioural models we use in the thesis as a

proxy. While such a proxy can sometimes be misleading, it remains a relevant estimate of information transfer, as depicted in information theory.

In trying to understand how bees optimise their foraging intake at these large spatial scales, many models relied on complex calculations of energetic returns (Pyke, 1978), route comparisons (Reynolds et al., 2013), or the existence of a cognitive map to navigate (Menzel et al., 2005; argued against in Cruse & Wehner, 2011; Collett et al., 2013). The mechanisms suggested in these strategies are computationally intense and impose heavy demands on learning and memory systems, hence we can describe them as imposing a high cognitive load on a bee. Whether such a load is reasonable is still not clear. Traplines, for example, present bees faithfully following learned routes between flowers. Although highly efficient and effective, bees have very small brains, and while they are capable of many things, their processing power and memory are likely size constrained (Chittka & Niven, 2009). While most studies trying to explain traplines measure the efficiency in terms of energetic returns, distance travelled, the cognitive load is often not considered. In this thesis, we argue that beyond considerations of whether a task is cognitively possible for a bee, we should also try to consider whether an improvement in efficiency is worth the cognitive load it demands. We suggest in this thesis that the optimisation behaviour of bees, so far explained through these cognitively intensive task solving behaviours, could also be the result of more parsimonious, very simple behavioural rules.

2 - Technological challenge

Despite the popularity of bees in studies of foraging behaviour, technology currently limits what can be observed. For a long time research on bee spatial behaviour has been made on small spatial scales (in inflorescences or flower patches; Pyke, 1978;1982; Williams, 1997). Researchers opportunistically focused on one bee at a time and recorded their behaviour by following until they lost it (Thomson et al., 1987). Tracking animals over long distances and

times has been a constant challenge, and while there has been huge progress in developing new technologies (Maggiore et al., 2019; Vo-Doan & Straw, 2020; Ratnayake et al., 2021), one of the main obstacles to their application to bees is how small they are, but also their large foraging range which is hard to cover even with current technology. When it comes to studies on spatial use and movements, tracking methods of bees fall into two categories: discrete tracking and continuous tracking.

Discrete tracking refers to methods where the points of interest (in our case, flowers or patches of flowers, natural or artificial) are monitored, and not the bee itself. These methods' aim is to record visits to a specific place of interest, by any individual or a specific bee (in which case bees are individually tagged). Recordings of visits can be done manually (i.e. observers) or automatically (RFID: reviewed in Nunes-Silva et al., 2019; computer vision with QR Codes: Wario et al., 2015; Gernat et al., 2018) using different technologies. This approach is useful if the behaviour studied is displayed at the monitored site (e.g. feeding behaviour, acceptance/rejection of flowers, interference competition, etc), as it can provide fine observations of behaviours (e.g. through the use of video recordings and pose analysis; Nath et al., 2019). Furthermore, video recordings are now being processed through machine learning programs to identify and track multiple individuals simultaneously, allowing the tracking of multiple untagged individuals within the scope of the recording (Lauer et al., 2021).

However, the discrete approach is ill-suited to studies of behaviours happening between the different resources the bees visit. Continuous tracking is addressing this issue by monitoring the bees themselves, allowing the study of fine movements in their environments such as the exploration behaviour. Such systems used to track bees are usually composed of a transponder attached to the bee, and of an antennae held by the observer (details of methods reviewed in Kissling et al., 2014). Yet, the application of these methods with insects is difficult. Bees are generally small, and lightweight (~3 grams),

meaning that the transponder would have to be very minimal to fit the bee without being invasive. Moreover, these methods are often expensive to deploy as they require heavy machinery (harmonic radar; Riley et al., 1996) or logistical support (use of planes to scout the area; Pasquet et al., 2008). Also, these methods are still quite limited in how often positions can be recorded, and how accurate these positions are. When studying the movements of an animal, the resolution of the observations will have a significant impact on the patterns that can be discerned (Pasquaretta et al., 2019). Lastly, tracking multiple bees at the same time through these methods is still difficult, as the signals detected from the bees are not specific to each individual.

To all these technological constraints are usually added the difficulties to follow animals in natural environments, such as an uneven terrain, disruptions from other signals (for telemetry and harmonic radar trackings) or the disappearance of the focal bee (either predated or a bee losing its transponder). Thus, most experiments on bee movements have been limited to controlled environments (flight cage, flight room in laboratory), and in doing so have reduced the complexity of the foraging environment while simplifying the tracking process by drastically limiting the space in which the bee can forage. Yet, these methods too are not without constraints or limits. In flight rooms, bees lose access to cues such as the polarised light or the sun's position, which are important elements in their navigation (Gould, 1998). By also reducing their environment to a set of minimal functional features (a nest and flowers in an otherwise empty room), it is reasonable to assume that they become deprived of cues and information from their environment that could affect their foraging behaviour, and would not be able to escape an imposed foraging situation (e.g. competition pressure or unprofitable patch) from a lack of other options in the limited space.

Finding a compromise between controlled experiments and studies in natural conditions is difficult. The emerging field of cognitive ecology is pushing towards the design

of experiments accounting for the social context of a cognitive task to bring controlled experiments to the field (Dukas & Ratcliffe, 2009; Muth et al., 2018; Lihoreau et al., 2019).

3 - Modelling approach

Because experiments with real bees in the field are challenging, a complementary approach is running experiments with simulated bees using computational models. The use of models in science has surged in the past decades with the developments in computational power and accessibility of computer languages. Models are mathematical descriptions of ecological systems, based on our knowledge of these systems. They allow, on the basis of this knowledge, to explicit specific hypotheses and virtually integrate them to the system to test them. Models, however, remain simplified representations of reality and as such are unfit to prove the hypotheses they test. Instead, they allow us to see if these hypotheses could have an effect on the system, or if an experimental situation would be appropriate to test this hypothesis, effectively guiding the experimental process. Models to study bees are numerous (e.g. Ohashi & Thomson, 2005; Reynolds et al., 2013; Becher et al., 2014;2016;2018; Olsson et al., 2015; Qu & Drummond, 2018), covering a large range of behaviours, as well as a range of purposes.

Models like BEEHAVE and further additions (Becher et al., 2014;2016;2018) are models including a wide range of variables covering most aspects of the life cycle of bees (colony dynamics, nutrition, predation, parasitism, foraging...). These models can simulate a very large range of scenarios, but the predictions resulting from it will generally not be generalisable to other contexts, meaning that their principal application will be to help decision-makers in conservation and agricultural fields looking for predictions to rely on when acting on specific field situations.

Most models on bees have otherwise focused on narrower aspects of their biology, hypothesising on the effect of parasitism (Sumpter & Martin, 2004; DeGrandi-Hoffman & Curry, 2004), pesticides (Thompson et al., 2005;2007) or nutrition (Schmickl & Crailsheim, 2007; Khoury et al., 2013) on the dynamics of the colony (reviewed in Becher et al., 2013).

Very few models focused on the process by which foraging strategies may emerge (Reynolds et al., 2013). This model by Reynolds et al. is to our knowledge the first to suggest a mechanism to explain the formation of traplines in bees, based on the idea that bees would compare multi-leg routes to find the shortest. Our own work is inspired by this initial model, as both rely on similar navigation systems based on probability matrices and reinforcement rules.

4 - Thesis prospectus

The work conducted in this thesis applied theoretical, modelling and experimental approaches to try to understand what traplines are, how they are able to form, and by answering these questions highlighted the role of foraging strategies in the broader context of the foraging behaviour of bees. The aim is to try to reconcile observations of foraging behaviour performed at small spatial scales and at larger spatial scales by finding mechanisms able to explain both.

In Chapter II, we first reviewed our knowledge on the foraging behaviour of bees, and advocated for the need to explain the emergence of foraging strategies through simpler processes than what has currently been suggested in the literature.

In Chapter III, we attempted to model these simpler processes in an attempt to showcase how traplines could emerge from very parsimonious foraging rules of positive and negative reinforcement in competitive environments.

In Chapter IV, we put our model to the test against a simple experiment for which the predictions of the models were very clear. These first results allowed us to refine further our models.

In Chapter V, we conducted multiple experiments to try to understand how groups of bees would be able to optimise their foraging efficiency at the scale of a single patch.

Finally, in Chapter VI, we conclude on our results and highlight the need to consider the foraging strategies of bees in their general context.

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Chapter II.

Understanding traplining as a foraging strategy

Abstract

Foraging bees often form traplines: stable, repeated routes between the flowers they visit during foraging, that minimise path length. In this review we explore how traplines form. This is of interest because forming traplines appears to pose a prodigious challenge, which has been likened to the famous Travelling Salesman problem, and understanding how foraging bees move between flowers has important consequences for the efficiency and performance of bees. In this review, we synthesise various studies on traplining in bees and other animals, and focus on understanding how the spatial and temporal constraints of their environment and behaviour can affect the formation of traplines, and also of similar foraging strategies such as resource partitioning. The definition of traplines limits us to recognise only a “perfected” form of traplines. These only occur in simplified controlled environments. In natural environments traplines do not show complete repeatability and reliability. The natural environments the routes of the bees are strongly influenced by constraints on resource availability in the environment, variability in resources and simple foraging strategies adopted by bees including floral constancy, nearest-neighbour movements and competitor avoidance. We argue that traplines emerge as a consequence of these constraints. By hypothesising that trapline formation mostly relies on these spatial and temporal constraints, we suggest that forming traplines would, under these assumptions, require little to no cognitive load. This new hypothesis opens a new opportunity to understand traplines and foraging strategies of bees in general under a new light, one that recognises them outside of their “perfected” form.

1 - Introduction

Bees have been studied for a long time in order to understand their foraging habits (von Frisch, 1915; Gould, 1990; Seeley 2010). A common observation has been that some species of bees develop stable, repeated routes between food sources. These routes are called “traplines” (Euglossine bees: Janzen, 1971; bumblebees: Thomson et al., 1997; Ohashi et al., 2007; Lihoreau et al., 2012b; honey bees: Buatois & Lihoreau, 2016).

Traplines have been observed in many species other than bees, including hummingbirds (Gill, 1988; Garrison & Gass, 1999), bats (Lemke, 1984), primates (Menzel, 1973; Cramer & Gallistel, 1997) and more (reviewed in Berger-Tal & Bar-David, 2015), suggesting this behaviour is common in animals foraging on renewable resources. Although traplining has been extensively studied, the very definition of traplines remains unclear. Indeed, their properties (i.e. stable, repeated and optimised) ask more questions than the definition answers. How stable does a route have to be to be a trapline? How often does a route have to be repeated to be a trapline? What is optimised in a trapline, and to what degree? To understand traplines we need to explore these questions, and through them learn about the development and function of traplines.

Defining traplines as stable, repeated and optimised routes poses a fundamental problem. We can only recognise a trapline if it is in its ideal, perfected form (Thomson et al., 1997) where an optimised route (assuming some criterion of optimisation, e.g. distance travelled or net energy intake) between food sources is repeated without any deviation from it. This is sometimes seen in experiments in controlled environments (bumblebees: “positive array” of Ohashi et al., 2007; Lihoreau et al., 2012a), but most observations of traplines in field conditions are far from perfect. In field conditions bees developed fidelities toward foraging areas (bumblebees: Thomson et al., 1982; Thomson et al., 1987; honey bees: Williams, 1997) or suboptimal foraging routes (euglossine bees: Janzen, 1971). Here, we hypothesise that the perfect traplines in controlled environments and the various behaviours

observed in field conditions are different outcomes of the same general foraging strategy applied in different foraging environments. We propose that traplines can form as a result of very simple decision-making processes that impose little cognitive load.

Through this review, we reconsider traplines in the context of evolved foraging strategies, and detail how a simple foraging strategy based on basic behavioural responses can explain the wide range of traplines observed in controlled and field conditions. We discuss the factors that constrain the foraging behaviour of bees spatially and temporally in their natural environment, and argue how constraints can shape the emergence of traplines.

2 - Spatial constraints

2.1 - Flower constancy

The resources available to generalist bees (honey bees, bumblebees...) usually come from a large range of different species of plants. Different plant species will display various floral cues that affect their attraction for bees, such as the shape and colour of flowers (Dyer et al., 2011), or odour (euglossine bees: Dressler, 1968). These cues have often co-evolved with the pollinators that enable their reproduction through their services, and thus have been selected to be easily recognisable and attractive for bees. Bees learn to associate the visual and odour stimuli of a flower and its nectar content and look for flowers with similar cues in the future. This can lead to a phenomenon called flower constancy (Grant, 1950; Chittka et al., 1999). Such learned foral preferences in conjunction with the distribution of these flowers in the foraging range will constrain the pool of flowers that will be visited.

2.2 - Nearest-neighbour movements

Flowers are typically distributed in a heterogeneous way, or “patchy” distribution (Kotliar & Wiens, 1990). Flowers can be aggregated on many levels: flowers in inflorescences, inflorescences on a single plant, plants aggregated in patches (Goulson, 2000). Patches can

also have a heterogeneous distribution. Patches are often far enough from each other to be out of sight, and require bees to learn and remember their positions. The foraging behaviour of bees is known to be affected by the distribution of flowers (i.e. patchy vs. uniform distributions; bumblebees: Creswell, 2000) and also the distance between patches (bumblebees: Burns & Thomson, 2006).

Navigating between distant patches of resources is one of the challenges bees face as they forage. How they move while exploring will determine which patches are found, in turn shaping how traplines are formed. Studies on animal navigation have used random walks as a way to approximate how animals explore their environment (Bovet & Benhamou, 1988; Bartumeus et al., 2005; Edwards et al., 2007). While recent studies are questioning the ability of random walks and Levy-flight algorithms to accurately capture the properties of animal movements (reviewed in Chapter 4 of Klages, 2018), no better alternative solution has been suggested so far. Correlated random walks still provide a decent approximation of bee movements. Any lack of accuracy of this model is compensated by its conservative underlying assumptions. In Chapter III of this thesis, we show how, at least in exploitation situations, random walks can be good predictors of the likelihood of visiting a flower.

One of the emergent properties of random walks is that closer points of interest have a higher chance of being found first (Bartumeus et al., 2005). Multiple studies, including our experiments (Chapter V), have shown empirically that bees favoured nearest-neighbour (hereafter “NN”) movements in both intra-patch (bumblebees: Saleh & Chittka, 2007; Chapter V) and inter-patch (bumblebees: Ohashi et al., 2007; Woodgate et al., 2017; Kembro et al., 2019) movements. In various studies on the development of traplines, bees were faster to establish traplines on the shortest route if it corresponded to the route established by doing NN movements (bumblebees: “Positive array” of Ohashi et al., 2007; Lihoreau et al., 2012b; honey bees: Buatois & Lihoreau, 2016), and either established suboptimal routes, or required a longer training phase to reach the shortest route, when it did

not correspond to the NN route (bumblebees: “Negative array” of Ohashi et al., 2007; Lihoreau et al., 2012a; Woodgate et al., 2017).

This preference for NN movements in both intra-patch and inter-patch foraging could be the result of simple decision-making processes. In intra-patch foraging, a large number of flowers are spatially close. At this scale, moving between two flowers has a negligible cost. It is fair to assume that given the complex 3D distribution of these flowers memorising the routes between many specific flowers would imply a high cognitive load for a negligible improvement in efficiency since travels costs are trivial. At a higher scale (i.e. inter-patch foraging), the observation of NN movements would influence the order of discovery of the flower patches, since for a random walk model closer points of interests are usually found first. The nearest neighbour movements constrain how bees might move within and between patches and impose an order on the sequence of flowers or patches visited, in turn affecting learning and influencing trapline development.

3 - Temporal constraints

3.1 - History of rewards

There will be variations in nectar content between individual flowers of a given species (Leiss & Klinkhamer, 2005) and even within the same flower (Pacini & Nepi, 2007) that will negatively impact flower constancy. Nectar varies, with the time of day, or the flower’s age. Everytime a bee visits a flower, it is able to quantify how much nectar is available and make comparisons with rewards offered by other flowers (bumblebees: Makino & Sakai, 2007). Bees establish with experience a history of reward for each flower type. Being able to discriminate which flowers are rewarding and which are not, or offer too little reward, is essential for bees to best exploit a renewable food source, and also for bees to adapt to temporal variation in resources. Bees form a stable memory of the association between floral cues and history of reward (honey bees: Menzel, 1993). While different bees

display differential learning speeds (bumblebees: Chittka et al., 2003), learning associations is fast, and can be updated quickly in situations where rewarding flowers shift throughout the observation period (honey bees: Gil et al., 2007; Gil, 2010).

If flowers vary temporally in the nectar reward they offer, bees cannot be expected to keep a same trapline indefinitely, but instead should adapt to this characteristic by updating their learning and as a consequence we would expect traplines to change. How fast the rewards change will thus be a defining factor for how stable a trapline can become before it becomes unprofitable. In such a context, we should expect bees to show a tradeoff between the trapline stability and profitability as they abandon routes that become unprofitable and create new routes from new options. Such a balance shows that traplines cannot be defined solely by their stability.

3.2 - Exploration to exploitation ratio

Bees need to explore their environment to find the resources they forage on. In most experiments on bees and their foraging strategies, the environment is controlled, limiting the space available to them (i.e. flight cages and rooms) and/or the number of flowers available to them. Because of this, bees have little to no need to keep exploring beyond their first few foraging bouts. Consequently, in most lab studies bees are in a accrued state of exploitation of the known resources. However, in natural conditions, space is rarely limiting, the rewarding flowers are constantly changing, and their nectar content is usually rare and sparse. These characteristics drive a need for bees to keep exploring new foraging options.

Exploration and exploitation are complementary parts of a solution for a problem of optimising nutrient intake. Exploration and exploitation need to be balanced to achieve efficient foraging: too much exploitation means missing new foraging opportunities and depleting your current resources, while too much exploration diminishes nutrient income and

risks wasting energy. How to balance these facets is a difficult problem, often based on incomplete information with degrees of uncertainty. Theoretical and empirical studies on this subject have led to the identification of two possible strategies regarding the exploration to exploitation ratio: probability maximising and probability matching strategies (tits: Krebs et al., 1978; honey bees: MaBouDi et al., 2020).

These strategies can be best understood in the theoretical context of the multi-armed bandit problem (Mahajan & Teneketzis, 2008; Morimoto, 2019). In this problem, the subject must allocate resources between a set of alternative rewarding choices (the arms of an imagined multi-armed bandit gambling machine). Each choice has a different probability of payout that is only partially known to the subject at the allocation time. For this problem, the probability maximising solution always picks the highest potential reward (i.e. if two choices have respectively a 80% and 20% probability of giving a reward, the bee will always choose the 80% option). In the probability matching solution, however, the allocation is made proportionally to the prospect of payout (i.e. if a flower rewards 80% of the time, the bee would visit this flower 80% of the time, regardless of other options).

While it feels intuitive to exclusively focus on the option giving the best odds of rewards, most studies done on bees found that they were following the probability matching solution (bumblebees: Keasar et al., 2002; Niv et al., 2002; honey bees: MaBouDi et al., 2020). Explanations for this preference can be found when looking at the underlying assumptions of both strategies. Probability maximising requires the subject to have knowledge of and confidence in all the options in order to choose the best one. However, in the case of bees, there is no *a priori* knowledge of the nectar content of new patches, except potentially extrapolated information from knowledge gathered from other patches. Probability matching means the bee will allocate efforts on each option proportionally to their expectations for each, but will not need to draw comparisons between their different options, making it a more parsimonious strategy. Exploration would emerge naturally from probability

matching since bees will not dedicate all their effort to a single known and exploited resources. Probability maximising inhibits exploration, and if the environment changes it risks locking bees onto a no longer favourable option.

How probability matching, which promotes visits to different species, and flower constancy, which promotes visits to a single species, interact is of great interest. Bumblebees observed during foraging were seen favouring same-species visits within a few seconds (i.e. flower constancy) of their last flower visit, and then expand to other species (Raine & Chittka, 2006). In the same paper, the authors suggest a similar phenomenon in honey bees, based on analogous observations in another paper (Zhang et al., 2005). Under the hypothesis that the other species the search is expanded to are chosen based on profitability, the probability matching strategy and the flower constancy could be used sequentially during the foraging process.

That foraging bees employ a probability matching strategy is further supported by studies in which artificial flowers were set in a field, thus allowing natural alternatives to be explored. In these experiments, while bees were seen mostly foraging on the artificial flowers (the best option given their higher nectar content), they were often observed exploring other options and never developed a “perfect” trapline (i.e. a trapline as defined in its ideal form; Woodgate et al., 2017; Kembro et al., 2019). Under the hypothesis that probability matching behaviours, which have only been observed in small scale experiments, can be applied to larger scales, this suggests the bees were still exploring alternative resources, even as they made use of the rewarding artificial flowers. While it could appear that exploring new foraging options takes the bee away from forming perfect traplines, it serves to help react to any changes in their exploited patches. Hence it facilitates traplines to adapt to changing nectar distributions. A similar behaviour was noted in bats, which developed repertoires of available cacti, composed of a core set of regularly visited cacti and “peripheral” cacti less often visited but kept in memory (Goldshtein et al., 2020).

4 - Social interactions

Social interactions, such as competitive interactions and general use of social cues, act as both spatial and temporal constraints. They involve the interactions of multiple individuals, each with their own foraging history and their own spatial and temporal constraints on their foraging.

4.1 - Competition and resource partitioning

Bees face strong competition when foraging affecting their foraging behaviour. This can be either interspecific or intraspecific, from other colonies as well as their own (Balfour et al., 2015). For nectar feeding animals competition takes on a very interesting form: a bee needs to visit a flower regularly enough to keep its content low and unattractive to competitors, but not so much that it would be unprofitable. From this requirement emerged a foraging strategy intimately linked with traplines: repeated revisits and resource partitioning.

Resource partitioning was described in species communities as adaptations to different dietary niches, or foraging behaviours to avoid competing with other species over the same resources (e.g. African grazers, Kleynhaus et al., 2011; squirrels, Wauters et al., 2002; bats, Emrich et al., 2014; Goldshtein et al., 2020; bumblebees, Morse, 1977; Inouye, 1978; seabirds, Kappes et al., 2011). In our context, we particularly focus on the development of a repertoire of patches a bee is revisiting regularly. This behaviour is closely related to traplines, as it can be described by its repeatability. In a competitive environment, we argue that these repertoires are the first step towards developing traplines through successive revisits of patches. These competitive interactions are thus crucial elements of the foraging environments, leading each different individual towards foraging areas with minimal competition.

Through these regular revisits to patches, bees also control the information and resources the other foragers will get from visiting the patch. While finding empty flowers is enough to drive a bee away from the patch (Gil et al., 2007; Gil, 2010), there are other cues bees use to judge the competition pressure within a patch. Bees passively secrete chemicals from their tarsi, which are commonly referred to as “scent marks” and act as a footprint (honey bees: Giurfa, 1993). These scent marks usually degrade in a few hours after being deposited, allowing other bees to assess the time that has passed since the last visit of a bee to a flower through the concentration of the chemical, and use this information to decide whether they should probe or not a flower (honey bees: Giurfa & Nunez, 1992). In many studies of the establishment of partitioning, bees were observed extending their area of exploitation as a result of a reduction of competition pressure (removal of competitors; bumblebees: Thomson et al., 1987; Ohashi et al., 2013; honey bees: Williams, 1997). This behaviour reflects their ability to continuously explore other options, and their ability to notice the disappearance of competitors, potentially through changes in scent marks.

4.2 - Social learning

Not all social interactions are for competition purposes. Some species of bees use social cues to improve their foraging intake (honey bees: Donaldson-Matasci & Dornhaus, 2014). Honey bees are well known to recruit nestmates to forage on an advertised patch, while other species such as bumblebees display passive learning through replication of behaviours of other foragers (Leadbeater & Chittka, 2007; Avargues-Weber & Chittka, 2014; Dunlap et al., 2016). In particular, two strategies emerging from the use of social cues have been identified: local enhancement (i.e. learning to forage on a flower on which a conspecific is) and stimulus enhancement (i.e. learning to forage on flowers with similar visual cues as the one on which a conspecific is). Bumblebees were observed using both strategies in different foraging contexts (Avargues-Weber & Chittka, 2014), but also weighing social information against their own (Dunlap et al., 2016).

Bees must then balance the use of social cues with their own knowledge, and balance the acquisition of information through social learning with the competition pressure it creates. In this regard, social learning appears to be intimately linked to the exploration behaviour of bees: a necessary deviation from exploitation of resources to gather information and anticipate changes in the environment.

5 - Conclusion

Traplins have been defined as stable, repeated and optimised routes between patches (Lihoreau et al., 2012a; Lihoreau et al., 2012b). It has been presumed that their emergence relies on cognitively intensive mapping and memorising processes. We present arguments supporting the idea that in natural conditions the spatial and temporal constraints that limit the foraging options of bees are able to explain the emergence of routes whose stability, repeatability and efficiency are far greater than random, and sufficient to be recognised as traplines.

Bees explore their environment with a clear NN preference, searching for profitable patches of flowers. Through their own experience, and also through social learning and learned flower constancy, bees develop a history of rewards for each flower type allowing them to discriminate profitable and non-profitable flower types and patches. Competitive interactions can force bees away from areas of high competition pressure, ultimately driving them towards the establishment of repertoires of flower patches where competition is low. Continuous exploitation of these patches in a regular fashion will meet the criteria of traplining behaviour. Because of the inherent spatial and temporal variability of resources, bees need to react quickly to a drop in reward in any of the patches they exploit. The degree of exploration afforded by a probability matching strategy will allow bees to explore new options while continuing to exploit their current patches. Low reward probabilities from

known patches will increase the degree of exploration, whereas high reward probabilities from known patches will increase the degree of exploitation and apparent trapline stability.

All these factors and constraints are expected to interact. How they do so will determine if and how traplines develop. In most controlled environments where they are observed, traplines emerge from bees foraging in environments with a low number of stable resources, with little to no other foraging option available to them. These limitations explain why traplines under such conditions are able to be fully stable and repeatable. However, such conditions are rarely found under natural conditions. This perspective does not invalidate the insights observed in controlled environments, but rather tries to bring our perspective back to the ecological role of traplines within the bigger picture of the foraging behaviour, a necessity shared with many other aspects in insect cognition (Lihoreau et al., 2019).

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Chapter III.

**A model of resource partitioning between foraging
bees based on learning**

Abstract

Central place foraging pollinators tend to develop multi-destination routes (traplines) to exploit patchily distributed plant resources. While the formation of traplines by individual pollinators has been studied in detail, how populations of foragers use resources in a common area is an open question, difficult to address experimentally. We explored conditions for the emergence of resource partitioning among traplining bees using agent-based models built from experimental data of bumblebees foraging on artificial flowers. In the models, bees learn to develop routes as a consequence of feedback loops that change their probabilities of moving between flowers. While a positive reinforcement of movements leading to rewarding flowers is sufficient for the emergence of resource partitioning when flowers are evenly distributed, the addition of a negative reinforcement of movements leading to unrewarding flowers is necessary when flowers are patchily distributed. In environments with more complex spatial structures, the negative experiences of individual bees on flowers favour spatial segregation and efficient collective foraging. Our study fills a major gap in modelling pollinator behaviour and constitutes a unique tool to guide future experimental programs.

1 - Introduction

Foraging animals are expected to self-distribute on food resources in order to minimise competition and maximise their individual net energy gain (Fretwell, 1969, Giraldeau et al., 2000). Resource partitioning between individuals of different species is well documented, and often results from functional (Fründ et al., 2010;2013) or behavioural (Nagamitsu & Inoue, 1997; Valdovinos et al., 2016) specialisations. By contrast, how individuals of the same species interact to exploit resources in a common foraging area is less understood (Johst et al., 2008; Tinker et al., 2012).

For pollinators, such as bees that individually exploit patchily distributed floral resources in environments with high competition pressure, efficient resource partitioning appears a prodigious problem to solve. It involves assessing the quality of food resources, their spatial distribution, their replenishment rate, and the activity of other pollinators. As central place foragers, bees often visit familiar feeding sites (plants or flower patches) in a stable sequence called a “trapline” (Janzen, 1971; Thomson et al., 1997). Individual bees with exclusive access to an array of artificial flowers tend to develop traplines minimising travel distances to visit all the necessary flowers to fill their nectar crop and return to the nest (e.g. bumblebees: Ohashi et al., 2008; Lihoreau et al., 2012a; Woodgate et al., 2017; honey bees: Buatois & Lihoreau, 2016). This routing behaviour involves spatial memories that can persist days (Lihoreau et al., 2010) or weeks (Thomson, 1996).

How bees partition resources, when several conspecifics exploit the same foraging area, is however an open question. Experimentally the problem is challenging to address as it requires monitoring the movements of numerous bees simultaneously over large spatial and temporal scales. In theory, bees should develop individualistic traplines that minimise travel distances and spatial overlap with other foragers, thereby improving their own foraging efficiency and minimising the costs of competition (Ohashi & Thomson, 2005; Lihoreau et al.,

2016). Best available data supporting this hypothesis come from observations of small numbers of bumblebees foraging on potted plants (Makino & Sakai, 2005; Makino, 2013) or artificial flowers (in effect mimicking inflorescences or plants; Lihoreau et al., 2016; Pasquaretta et al., 2019) in large flight tents. In these experimental foraging conditions with limited numbers of bees and feeding sites, foragers tend to avoid spatial overlaps as a consequence of competition by exploitation (when bees visited empty flowers) and interference (when bees interacted on flowers) (Pasquaretta et al., 2019).

Computational modelling is a powerful approach to further explore the mechanisms by which such partitioning might emerge from spatial learning and competitive interactions. At the individual level, trapline formation has been modelled using an iterative improvement algorithm where a bee compares the net length of the route it has just travelled (sum of the lengths of all transitions between two flowers, or the nest and a flower, comprising the flower visitation sequence) to the length of the shortest route experienced so far (Lihoreau et al., 2012b). If the new route is shorter (or equivalent), the bee increases its probability of using all the transitions composing this route in its subsequent foraging bout. After several iterations, this route-based learning heuristic typically leads to the discovery and selection of a short (if not the shortest possible) trapline, thereby replicating observations in bees across a wide range of experimental conditions (Reynolds et al., 2013). Note however that this model makes the strong assumption that bees can compute, memorise and compare the lengths of multiple routes upon return to their nest. To address this issue, it was proposed that trapline formation could also emerge from vector-based learning (Le Moël et al., 2019), in which the bee remembers independent vectors instead of complete routes. This form of learning was thought to be more parsimonious and plausible considering the current understanding of spatial computation in the insect brain (Stone et al., 2017). So far, however, none of these traplining algorithms have accounted for social interactions and current models that include bee foraging either did not consider individual specificities of movements based on learning and memory (Qu & Drummond, 2018; Everaars et al., 2018; Rands, 2014;

Becher et al., 2014;2016), or implemented them very succinctly without being the focus of the model (Becher et al., 2018). Thus presently, there has been no formal exploration of how resource partitioning between interacting bees might form.

Here, we investigated the behavioural mechanisms underlying resource partitioning among traplining bees by comparing predictions of three agent-based models to each other. The different models integrate learning behaviour and social interactions in slightly different ways. Recent work showed that resource partitioning in bats foraging on patchily distributed cacti can be explained by basic reinforcement rules, so that a bat that finds an abundant feeding site tends to return to this site more often than its conspecifics (Goldshtein et al., 2020). Since bees extensively rely on associative learnings to recognise flowers and develop foraging preferences (Giurfa, 2013), we hypothesised that the combination of positive experiences (when a flower is full of nectar) and negative experiences (when a flower is unrewarding) could lead to the emergence of resource partitioning when different bees learn to use spatially segregated routes (Lihoreau et al., 2016; Pasquaretta et al., 2019). While the role of positive reinforcement in forming traplines has often been shown (Reynolds et al., 2013; Goldshtein et al., 2020), the potential role of the negative reinforcement has not been addressed, most likely as a result of trapline studies focusing on non-competitive situations.

First, we developed models implementing biologically plausible navigation (derived from vector-based learning) based on positive and negative reinforcements of transition probabilities between flowers and tested the independent and combined influences of these feedback loops on trapline formation by comparing simulations to published experimental data. Next, we explored how these simple learning rules at the individual level can promote complex patterns of resource partitioning at the collective level, using simulations with multiple foragers in environments with different resource distributions.

2 - Results

2.1 - Overview of models

We designed models of agents (bees) foraging simultaneously in a common set of feeding sites (flowers) from a central location (colony nest) (see summary in Fig 1). A full description of the models is available in the ODD protocol (S1 Text). Briefly, each bee completes a succession of foraging trips (foraging bouts) defined as the set of movements and flower visits between the moment it leaves the nest until the moment it returns to it. Each bee initially moves between the different flowers using a distance-based probability matrix (Lihoreau et al., 2012b; Reynolds et al., 2013). The probability to move between each flower is then modulated each time the bee finds the flower rewarding (positive reinforcement) or unrewarding (negative reinforcement). Learning occurs after each flower visit (online learning). We implemented three models to explore different combinations of positive and negative reinforcements: model 1: positive reinforcement only (hereafter “Model 1[+]”), model 2: negative reinforcement only (Model 2[-]), model 3: positive and negative reinforcements (Model 3[+/-]). Model comparison thus informed about the effect of each of the rules separately and in combination.

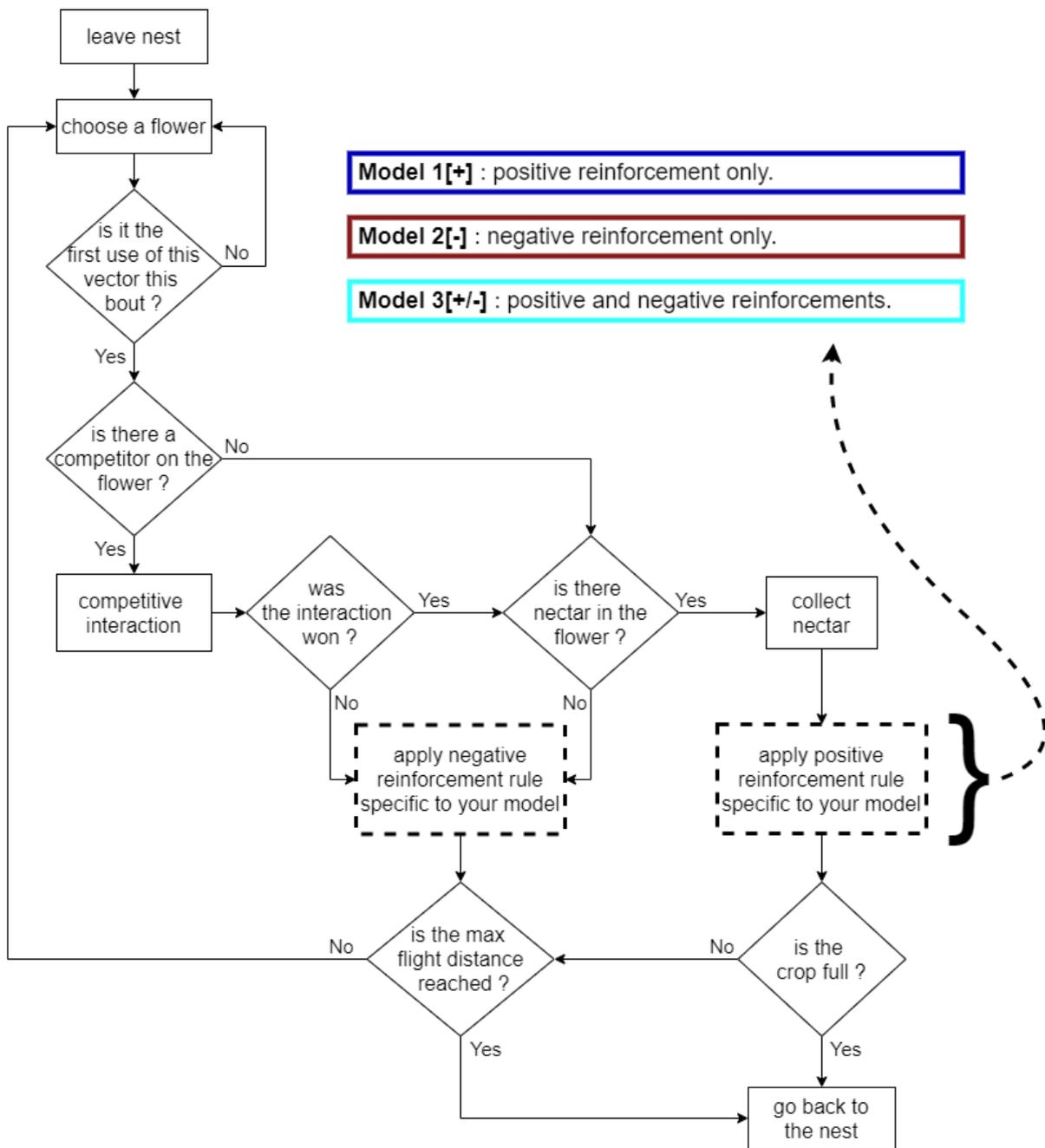


Fig 1. Flowchart summarising the agent-based models.

Rectangles represent actions performed by a bee. Diamonds indicate conditional statements. Arrows connect the different modules. The dashed rectangles are subject to the different rules of the three models.

2.2 - Simulations with one forager

We first tested the ability of our models to replicate trapline formation by real bees, by comparing simulations of a single forager to published experimental data in which individual bumblebees were observed developing traplines between five equally rewarding artificial flowers in a large open field (Woodgate et al., 2017; Lihoreau et al., 2012b). Lihoreau *et al.* (Lihoreau et al., 2012b) tested seven bumblebees in a regular pentagonal array (S1A Fig), which we judged enough to run quantitative comparisons with model simulations. While Woodgate *et al.* (Woodgate et al., 2017) tested six bees in a narrow pentagonal array (S1B Fig), only three of them presented enough successive foraging bouts in a single day to allow statistical comparisons with our model. Thus, for this dataset only qualitative comparisons were made with the model simulations. All statistical results are presented in Table 1.

We assessed the ability of bees to develop efficient routes by computing an index of route quality (i.e. the squared volume of nectar gathered divided by the distance travelled; see Methods). For real bees, route quality increased significantly with time in the regular pentagonal array of flowers (Fig 2A). When comparing simulations to experimental data, there were no significant differences in trends with models 1[+] and 3[+/-] (Table 1), meaning that simulated bees developed routes of similar qualities as real bees. However, route qualities predicted by model 2[-] were significantly lower than the experimental data. Similar trends were observed in the narrow pentagonal array of flowers (S3 Text).

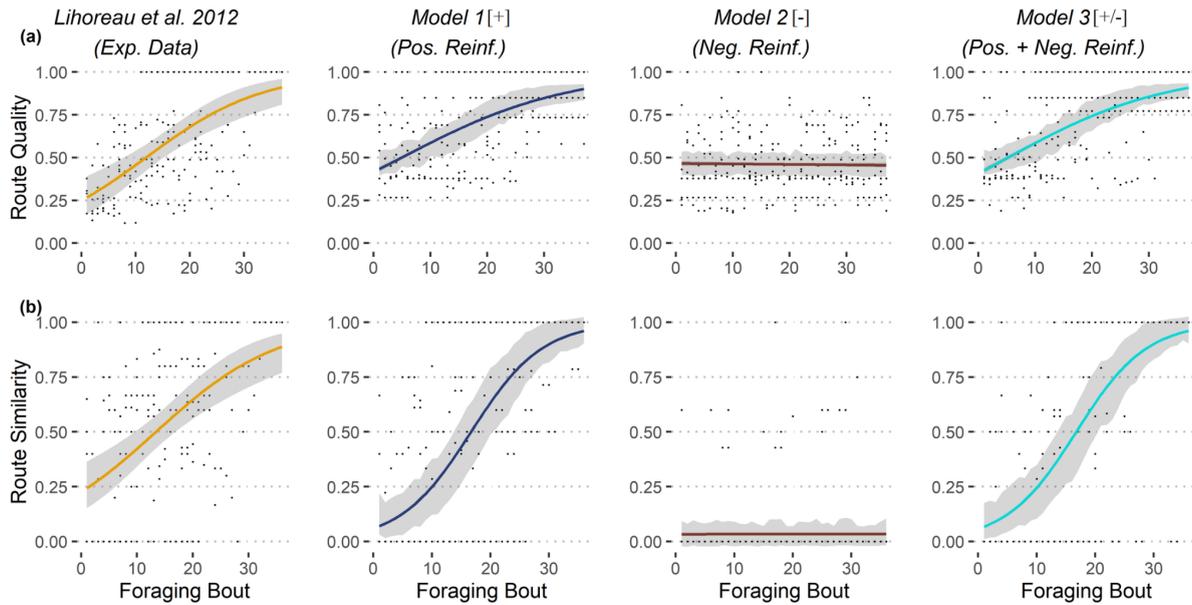


Fig 2. Comparisons of experimental and simulated route qualities.

Comparisons of route qualities (a) and route similarities (b) between simulations and experimental data (regular pentagonal array of flowers as in Lihoreau et al., 2012b). See details of models in Fig 1. For each dataset, we show the estimated average trends across foraging bouts (coloured curves), along the standard error (grey areas) of the mean. For the sake of eye comparison, in the simulation plots the standard error of the mean is estimated from a sample of 7 simulations ($N = 7$ bees in Lihoreau et al., 2012b). Average trends were estimated over 500 simulation runs, using GLMM Binomial model with bee identity as random effect (bee identity nested in simulation identity for simulated data).

We assessed the ability of bees to develop stable routes using an index of route similarity (i.e. computing the number and percentage of transitions between two flowers (or the nest and a flower) shared between two successive routes; see Methods). Route similarity is set between 0 (the two routes are completely different) and 1 (the two routes are completely identical). For real bees, route similarity increased with time in the regular pentagonal array (Fig 2B). When comparing simulations to experimental data, route similarity increased significantly more in models 1[+] and 3[+/-] than for real bees. However, route similarity in model 2[-] was significantly lower than for real bees. Similar trends were observed in the narrow pentagonal array (S3 Text).

Thus overall, positive reinforcement is necessary and sufficient to replicate the behavioural observations (although with a significant difference found for route similarity between the experimental data and the models 1[+] and 3[+/-]), while negative reinforcement has no detectable effect.

Table 1. Statistical output for simulations with one individual.

Comparisons of route quality and route similarity through Binomial GLMMs using bee identity as a random effect (bee identity nested in simulation identity for simulated data).

<i>Variable</i>	<i>Data</i>	<i>Estimate</i>	<i>P</i>
Route Quality	Exp. Data (Intercept)	0.153 ± 0.023	0.001
	Model 1[+]	-0.027 ± 0.023	0.224
	Model 2[-]	-0.155 ± 0.023	0.001
	Model 3[+/-]	-0.022 ± 0.023	0.339
Route Similarity	Exp. Data (Intercept)	0.110 ± 0.020	0.001
	Model 1[+]	0.088 ± 0.020	0.001
	Model 2[-]	-0.109 ± 0.020	0.001
	Model 3[+/-]	0.086 ± 0.020	0.001

2.3 - Simulations with two foragers

Having tested our models with one forager, we next explored conditions for the emergence of resource partitioning within pairs of foragers. Here experimental data are not available for comparison. We thus simulated foraging patterns and interactions of bees in different types of environments defined by flower patchiness. Each environment contained 10 flowers that were either distributed in one patch, two patches, or three patches (see examples in S2 Fig; for details, see Methods). Each bee had to visit five rewarding flowers to fill its crop to capacity. All the statistical results of this part are presented in Table 2.

Exploitation and interference competition

We first analysed exploitation competition by quantifying the frequency of visits to non-rewarding flowers by each bee during each foraging bout. The frequency of visits to non-rewarding flowers decreased for simulated bees in models 2[-] and 3[+/-] (Fig 3A and Table 2), irrespective of the environment they were tested in. However, in model 1[+], bees behaved differently in the different environments. In the one patch environment, bees decreased their visits to non-rewarding flowers, whereas in the two and three patch environments, bees tended to increase their visits to non-rewarding flowers. The increase of non-rewarding visits in environments with patchily distributed resources can be explained as follows. If bees start reinforcing visits to flowers of a shared patch, they will become more likely to visit the same patch. Given the much larger space between flowers of different patches than between flowers of the same patch, the probability to switch from one patch to the next (without the help of the negative reinforcement) is low, leading to bees flying between the empty flowers of a patch repeatedly. This process ultimately increases visits to empty flowers, and also occurrences of interference between the two bees if they are both at the same patch.

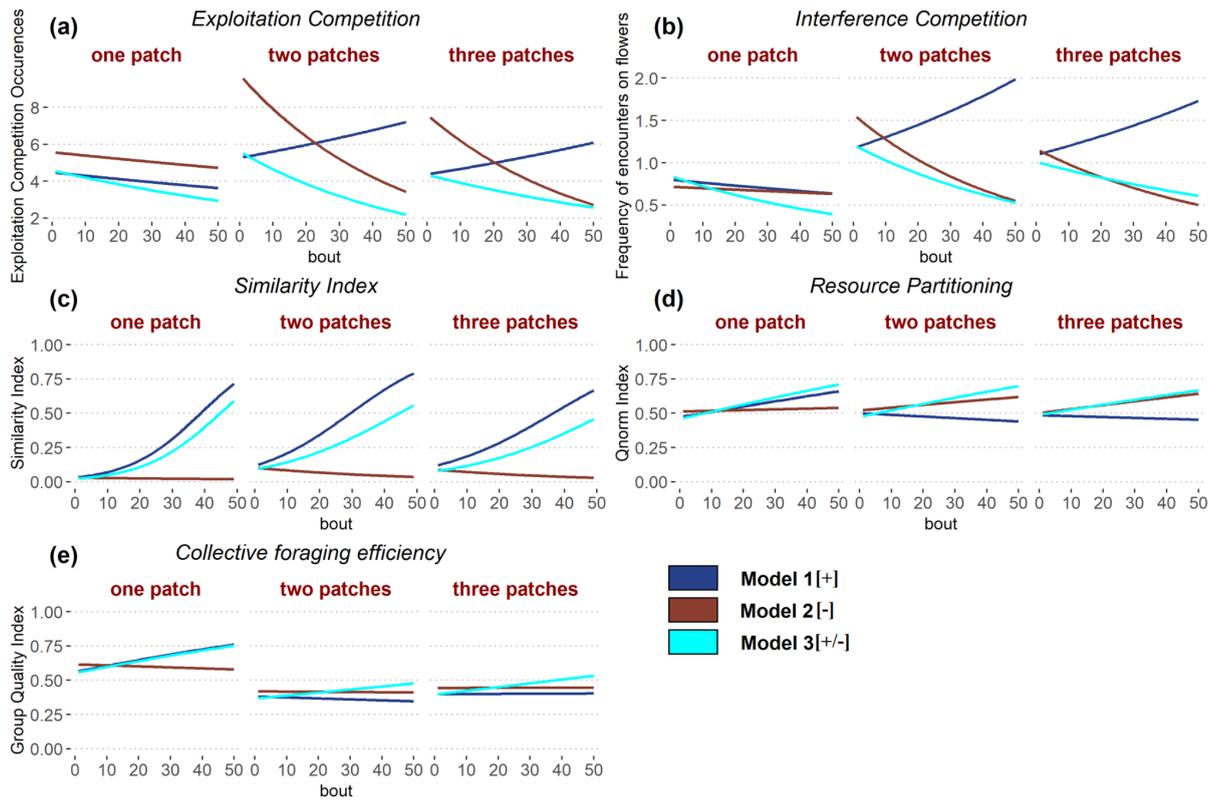


Fig 3. Model comparisons for observed variables.

Results of simulations with two foragers in environments with 10 flowers. See details of models in Fig 1. The x axis is the number of completed foraging bouts by the two foragers. The y axis represents respectively: (a) the estimated mean frequency of visits to empty flowers; (b) the estimated mean frequency of encounters on flowers; (c) the similarity index SI_{ab} between two successive flower visitation sequences; (d) the index of resource partitioning Q_{norm} (0: both bees visit the same set of flowers; 1: bees do not visit any flower in common); (e) the collective foraging efficiency index QL_{group} . Average trends for each model are estimated across all types of environments (one patch, two patches and three patches; see S2 Fig).

We analysed interference competition by quantifying the number of interactions on flowers at each foraging bout between the two bees. The frequency of encounters on flowers decreased with time for both models 2[-] and 3[+/-] (Fig 3B and Table 2), irrespective of the type of environment. Here again, bees of model 1[+] behaved differently in the different environments. In the one patch environment, bees decreased their frequency of encounters on flowers, whereas in the two and three patches environments they increased their frequency of interactions. Again this is likely due to the absence of negative reinforcement, leading bees to be trapped in an empty patch.

These differences in the occurrence of exploitation and interference competition correlate to a variation in the total number of visits to flowers, effectively improving the bees' foraging efficiency. The strength of this effect is greater for the exploitation competition as it is occurring much more often (exploitation: 2 to 10 occurrences in average; interference: 0 to 2 occurrences in average; Fig 3A and 3B).

Thus, overall negative reinforcement was necessary for reducing exploitation and interference competition. By allowing bees to avoid empty flowers, negative reinforcement facilitated the discovery of new flowers and thus gradually relaxed competition. In the absence of negative reinforcement, both types of competition increased in environments with several flower patches.

Route similarity

We analysed the tendency of bees to develop repeated routes by comparing the similarity between flower visitation sequences of consecutive foraging bouts for each individual (Fig 3C). Bees increased route similarity through time in all types of environments in models 1[+] and 3[+/-] (Table 2). By contrast, in model 2[-], route similarity did not vary in the one patch environment and decreased through time in the other environments. The presence of

negative reinforcement in models 2[-] and 3[+/-] reduced the final level of route similarity compared to trends found in model 1[+]. In these conditions, bees learned to avoid revisits to empty flowers and showed greater variation in their visitation sequences as a result of searching for new flowers.

Resource partitioning

We analysed the level of resource partitioning by quantifying the tendency of the two bees to use different flowers. This index varies between 0 (the two bees use the same set of flowers) and 1 (the two bees use completely different sets of flowers; see Methods).

In model 1[+], bees showed an increase of resource partitioning with time in environments with one patch, and a decrease in environments with two or three patches (Fig 3D and Table 2). By contrast, in model 2[-] and model 3[+/-], bees showed an increase of resource partitioning with time in all types of environments. Model 3[+/-] displayed similar levels of partitioning in all the different environments where models 1[+] and 2[-] showed a greater variance. Model 1[+] had greater partitioning only in the one patch environment, while model 2[-] had greater partitioning in the two and three patch environments. This suggests positive and negative reinforcements contributed unevenly but complementarily in the model 3[+/-] with different spatial distributions of flowers. Positive reinforcement would be the main driver for partitioning in the one patch environment, while negative reinforcement would be the main driver in the two and three patches environments.

Collective foraging efficiency

To quantify the collective foraging efficiency of bees, we analysed the capacity of the two foragers to reach the most efficient combination of route qualities (i.e. minimum distance travelled by a pair of bees needed to visit the 10 flowers; see Methods).

In model 1[+], pairs of bees increased their collective foraging efficiency with time in environments with one and three patches (Fig 3E and Table 2). By contrast, bees decreased their level of foraging efficiency in the environment with two patches. In model 2[-] pairs of bees decreased their collective foraging efficiency with time in all types of environments. In model 3[+/-] bees increased their collective foraging efficiency with time in all types of environments. Positive reinforcement seems to be the main driver for collective foraging efficiency in the one patch environment. However, neither the positive or negative reinforcements alone managed to increase foraging efficiency in the two and three patch environments. Only their interaction, as seen in the model 3[+/-], brought an increase in collective foraging efficiency. Collective efficiency is generally higher in the one patch environment than in the two and three patches environments because the difference between the best possible path (for which the collective foraging efficiency is equal to 1) and a typical suboptimal path of a simulated bee is lower due to the absence of long inter-patch movements.

Table 2. Statistical output for simulations with two individuals.

Comparisons of (i) exploitation competition, (ii) interference competition, (iii) route similarity, (iv) route partitioning and (v) collective foraging efficiency through GLMMs using bee identity as a random effect (bee identity nested in simulation identity for simulated data). The results presented are the slope estimate along with a 95% confidence interval of the mean, for each type of environment tested (See Methods for details).

Variable	Model	Estimate (1 patch)	Estimate (2 patches)	Estimate (3 patches)
Exploitation Competition	Model 1[+]	-4.26e-03 ± 2.10e-04	6.27e-03 ± 1.80e-04	6.65e-03 ± 1.90e-04
	Model 2[-]	-3.32e-03 ± 1.90e-04	-2.10e-02 ± 2.00e-04	-2.06e-02 ± 2.00e-04
	Model 3[+/-]	-8.94e-03 ± 2.20e-04	-1.88e-02 ± 3.00e-04	-1.05e-02 ± 2.00e-04
Interference Competition	Model 1[+]	-4.57e-03 ± 7.20e-04	1.05e-02 ± 4.00e-04	9.16e-03 ± 5.20e-04
	Model 2[-]	-2.49e-03 ± 7.40e-04	-2.10e-02 ± 6.00e-04	-1.68e-02 ± 7.00e-04
	Model 3[+/-]	-1.53e-02 ± 8.00e-04	-1.66e-02 ± 7.00e-04	-1.01e-02 ± 6.00e-04
Route Similarity	Model 1[+]	1.34e-01 ± 2.00e-03	9.56e-02 ± 1.30e-03	7.76e-02 ± 1.20e-03
	Model 2[-]	7.46e-04 ± 6.65e-03	1.91e-02 ± 2.90e-03	-3.20e-02 ± 3.10e-03
	Model 3[+/-]	1.33e-01 ± 2.00e-03	6.95e-02 ± 1.20e-03	6.14e-02 ± 1.30e-03
Route Partitioning	Model 1[+]	2.90e-02 ± 1.30e-03	-1.02e-02 ± 1.30e-03	-8.26e-03 ± 1.26e-03
	Model 2[-]	1.22e-02 ± 1.30e-03	1.28e-02 ± 1.20e-03	1.82e-02 ± 1.20e-03
	Model 3[+/-]	3.55e-02 ± 1.30e-03	3.17e-02 ± 1.30e-03	2.19e-02 ± 1.30e-03
Collective Foraging Efficiency	Model 1[+]	4.20e-02 ± 1.50e-03	-4.61e-03 ± 1.27e-03	3.04e-03 ± 1.25e-03
	Model 2[-]	-5.08e-03 ± 1.24e-03	-8.03e-03 ± 1.27e-03	-4.24e-03 ± 1.24e-03
	Model 3[+/-]	4.12e-02 ± 1.50e-03	8.77e-03 ± 1.25e-03	1.83e-02 ± 1.30e-03

3 - Discussion

Central place foraging animals exploiting patchily distributed resources that replenish over time are expected to develop foraging routes (traplines) minimising travel distances and interactions with competitors (Ohashi & Thomson, 2005; Lihoreau et al., 2016; Possingham, 1989). Here we developed cognitively plausible agent-based models of probabilistic navigation to explore the behavioural mechanisms leading to resource partitioning between traplining bees. In the models, bees learn to develop routes as a consequence of feedback loops that modify the probabilities of moving between flowers. Simulations show that, in environments where resources are evenly distributed, bees can reach high levels of resource partitioning based on positive reinforcement only, but cannot do so based on negative reinforcement only. However, in environments with patchily distributed resources, both positive and negative reinforcements become necessary.

We developed our hypotheses and models based on observations on single foraging bees (Woodgate et al., 2017; Lihoreau et al., 2012b). Our first step was therefore to test how the models compared to existing data. Models with positive reinforcement showed a good general fit to the experimental data (Fig 2 and Fig A in S3 Text), although they often overestimated the increase of route similarity with experience in real bees. This imperfect match could be due to the low amount of available experimental data in the original studies (seven individuals in Lihoreau et al., 2012b, three individuals in Woodgate et al., 2017), but also a result from the limitations of our models. First, the model bees navigate with the only intent of finding resources, while real bees sometimes show phases of stochastic exploration during and after the trapline formation (Woodgate et al., 2017; Kembro et al., 2019). Second, real bees do not always find a flower when exploring their environment, especially when naïve. On the contrary, there is no probability of not finding a flower for the model's bees, which then visit on average more flowers from the first foraging bout. The resulting routes are of higher route quality as they visit more different flowers, but of lower similarity as they

also use different transitions between flowers, while real bees navigate back and forth between the same few flowers.

We then used our models for predicting behaviours of two competing bees in different types of environments. To develop a trapline in this competitive situation, the bees needed to find rewarding flowers but also avoid competitors. These two goals were independently fulfilled by the positive reinforcement and the negative reinforcement. Simulated bees were fastest to develop a trapline when using the positive reinforcement only, and unable to follow any stable route when solely using the negative reinforcement. However, this does not indicate that the use of both reinforcements was less effective than just positive reinforcement. Simulated bees were indeed more likely to establish a stable route with positive reinforcement only, but these routes most likely contained contested flowers that the bees were not able to give up on, as they did not change their behaviour after experiencing unrewarded visits. This assumption is supported by the fact that both reinforcements (model 3[+|-]) leads to a greater resource partitioning and a higher collective foraging efficiency.

When foraging in uniformly distributed plant resources (one patch), it is easiest to encounter all the resources available as none of them are isolated far from any other (with thus a low probability of being reached). Consequently, two bees are very likely over time to learn non-overlapping foraging routes and show resource partitioning. However, in environments with non-uniformly distributed resources (two or three patches), the added spatial complexity can interfere with this process. The initial likelihood of moving between distant patches is relatively low. Thus, the sole implementation of positive reinforcement often does not allow bees to explore all possible patches, so that the paths of competing bees overlap and interfere within a subset of the available patches. Adding a negative reinforcement for movement transitions leading to unrewarded flowers increases aversion for these empty flowers, the spatial segregation of foraging paths between competing bees and

the collective exploitation of all available patches, even if the initial probabilities of moving to distant patches are low. This interplay between the influences of positive and negative experiences at flowers on the spatial and competitive decisions of bees is in accordance with the behavioural observations that bees tend to return to rewarding flowers and avoid unrewarding flowers, either because flowers were found empty or because the bees were displaced by a competitor during a physical encounter (Lihoreau et al., 2016; Pasquaretta et al., 2019).

The need for a negative reinforcement to enhance discrimination between different options or stimuli is well-known in learning theory and behavioural studies (Beshers & Fewell, 2001; Garrison et al., 2018; Kazakova et al., 2020). At the individual level, negative experiences modulate learning. For both honey bees and bumblebees, adding negative reinforcement to a learning paradigm (e.g. quinine or salt in sucrose solution) enhances fine scale colour discrimination (Avergues-Weber et al., 2015) and performance in cognitive tasks requiring learning of rules of non-elemental associations (Giurfa, 2004). The insect brain contains multiple distinct neuromodulatory systems that independently modulate negative and positive reinforcement (Schwaerzel et al., 2003) and the ability of bees to learn negative consequences is well-established (Vergoz et al., 2007). At the collective level, negative feedbacks are also known to modulate social and competitive interactions. This is especially notable in collective decisions making by groups of animals and robots (Sumpter, 2010), where negative feedbacks enable individuals to make fast and flexible decisions in response to changing environments (Robinson et al., 2005; Seeley et al., 2012). Even so, the utility of negative reinforcement to enhance efficient trapline formation and the consequences of this for the emergence of effective resource partitioning has not been commented on previously. It may be that this is a general phenomenon with applicability to other resource partitioning systems.

Our study implies that some very basic learning and interaction rules are sufficient for trapline formation and resource partitioning to emerge in bee populations, providing a solid basis for future experimental work. Nonetheless, several improvements of the model could already be considered for further theoretical investigations of bee spatial foraging movements and interactions. These could include adding to the model the documented inter-individual variability in cognitive abilities (Chittka et al., 2003; Raine et al., 2012) and spatial strategies (Klein et al., 2017) of bees, the variability in the nutritional quality of resources (Wright et al., 2018; Hendriksma et al., 2019) and the specific needs of each colony (Kraus et al., 2019), or the well-known ability of bees to use chemical (Leadbeater & Chittka, 2005), visual (Dunlap et al., 2016) and social information to decide whether to visit or avoid flowers. For instance, foragers of many bee species leave chemical cues as footprints on the flowers they have visited (bumblebees and honeybees: Stout & Goulson, 2001; solitary bees: Yokoi & Fujisaki, 2009). Bees learn to associate the presence or absence of a reward to these footprints and to revisit or avoid scented flowers (Leadbeater & Chittka, 2011). Such a pheromone system is a beneficial signal for all participants in the interaction (Stout & Goulson, 2001). This additional information could significantly enhance the positive or negative experiences of bees visiting flowers and thus increase resource partitioning to the benefit of all bees coexisting in the patch (S4 Text). Even different species of bee can learn to use these cues (Stout & Goulson, 2001; Dawson & Chittka, 2012). More exploration could also be done in the future in regards to the probability of winning a competitive interaction on flower. While we considered all individuals to have similar probabilities to access floral nectar when bees encounter on flowers, resource partitioning has been suggested to be favoured by asymmetries in foraging experiences (Ohashi et al., 2008; Lihoreau et al., 2016). Differences in experience or motivation would ultimately affect the outcome of competition, both passively (more consistent depletion of the flowers in a trapline) and actively (active displacement of other bees from one's established trapline).

Our study fills a major gap in our understanding of pollinator behaviour and interactions by building on recent attempts to simulate trapline foraging by individual bees (Lihoreau et al., 2012b; Reynolds et al., 2013; Le Moël et al., 2019). It constitutes a unique theoretical modelling framework to explore the spatial foraging movements and interactions of bees in ecologically relevant conditions within and between plant patches, thereby holding considerable premises to guide novel experiments. Further developments of the model could be used to test predictions with more than two bees (see examples S1 Video and S4 Text), several colonies, or even different species of bees (e.g. honey bees) and thus complement current predictions about pollinator population dynamics (Becher et al., 2014;2016;2018). Ultimately, the robust predictions of the spatial movements and interactions of bees over large spatio-temporal scales, through experimental validations of the model, have the potential to show the influence of bee movements on plant reproduction patterns and pollination efficiency (Ohashi & Thomson, 2009; Pasquaretta et al., 2017).

4 - Methods

4.1 - Description of models

We built three agent-based models in which bees learn to develop routes in an array of flowers (see summary diagram in Fig 1). The environment contains flowers each delivering the same quality and volume of nectar. At each foraging bout (flower visitation sequence, beginning and ending at the colony nest entrance as the bee starts and finishes a foraging trip, respectively), each bee attempts to collect nectar from five different flowers in order to fill its nectar crop (stomach) to capacity. Flowers are refilled between foraging bouts. In simulations with two bees, the two individuals start their foraging bout synchronously, and the flowers are refilled with nectar after the last bee has returned to the nest. For each bee, flower choice is described using movement transitions (orientated jumps between two flowers or between the nest and a flower). The initial probability of using each possible transition is based on the length of the movement, so that short transitions have a higher

probability than longer ones. This probability is then modulated through learning when the bee used a transition for the first time during a bout.

We implemented two learning rules: (i) a positive reinforcement, i.e. if the flower at the end of a transition contains nectar and the bee feeds on it, it is set as a rewarding experience and the probability to reuse the transition later is increased; (ii) a negative reinforcement, i.e. if the flower is empty or if the bee is pushed away by competitors, it is set as a non-rewarding experience and the probability to reuse the transition later is decreased. The three models implemented either one of these two rules (model 1[+]: positive reinforcement only; model 2[-]: negative reinforcement only) or both rules (model 3[+/-]).

A flower is empty if it had previously been visited in the same foraging bout by the same or another bee (exploitation competition). If multiple bees visit a flower at the same time (interference competition), only one bee (randomly selected) is allowed to stay and take the reward if there is one. The other bees react as if the flower was empty. After each flower visit, all bees update their probabilities to reuse the movement transitions accordingly.

Trapline formation thus depends on the experience of the bee and its interactions with other foragers. For simplicity, we restricted our analysis to two bees. Working with pairs of bees facilitates future experimental tests of the models' predictions by reducing the number of bees to manipulate and control in experiments (Ohashi et al., 2008; Lihoreau et al., 2016). Note, however, that the same models can be used to simulate interactions among more bees (see examples with five bees in S1 Video, S4 Text).

A detailed description of the model is provided in S1 Text, in the form of an Overview, Design concepts and Details (ODD) protocol (Grimm et al., 2006;2020). The complete R code is available at

<https://gitlab.com/jgautrais/resourcepartitioninginbees/-/releases>.

4.2 - Environments

Simulations with one forager

Our first goal was to test the ability of our models to replicate observations of real bees. To do so, we ran simulations in environments replicating published experimental studies in which individual bumblebees (*Bombus terrestris*) were observed developing traplines between five equally rewarding artificial flowers in a large open field (Woodgate et al., 2017; Lihoreau et al., 2012b). To our knowledge, these studies provide the most detailed available datasets on trapline formation by bees. Lihoreau *et al.* (Lihoreau et al., 2012b) used a regular pentagonal array of flowers (S1A Fig) in which they tracked seven bumblebees. We judged this sample size enough to run quantitative comparisons with model simulations (raw data are available in the supporting information of [22]). Woodgate *et al.* (Woodgate et al., 2017) used a narrow pentagonal array of flowers (S1B Fig). Here, however, the small sample size of the original dataset (three bumblebees, data shared by J. L. Woodgate) only enabled a qualitative comparison with the model simulations (S3 Text).

Simulations with two foragers

We then explored conditions leading to resource partitioning by running model simulations with two foragers. Here we simulated environments containing 10 flowers, in which each bee had to visit five rewarding flowers to fill its crop to capacity. The simulated flowers should thus be considered as feeding sites such as plants or inflorescences, which are more likely to contain such large amounts of resources (20% of the bee's crop). To test whether model predictions were robust to variations in spatial distributions of resources we simulated three types of environments characterised by different levels of resource patchiness: (i) a patch of 10 flowers, (ii) two patches of five flowers each, and (iii) three patches of five, three and two flowers respectively (see examples in S2 Fig). We generated flower patches in a spatial configuration comparable to the one used in both experimental setups (Woodgate et al.,

2017; Lihoreau et al., 2012b). In a 500m x 500m plane, a nest was set as the centre (coordinates 0,0). Then, patch centres were placed with a minimum distance of 160m between each, and at least 20m from the nest. Within a patch, flowers were randomly distributed according to two constraints: (i) flowers were at least 20m apart from each other and from the nest, (ii) the maximum distance of each flower from the centre of their patch was 40m. This ensured that each patch had a maximum diameter of 80m and inter-flower distances were smaller between all flowers of the same patch than between all flowers of different patches (See ODD Protocol for more details, S1 Text, Ch.7 “Submodels”). The distances used in the simulated environments were chosen to replicate the experimental data used to test the model (Woodgate et al., 2017; Lihoreau et al., 2012b) where closest flowers were spaced by 25 metres. In our model, however, only the relative distance between the different elements of the environment mattered as all distances were normalised in the process of creating the probability matrix (S1 Text).

4.3 - Movements

At each step, a bee chooses to visit a target location (flower or nest) based on a matrix of movement probabilities. This matrix is initially defined using the inverse of the square distance between the current position of the bee and all possible target locations (Lihoreau et al., 2012b; Reynolds et al., 2013). The probability of moving from location i to location j among multiple possible targets, is initially set to:

$$(1) \quad P(i \rightarrow j) = \frac{\frac{1}{d_{ij}^2}}{\sum_j \frac{1}{d_{ij}^2}}$$

Where d_{ij} is the distance between locations i and j . The use of a movement probability matrix is justified by its capacity to approximate accurately the probability to reach a flower

using a random walk, although it is significantly dependent on what exponent is used in the formula transforming distances to probabilities (See S6 Text for details). Thus, while the probability matrix allows unexperienced bees (during their first foraging bout) to move between all flowers, it should not be interpreted as a knowledge of their positions, but rather a probability of finding them by chance.

Before choosing its destination, the bee lists all possible target locations. For simplicity, the bee excludes its current location, thus preventing looping flights to and from the same target (flower or nest), which are rare in experienced bumblebee foragers (Saleh & Chittka, 2007) and provide little information about bee routing behaviour. The bee also excludes the location it had just come from to simulate the tendency of bumblebees to avoid recently visited (and thus depleted) flowers (Saleh & Chittka, 2007). The foraging bout ends if: (i) the bee fills its crop to capacity, (ii) the bee chooses the nest as a target destination, or (iii) the bee reaches a maximum travelled distance of 3000 m. The latest was added to avoid endless foraging trips in the model. The maximum distance was chosen based on the observation that bumblebees typically forage within a distance of less than 1km from their nest (Osborne et al., 1999; Wolf & Moritz, 2008; Woodgate et al., 2016).

4.4 - Learning

Learning modulates the probability of using transition movements as soon as the bee experiences the chosen target and only once within a foraging bout (the first time the transition is used during the foraging bout; Fig 1). This approach has the advantage of implementing vector navigation (Le Moël et al., 2019; Stone et al., 2017) (S6 Text) and thus avoids assumptions about computation and comparison of complete routes (Lihoreau et al., 2012b; Reynolds et al., 2013), but it makes new assumptions about bees remembering a large number of locations and distances of flowers. Bees are known to be able to learn few independent feeding sites, and even to create shortcuts between these locations (Menzel et

al., 2005;2011). By keeping a low number of flowers, we ensured the number of transitions to remember would be low so that this hypothesis was reasonable. In comparison, bees in the wild can be expected to visit 50 to 100 flowers on average, although this number can vary greatly depending on many factors such as the flower species available, the competition pressure, the seasons, the weather or the time of the day. As such, the “flowers” used in our study would be closer to plants featuring multiple flowers.

Positive reinforcement was implemented in models 1[+] and 3[+/-]. It occurred when a bee used a transition leading to a rewarding flower. The probability of using this transition was then multiplied by 1.5, then normalised among other transition probabilities to ensure that all sum up to 1 and no single probability goes beyond a value of 1, as in Reynolds *et al.* (Reynolds et al., 2013). This positive reinforcement is based on the well-known tendency of bumblebees to return to nectar-rewarding places through appetitive learning (Goulson, 2010). Negative reinforcement was implemented in models 2[-] and 3[+/-]. It occurred when a bee used a transition leading to a non-rewarding flower. The bee reduced the probability of using that transition by multiplying it by 0.75 (here also rescaling the probabilities after application of the reinforcement). This negative reinforcement rule was based on the tendency of bumblebees to reduce their frequency of revisits to unrewarded flowers with experience (Pasquaretta et al., 2019). We applied a lower value to negative reinforcement because bees are much more effective at learning positive stimuli (visits to rewarding flowers) than negative stimuli (visits to non-rewarding flowers) (review in Menzel, 2014). Sensitivity analyses of these two parameters show that increasing positive and/or negative reinforcement increases the speed and level of resource partitioning (S2 Text). However, only positive reinforcement has a significant effect on route similarity (Fig C in S2 Text).

4.5 - Competitive interactions

We implemented competitive interactions between foragers in the form of exploitation and interference (Fig 1). Exploitation competition occurred when a bee landed on a flower whose nectar reward had already been collected by another bee. If the flower was empty, the probability to reuse the transition was either left unchanged (Model 1[+]) or decreased (negative reinforcement, Models 2[-] and 3[+/-]). Interference competition occurred when two bees arrived simultaneously on a flower. Only one bee could stay on the flower and access the potential nectar reward with a random probability ($p=0.5$). After the interaction, the winner bee took the reward if there was one. The loser bee reacted as it would for an empty flower. To our knowledge, there is no empirical data suggesting that bees would react differently to these types of competitive interactions. Therefore, we made the parsimonious assumption that the effect was the same. We note, however, that the model assumes all direct interactions are of competitive nature, while in nature whether bees would engage in a competitive interaction or share the resource would likely depend on different factors such as flower size, amount of reward, or the species and physiology of the interacting bees. This simplification was made to keep the focus of the model towards competition, and to avoid adding too much complexity to the model.

4.6 - Data Analyses

All analyses were performed in R version 3.3 (R Core Team, 2018).

Simulations with one forager

For each model, we compared the results of the simulations to the reference observational data, either quantitatively (for Lihoreau et al., 2012b) or qualitatively (for Woodgate et al., 2017; S3 Text). We stopped the simulations after the bees completed a number of foraging bouts matching the maximum observed during the experimental conditions of the published data (37 foraging bouts in Lihoreau et al., 2012b; 61 foraging bouts in Woodgate et al.,

2017). We ran 500 simulations for each model and we estimated how models fitted the experimental data using two main measures:

(i) the quality of each route, QL , calculated as:

$$(2) \quad QL = \frac{\frac{F^2}{d}}{QL_{opt}}$$

Where F is the number of rewarding flowers visited during a foraging bout and d is the net length of all transition movements travelled during the foraging bout. QL is standardised between 0 and 1 by the quality of the optimal route in each array QL_{opt} (shortest possible route to visit all 5 flowers).

(ii) a similarity index SI_{ab} between flower visitation sequences experienced during two consecutive foraging bouts a and b as follows:

$$(3) \quad SI_{ab} = \frac{s_{ab}}{2l_{ab}}$$

Where s_{ab} represents the number of flowers in transitions found in both sequences, and l_{ab} the length of the longest flower visitation sequence between i and j multiplied by 2 to make sure that $SI_{ab} = 1$ occurs only when two consecutive sequences sharing the same transitions also have the same length (more details and examples in S5 Text).

We applied generalised linear mixed effect models (GLMM) with binomial error, using the *glmer* function in 'lme4' package (Bates et al., 2015), to assess whether the estimated trends across foraging bouts for QL and SI_{ab} obtained from model simulations with one forager differed from trends obtained from experimental data. In each model, we used a random structure to account for the identity of bees.

Simulations with two foragers

We generated 10 arrays of flowers for each of the three types of environments (one patch, two patches and three patches) and ran 100 simulations for each of the three models (9000 simulations in total). We compared the simulation outcomes of the models using four measures:

i) the frequency at which each bee experienced exploitation competition (i.e. flower visits when the reward has already been collected) and interference competition (i.e. flower visits when two bees encounter on the flower).

ii) the similarity index SI_{ab} between successive foraging bouts by the same bee.

iii) the degree of resource partitioning among bees, based on network modularity Q (Pasquaretta & Jeanson, 2018; Pasquaretta et al., 2019). Q is calculated using the *computeModules* function implemented in the R package 'bipartite' (Dormann et al., 2008) using the *DIRTLPAwb+* algorithm developed by Beckett (Beckett, 2016). Although Q ranges between 0 (the two bees visit the same flowers) and 1 (the two bees do not visit any flower in common), the comparison of modularity between networks requires normalisation because the magnitude of modularity depends on network configuration (e.g., total number of flower visits) (Beckett, 2016; Dormann & Strauss, 2014). For each network, we calculated:

$$(4) \quad Q_{norm} = \frac{Q}{Q_{max}}$$

where Q_{max} is the modularity in a rearranged network that maximises the number of modules (Pasquaretta & Jeanson, 2018).

iv) an index of collective foraging efficiency, QL_{group} , computed for each foraging bout b , to estimate the collective efficiency of all foraging bees, as:

$$(5) \quad QL_{group,b} = \frac{\sum_{p=1}^n QL_{p,b}}{QL_{optimal}}$$

where $QL_{p,b}$ is the route quality of the individual p during bout b , n the number of bees, and $QL_{optimal}$ is the maximum value of all the possible sums of individual route qualities. $QL_{optimal}$ was calculated in each environment by computing all possible combinations of two routes visiting five flowers each and extracting the combination with the highest quality.

To assess whether the trends across foraging bouts obtained from simulations with two bees differed between models (Fig 1) and types of environments (S2 Fig), we applied GLMMs for each of the following response variables: (i) frequency of competition types (Poisson error distribution), (ii) SI_{ab} (Binomial error distribution), (iii) Q_{norm} (Binomial error distribution) and (iv) QL_{group} (Binomial error distribution). In each model, we used a random structure to account for bee identity nested in flower arrays (i.e. 100 simulations of each spatial array for each model). To statistically compare the trends across foraging bouts, we estimated the marginal trends of the models, as well as the 95% confidence intervals of

the mean using the *emtrends* function in 'emmeans' package (Lenth et al., 2019). When the 95% confidence intervals of the estimated trends included zero, the null hypothesis was not rejected. Statistical models were run using the *glmer* function in 'lme4' package (Bates et al., 2015).

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Supporting Information

S1 Text. ODD Protocol.

Below we provide a description of our models following the ODD (Overview, Design concepts, Details) protocol (Grimm et al., 2006;2020).

1 – Purpose and patterns

The purpose of the models is to offer an explanation to how multiple bees learn to optimise their foraging efficiency. Bees are expected to do so by developing efficient routes (traplines) minimising spatial overlaps with other foragers (resource partitioning) (Lihoreau et al., 2016). We suggest such process can be achieved through combinations of positive and negative reinforcements during flower visits.

The patterns we look at are the development, partial or complete, of stable partitioned routes between flowers by bees, which are observed in most studies on bee foraging strategies. Sensitivity of this behaviour to the parameters of the model is key to determine the usefulness of the model.

2 - Entities, state variables and scales

The models depict three kinds of entities: bees, flowers and the colony nest. Bees go out foraging for nectar rewards on flowers and return to the nest. Numbers of bees and flowers can be adjusted in the model. In our simulations we set a ratio of five flowers per bee as to fit the experimental data we replicated (Woodgate et al., 2017; Lihoreau et al., 2012). A single colony nest is represented, from which all bees forage and come back to. The bees are defined by the following set of state variables:

Variable name	Variable type and units	Meaning
<i>ID</i>	Integer, constant	A simple number to identify each bee in the model.
<i>Crop</i>	Integer, dynamic	A count of how many flower rewards (volume of nectar) the bee has collected since its departure from the nest.
<i>maxCrop</i>	Integer, constant	A maximum number of rewards (volume of nectar) a bee can hold at a time.
<i>probabilityMatrix</i>	Matrix of probabilities, dynamic	A square matrix of length equal to the sum of number of flowers and the nest, depicting the probability to move between each entity.
<i>indPos</i>	Integer, dynamic	An integer showing the ID of the flower currently visited by the bee.
<i>winProbabilities</i>	Float, constant	Value indicative of the probability of winning an encounter on a flower (competition by interference).
<i>indFlowerOutcome</i>	Matrix of integers, dynamic	A square matrix of length equal to the sum of number of flowers and the nest, depicting a transition's outcome (1: rewarding; 0: not used; -1: non-rewarding)

The flowers are static entities placed in the environment. They are defined by:

Variable name	Variable type and units	Meaning
<i>ID</i>	Integer, constant	A simple number to identify each flower in the model.
<i>resourceOnFlower</i>	Integer, dynamic	Variable showing the resource availability on the flower (0 or 1).
<i>x,y</i>	Floats, constant	Coordinates of the flower in the environment.

The nest is a unique entity which is represented by the following variables:

Variable name	Variable type and units	Meaning
<i>x,y</i>	Floats, constant	Coordinates of the nest in the environment.

Both the spatial and temporal scales are represented. Space is represented by the relative distance between the different flowers. Internal parameters define min/max ranges in which each entity can be placed, relative to other entities (see *CreateEnvironment* submodel). Time is represented abstractly. At each step, the bees visit a flower, and possibly feed on it. We assume all travel and flower manipulation times are identical. The use of a movement probability matrix is justified by its capacity to approximate accurately the probability to reach a flower using a random walk, although significantly dependent on what exponent is used in the formula transforming distances in probabilities (See S5 Text for details).

3 - Process overview and scheduling

The model covers the execution of a series of foraging bouts (foraging trip starting and ending at the colony nest) by all the bees. During a foraging bout, the model keeps running as long as one bee is foraging. The number of foraging bouts performed by the bees is set as a parameter of the simulation. Each foraging bee chooses its next destination, using the *ChooseDestination* submodel, and updates its position to this new destination. This action is executed in the order of the bees' *ID* value. If a bee chooses the nest as next destination, its bout is over. Before feeding, the bees resolve any competition occurrences (if more than one bee is on a single flower), in the order of the flower's *ID* on which the competition occurs. A weighed sampling of the *winProbabilities* decides which individual wins the interaction (default values are uniform for all bees). Bees that either were alone on a flower or won an interaction feed on the flowers, using the *Feeding* submodel. All foraging bees update their movement probability matrix according to their recent experience (details in the *Learning* submodel). Finally, each foraging bee checks if it has reached one of the conditions to return to the nest (crop full or maximum distance of travel), and if so, finishes its foraging bout.

4 - Design concepts

a - Basic principles

Animals are expected to distribute themselves among different resources to optimise the energetic intake, following what is commonly called the Ideal Free Distribution theory (Fretwell, 1969). The environment in which bees forage present multiple constraints. Flowers provide a renewable resource in very low amounts and have a fast turnover changing the distribution of resources. Competition with other bees is strong but also very dynamic as new foragers arrive daily while older ones die.

Bees tend to optimise their foraging activity (Lihoreau et al., 2016; Lihoreau et al., 2012; Ohashi et al., 2007) by traplining (developing stable routes between feeding sites) and

partitioning resources (exploiting feeding sites in different areas of the environment to minimise competition). The aim of the model is to explore the conditions under which traplining emerges from the behaviour of independent bees interacting with the resources and other agents according to the principles of the Ideal Free Distribution. The models reuse parts of a previously published model (Lihoreau et al., 2012; Reynolds et al., 2013) which has been to our knowledge the only model trying to represent the ontogeny of the traplining behaviour to this date.

b - Emergence

We focused on how both optimisation strategies (traplining and partitioning) can emerge from simple rules of positive and negative reinforcements derived from the foraging experience of individual bees. We explored how these outcomes change when we alter the spatial distribution of the flowers, or remove either type of reinforcement. When bees partition resources, the partitioning index Q_{norm} (Pasquaretta & Jeanson, 2018) reaches a maximum value of 1. When bees follow traplines, the cumulated foraging efficiency and route similarity index of the bees approaches a maximum value of 1.

Resource partitioning and traplining are emergent and vary depending on the parameters of the simulation. While traplining could be achieved with a positive reinforcement only, resource partitioning required both positive and negative reinforcements to emerge.

c - Adaptation

Bees follow a heuristic in the form of a matrix of movement probability to choose their next destination. This matrix changes with experience to favour the flowers where the bees had a positive experience (collection of nectar). Bees thus learn to fill their nectar crop to capacity. More information on the probability matrix is given in the *ChooseDestination* submodel.

d - Objectives

The bees have a unique goal: finding a set amount of nectar to fill their crop (whose capacity is set by the *maxCrop* parameter). In all the conditions explored, this crop capacity was set to 5 resources (units of nectar volume): an arbitrary value used to fit the experimental data of Lihoreau et al. (Lihoreau et al., 2012). The decision-making process is altered by this crop filling (described by the *crop* variable) if it reaches the same value as *maxCrop*. If so, the foraging bout is over, and the bee returns to the nest. This behaviour has been observed in experimental conditions (Woodgate et al., 2017; Lihoreau et al., 2012).

e - Learning

The learning process occurs as changes made to the probability matrix during the simulation through two components: a positive and a negative reinforcement. Positive reinforcement is triggered when a bee finds a resource on a flower. When it happens, the bee increases the probability of reusing the transition it just used. Values of the positive reinforcement factor are typically set to be superior or equal to 1. Negative reinforcement is similar but reduces the probability of reuse of the transition. Negative reinforcement occurs when there is either no resource on the flower or if the bee has been evicted from the flower by a competitor. Values of the negative reinforcement factor are typically set to be less than or equal to 1.

f - Prediction

In the models the movement probability matrix acts as a prediction tool for the bees. After some experience in the environment, these probabilities become proxies for the probability of finding nectar in flowers, as the positive and negative experiences shape the matrix. The choice of visiting a flower is done prior to the knowledge of presence of a reward on the flower, and only relies on the previous experience on this flower. More information on the movement probability matrix is given in the *ChooseDestination* submodel.

g - Sensing

When the model is initialised, the movement probability matrix is based on the distances the bees have to travel between each flower. However, the model does not depict a probability of “not finding any flower”. In experimental situations, it is not rare that the bees would not find all the flowers during their first bout (Woodgate et al., 2017; Lihoreau et al., 2012). However, in our models it is assumed that the bee always finds a flower, even if it has never visited it. Moreover, it is assumed that the bee knows how to link all the flowers together (knowledge of all the existing transitions) even if these links have never been performed. The use of distance-weighted probabilities is a good approximation of the probabilities obtained by a random walk. As bees always keep tracks of their successive movements after leaving the nest, they can sum these movements to know the direction and approximate distance of the nest.

h - Interactions

Two types of interactions were included: exploitation competition and interference competition. Exploitation competition occurs when a bee visits a flower that has already been depleted by a competitor or by itself during a prior visit. Interference competition occurs when two or more bees are simultaneously present on the same flower. When this direct interaction happens, only one bee can access the nectar reward. The winning bee is selected using uniform probabilities.

i - Stochasticity

Stochasticity is included in three parts of the model: to place all the flowers in the environment, to choose a bee’s next destination, and to choose a winner from interference competition. When generating a new environment, the submodel *CreateEnvironment* sets rules for placing the flowers. The algorithm will repeatedly try to place a flower at a random position until it fits all the conditions.

The choice of the bee's next destination (described in the *ChooseDestination* submodel) is done by consulting the movement probability matrix of the agent. As this choice relies on probabilities, we used a stochastic process to choose the next destination, according to the weights of the different destinations' probabilities. The reason behind this choice is explained in the *Learning* and *Sensing* part of this ODD. Finally, the winner of an interference interaction is decided by choosing with uniform probabilities which bee wins.

j - Collectives

The model includes no collectives.

k - Observation

There are two observations collected from the bees: visitation sequences and occurrences of competition.

Every time a bee visits a flower during a foraging bout, the flower's *ID* is reported into a vector containing all the visits in order during the bout. Every time a bee finds an empty flower or finds a competitor on the same flower, these occurrences are reported throughout the foraging bouts. This information gives the number of competitive interactions throughout the bouts. We also save the spatial positions of the flowers to compute the distances between them and find the theoretically optimal routes for the agents. This allows to compute the route qualities of the agents' successive bouts.

5 - Initialisation

The first part of the initialisation of the models is to create the environment. This part, described in details in the *CreateEnvironment* submodel, places all the flowers and the nest. The environment can be initialised in two ways: either by calling for an existing one (all the environments are stocked in a folder called *Arrays*, inside the main folder where the R script is), or by using the models to generate one.

The variables of the bees that will be dynamic during the simulation are then initialised: *crop*, the number of resources gathered during the ongoing foraging bout; *distanceTravelled* the distance travelled by the bee during this bout; and then *boutFinished*, a Boolean indicating if the bee has finished its bout or not. The movement probability matrix is then initialised for each bee. Finally, we initialise the output objects to store the visitation sequences and resources gathered by each bee during each bout, and store all the information we inputted as parameters to be able to identify what were the parameters used for this simulation later.

6 - Input data

The model has no input data.

7 - Submodels

Our models can be decomposed in multiple different submodels, which are described here.

a - Input Parameters

The model contains an R script dedicated to the input parameters. While all parameters have comments to help the user, a description of the important parameters is given here.

environmentType: Can either contain “generate” or the name of an existing and valid environment found in the *Arrays* folder (the *Arrays* folder will only be created after a first simulation, unless it is created manually beforehand). If using an existing environment, the parameters in part 3.1. will be ignored.

numberOfResources: Total number of flowers in the environment.

numberOfPatches: In how many patches these flowers should be distributed.

patchinessIndex: Deprecated index, should be kept at 1. If multiple patches are created, a value of 1 will ensure the patches are set very distinctly from each other. Values closer to 0 will create a less defined limit between patches. Inputs between 0 and 1 are accepted.

envSize: The size of the environment. It defines a square environment in which the flowers will be placed. However, if it is impossible to place all the flowers, this value will be incrementally increased to give enough space.

flowerPerPatch: Should contain as many values as the number of patches. If only one patch, it must be set to NULL. Otherwise, it must take a succession of values following the syntax $c(a, b, c, \dots)$, a , b and c being integer values whose sum equals to *numberOfResources*.

numberOfArrays: The number of different environments the model should generate using these parameters.

reuseGeneratedArrays: Can take TRUE or FALSE. If TRUE, the model will look into the *Arrays* folder for environments fitting all the parameters. If it does find similar ones, it will use them instead of generating new ones.

numberOfBees: Number of bee agents in the model.

numberOfSimulations: How many simulations the model will do for each set of parameters.

numberOfBouts: How many foraging bouts each bee will do during a simulation.

distFactor: Weight given to distance when generating the movement probability matrix. The probability for a transition movement of distance d is computed as probability = $1/d^{distFactor}$. Changing this number will change the initial probability matrix.

param.useRouteCompare: deprecated, used to switch between our *Learning* submodel and the route comparison model of Reynolds et al. (Reynolds et al., 2013). Should be left at FALSE.

param.learningFactor: the value used for the positive reinforcement process. Values should be greater than or equal to 1. Requires at least one value. If multiple values are inputted, the model will run the simulations for each value.

param.abandonFactor: The value used for the negative reinforcement process. Values should be between 0 and 1.

maximumBoutDistance: Maximum distance a bee can travel during a foraging bout.

In the “Advanced parameters” category different rules can be enforced on the bees. In the following we detail the ones used in our simulations:

allowNestReturn: Allows the bee to select the nest as its next destination in the *ChooseDestination* submodel, based on the distance-weighted probabilities. If the bee does so, the foraging bout is finished.

forbidReverseVector: This rule forbids the bees to use the reverse movement transition from the one they just used. If the bee has just moved from flower 2 to flower 3, for its next movement this bee will not be given the choice to go from flower 3 to flower 2. This interdiction only applies for the last transition executed.

onlineReinforcement: This rule forces the trigger of the *Learning* submodel after each encounter of a flower, instead of only triggering it when the bee had finished its bout. Movement probabilities are thus altered directly after the execution of a movement transition.

b - CreateEnvironment

The creation of an environment happens first in the initialisation of the model. The code relating to this process is found in the Functions script, in a function of the same name. If the user chooses an *environmentType* different than “generate” the model will import the user’s selected environment. The creation of the environment using the “generate” option calls an algorithm we designed to create flower patches. It follows arbitrary rules without any ground in experimental data or theoretical background. In this function, all the parameters inputted in the 3.1.1 part of the Parameters script are being used. Refer to their description in the Input Parameters submodel for their meaning. The basic distance unit between entities is set by an internal parameter, *perceptionRange*, whose default value is 10.

The nest is set first at the centre of the environment (coordinates (0,0)). The different patch centres are placed between $2 * \textit{perceptionRange}$ and *envSize* from the nest. Every time a patch is placed, the algorithm checks if this patch centre is at least $16 * \textit{perceptionRange}$ away from any other patch centre. The algorithm reiterates this process until the condition is verified. The patch centres act as the first flower of each patch.

Flowers are then placed around the patch centres, respecting the distribution specified in *flowerPerPatch*. All flowers are tentatively placed between $2 * \textit{perceptionRange}$ and $4 * \textit{perceptionRange}$ of the patch centre, and must verify the condition that each flower has to be at least $2 * \textit{perceptionRange}$ from any other flower. The algorithm reiterates this process until the condition is verified. If the algorithm fails to place a flower 200 times in a row, the range at which the flowers can be placed around the patch centre becomes between $2 * \textit{perceptionRange}$ and $4 * \textit{perceptionRange} + (\textit{envSize}/20)$.

Once all the flowers are placed in all the patches, a table containing the flowers' *ID*, coordinates *x* and *y*, and the patch they belong to (numbered from 1 to *numberOfPatches*) is created. The nest is also represented in this table, and takes the *ID* 0, and is part of its own patch.

c - ChooseDestination

In order to choose among the possible destinations, the bees refer to a movement probability matrix they are given at the beginning of the simulation. This matrix has *n* rows and columns, *n* being the number of entities (flowers and nest). It is created by extracting in a similar matrix the distance between each entity, and from then applying the following formula for each cell:

$$(1) \quad P(i \rightarrow j) = \frac{\frac{1}{d_{ij}^n}}{\sum_j \frac{1}{d_{ij}^n}}$$

Where d_{ij} is the distance between locations *i* and *j*, and *n* is an integer parameter, whose default value is 2. See the Input Parameters submodel for more information about *distFactor*, which sets the value of *n* in this equation. The probability to go from a flower to itself (immediate revisit) was set to 0. Visiting the same flower twice in a row happens when bees come back to the last departed flower if their search for another flower was unsuccessful, or if they do short orientation flights on the flower. However, these revisits have little importance for the establishment of a stable route (Lihoreau et al., 2010), and were thus ignored. All rows of the probability matrix are normalised so that their sum is equal to 1. To choose a bee's next destination, it looks at the matrix's row matching the flower ID of its current position. As the use of a "reverse transition" is forbidden (see the *forbidReverseVector* parameter described in the Input Parameters submodel), the bee's

previous position is removed from the possible destinations. This prevents an artificial situation the model could create when two flowers are very close to each other, and the probability to move between them is much higher than any other probability. Without this rule, bees would often get stuck navigating back and forth between both flowers. If the *allowNestReturn* is used, the nest is kept in the possible destinations. Otherwise, it is removed. A weighed sample is made between all the remaining potential destinations to choose the one that the bee will use.

d - Feeding

This submodel takes care of all matters that happen when a bee lands on a flower, i.e. all competition occurrences and the collection of resources. If two or more bees choose the same destination on the same step, one of them is chosen randomly to access to the resource. The losing bees will still depart from this flower for the next step, but will not feed.

e - Learning

As bees finish to go through the *Feeding* module, they have five possible outcomes: (i) the bee has landed alone on a flower, and found resources; (ii) the bee has landed alone on a flower, and did not find any resource; (iii) the bee has landed with competitors on a flower, and has lost the competition; (iv) the bee has landed with competitors on a flower, has won the competition, but found no resource; (v) the bee has landed with competitors on a flower, has won the competition, and found resources. These outcomes can be placed into two categories: the positive (i and v) or negative (ii, iii and iv) outcomes.

Each bee has a square matrix named *indFlowerOutcome*, with n rows and columns, n being the combined number of flowers and nest. Every bout it is initialised with 0s in all cells, and then altered every time a transition is used during the bout. If the outcome of the transition performed by the bee is positive (rewarding), the cell corresponding to this transition in the *indFlowerOutcome* matrix takes a value of 1. Similarly, if the outcome in

negative (non-rewarding), it takes the value -1. Only the first use of a transition during a bout will alter this matrix. This matrix is then used as a reference to change the movement probability matrix as it contains a trace of all the transitions that receive a change.

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S2 Text. Sensitivity analysis of positive and negative reinforcements.

We ran a sensitivity analysis for the two main parameters: the positive and negative reinforcements. As we had no *a priori* understanding of how the model behaved with different values of reinforcements, we ran simulations on ranges of positive (1, 1.2, 1.4, 1.6, 1.8, 2) and negative (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1) reinforcements for a total of 66 sets of parameters, for each environment type (one, two and three patches; S2 Fig). We simulated 10 environments for each environment type and computed 100 simulations of 50 foraging bouts per iteration (i.e. 1000 simulations per environment type and set of parameters).

Since our study focused on resource partitioning, we extracted the Q_{norm} index values for these simulations and compared them. We plotted a heatmap showing the value of the mean Q_{norm} index at the last foraging bout for each set of parameters and environment type (Fig A). In all types of environments, positive reinforcement had a strong effect on the final Q_{norm} index value. High values of resource partitioning were obtained for positive reinforcement values > 1.5 . By contrast, negative reinforcement only had an impact in environments with two or three patches. High values of resource partitioning were obtained for negative reinforcement values larger than 0.75.

For each pair of bees, we also looked at how this same index evolved over successive foraging bouts. Fig B shows the dynamics of mean Q_{norm} index across 50 foraging bouts for each combination of positive reinforcement (1.0, 1.2, 1.4, 1.6, 1.8, 2.0) and negative reinforcement (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1) parameters, and each environment type (one patch, two patches, three patches). Higher values of both positive and negative reinforcements most often lead to faster resource partitioning (with

some uncertainty due to the probabilistic nature of the model). Combinations of values in which the negative reinforcement factor was missing (violet gradient curves) led to a decrease in partitioning.

Finally, we also looked at how the Similarity Index was affected by these ranges of parameters. We drew a similar heatmap showing the Similarity Index at the last foraging bout for each parameter set and environment type (Fig C). It appeared that positive reinforcement had a strong impact on route similarity in all environment types, while negative reinforcement only seemed to have a small effect only on the two and three patches environments.

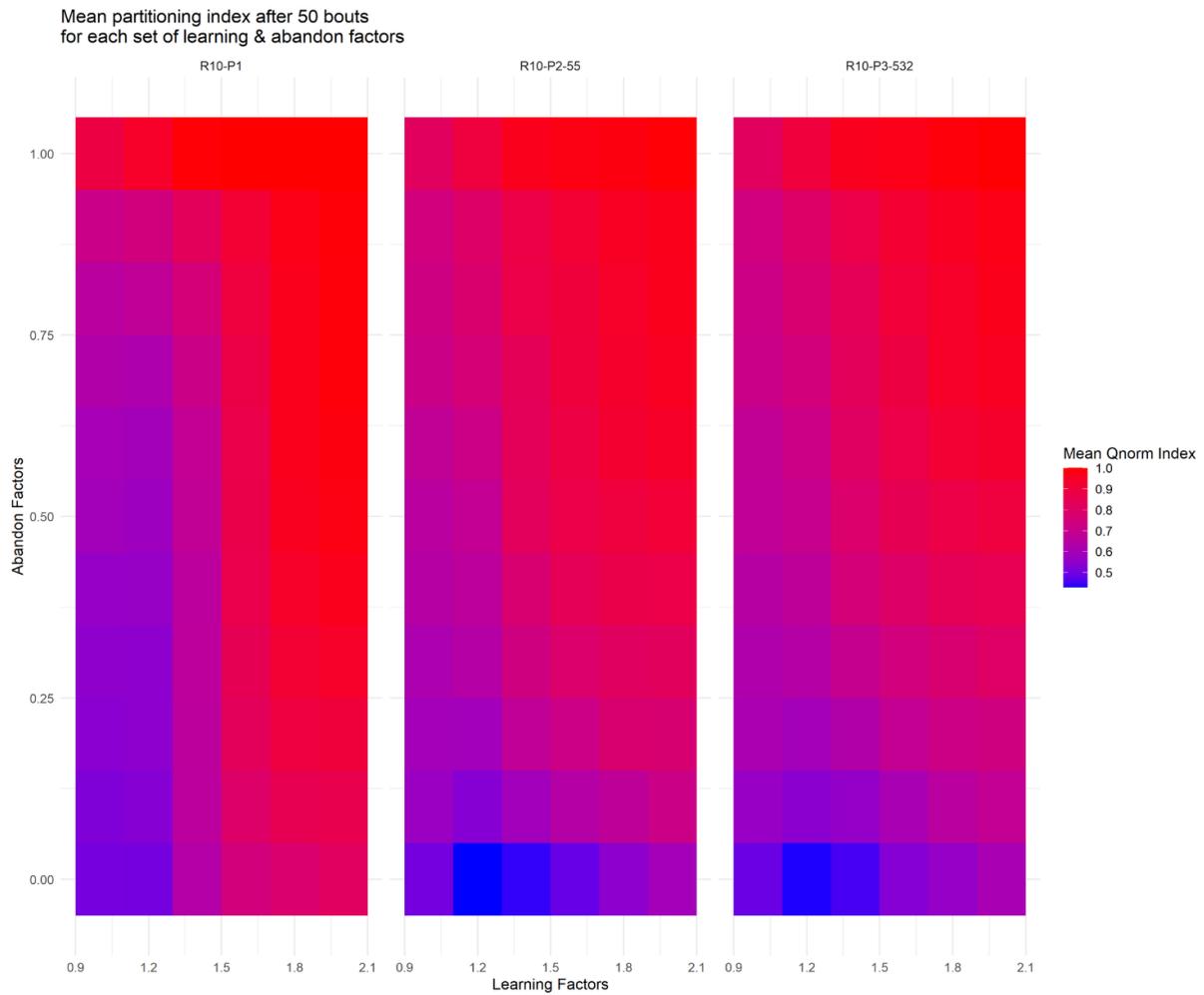


Fig A. Heatmap graph of sensitivity analysis of partitioning index to parameters.

Heatmap showing the mean Q_{norm} Index value after 50 foraging bouts (mean over 1000 simulations on 10 arrays of the same environment type), for each combination of positive reinforcement (1.0, 1.2, 1.4, 1.6, 1.8, 2.0) and negative reinforcement (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1) parameters, and for each environment type (one patch, two patches, three patches). For simplicity, we inverted the values of negative reinforcement. 0 indicate models without negative reinforcement.

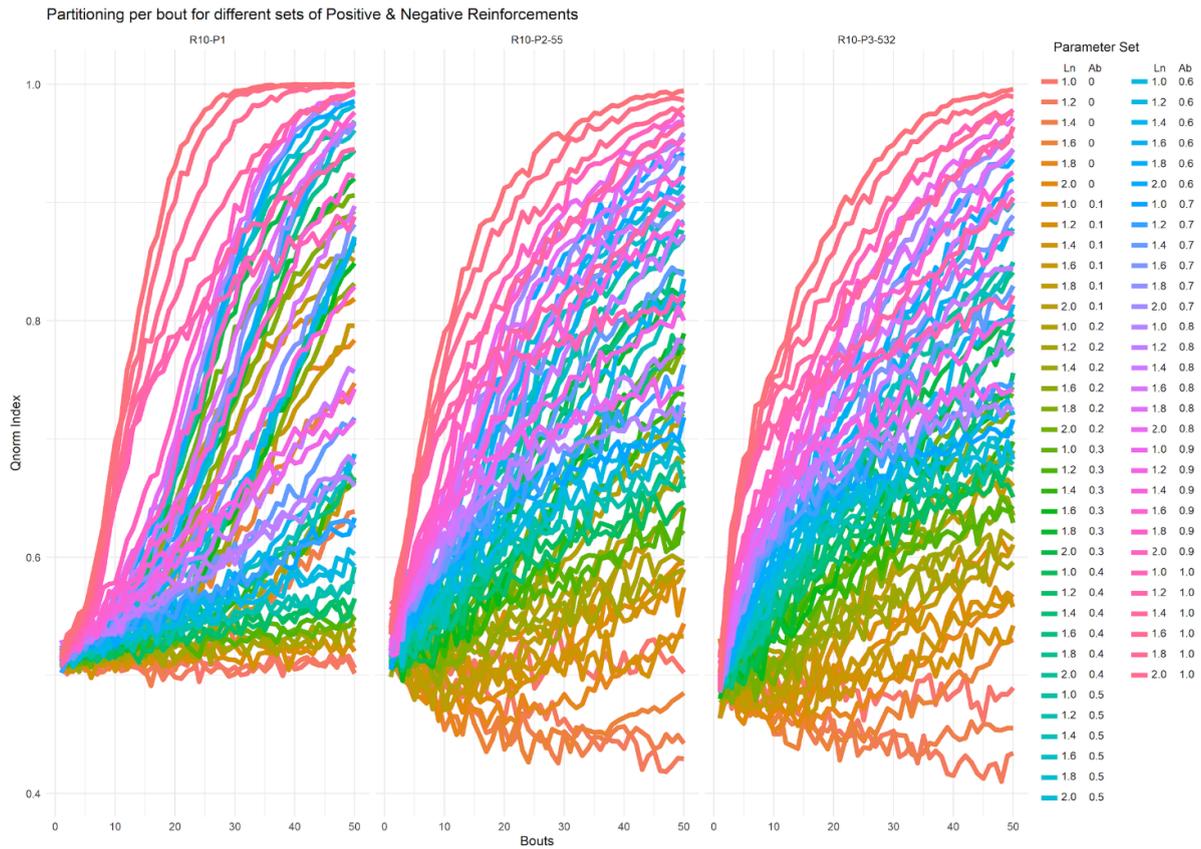


Fig B. Partitioning dynamic across the ranges of reinforcement factors. Dynamic of the mean Q_{norm} Index across foraging bouts for each combination of positive (1.0, 1.2, 1.4, 1.6, 1.8, 2.0) and negative (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1) reinforcement factors and for each environment type (one patch, two patches, three patches). For simplicity, we inverted the values of negative reinforcement here. 0 being models without negative reinforcement.

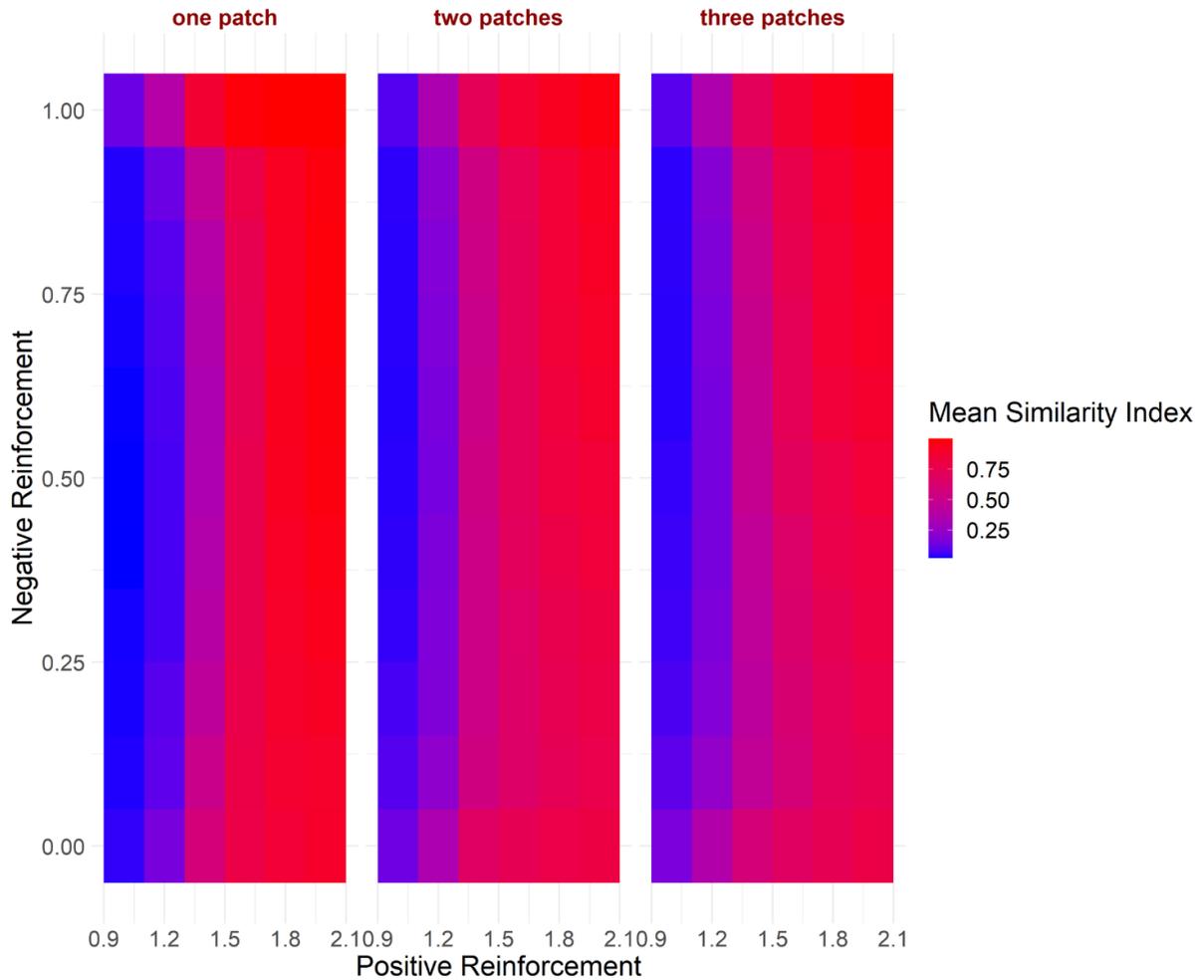


Fig C. Heatmap showing the mean Similarity Index value after 50 foraging bouts (mean over 1000 simulations on 10 arrays of the same environment type), for each combination of positive reinforcement (1.0, 1.2, 1.4, 1.6, 1.8, 2.0) and negative reinforcement (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1) parameters, and for each environment type (one patch, two patches, three patches). For simplicity, we inverted the values of negative reinforcement. 0 indicate models without negative reinforcement.

S3 Text. Qualitative comparison between simulations and observations in the narrow pentagon.

We ran simulations with one forager to compare model outcomes to observational data using a second reference field study (Woodgate et al., 2017). In this study, the authors used five artificial flowers arranged in a narrow pentagon (S1B Fig). Four bumblebees were tested during 27 to 61 foraging bouts each (flower visitation sequences were kindly provided by Joe Woodgate). One of these bumblebees was tested over different days and was therefore removed from the analyses (as bees' experience memory drops overnight; Lihoreau et al., 2010). In these conditions, none of the bumblebees developed a stable trapline, although all significantly increased their foraging efficiency with time (e.g. reduced travel distance and duration, increased similarity between two consecutive flower visitation sequences).

The sole implementation of positive reinforcement in Model 1[+] was sufficient to replicate the observations. While the use of the negative reinforcement alone in Model 2[-] showed drastically different results, its addition with the positive reinforcement in Model 3[+/-] had no major effect on route quality nor on route similarity trends (Fig A). Overall, simulations of Models 1[+] and 3[+/-] showed good qualitative fit to the traplining behaviour observed in real bees – i.e. there is a trend of increasing route similarities across foraging bouts. Note however that the models tend to overestimate the bee ability to develop stable routes. This imperfect match could be due to the low amount of available experimental data in the original study (three individuals in Woodgate et al., 2017). Alternately, the model has been shown to overestimate the increase of the similarity index and underestimate the initial similarity of the first few bouts (See Discussion).

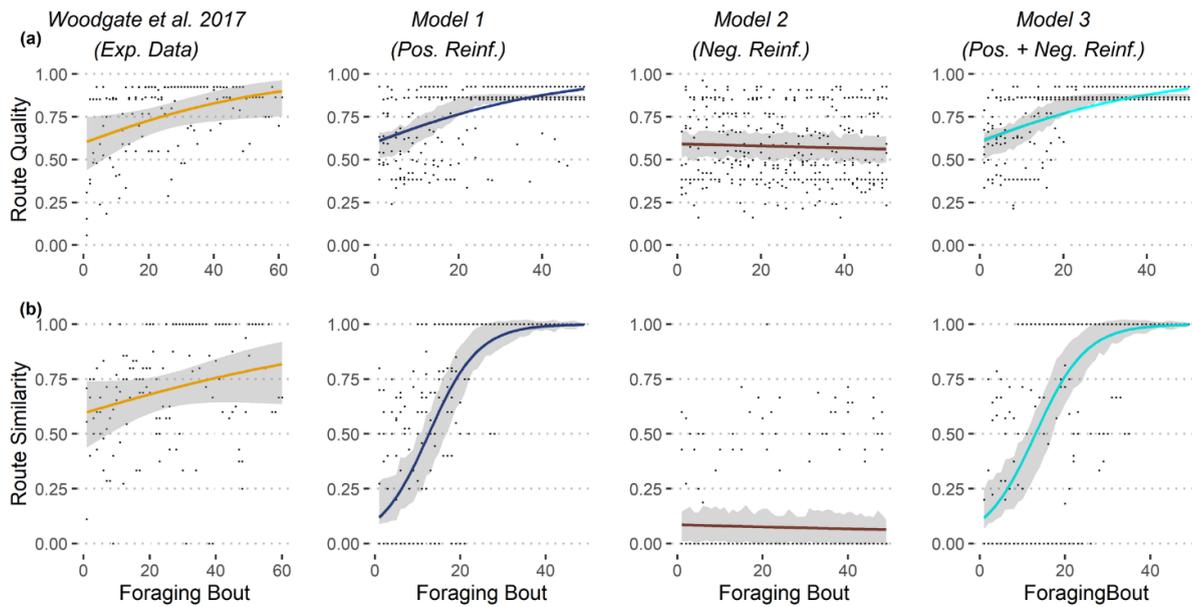


Fig A. Qualitative comparisons of route qualities (A) and similarities (B) between simulations and experimental data (narrow pentagon of Woodgate et al., 2017) for one forager (see details of the models in Fig 1). For each dataset, we show the estimated average trends across foraging bouts (colored curves), along with the standard error (gray areas). For the sake of eye comparison, in the simulation plots the standard error of the mean is computed from a sample of 3 simulations ($n = 3$ bees in our sample taken from Woodgate et al., 2017). Average trends were estimated over 500 simulation runs, using GLMM Binomial model with bee identity as random effect (bee identity nested in simulation identity for simulated data).

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S4 Text. Predictions with more than two bees.

We explored the emergence of resource partitioning in groups of 5 bees, and how this varies in environments containing 20, 25, 30, 40, 50, 70 and 100 flowers, thus encompassing a gradient of competition pressures from conditions where there are not enough flowers for all bees (20) to conditions where there are four times more flowers than necessary for all bees (100). For simplicity, flowers were evenly distributed (i.e. environment with one patch). The model used for these simulations is Model 3[+/-]. For each flower density, we generated 10 environments, and ran 100 simulations of 100 foraging bouts, for a total of 1000 simulations per density value. We computed the resource partitioning index (Q_{norm}) at each foraging bout.

The mean final Q_{norm} was higher in environments with most flowers (Fig A). Plotting the mean final partitioning index (final foraging bout) as a function of the number of available flowers confirmed that bees converge to a plateau when increasing the number of flowers up until around 50 flowers (Fig A). As the number of flowers increases, positive reinforcement became more prevalent in driving partitioning, while negative reinforcement became less relevant. This result simply reflects how unlikely it becomes to come across competition as the resources become more available.

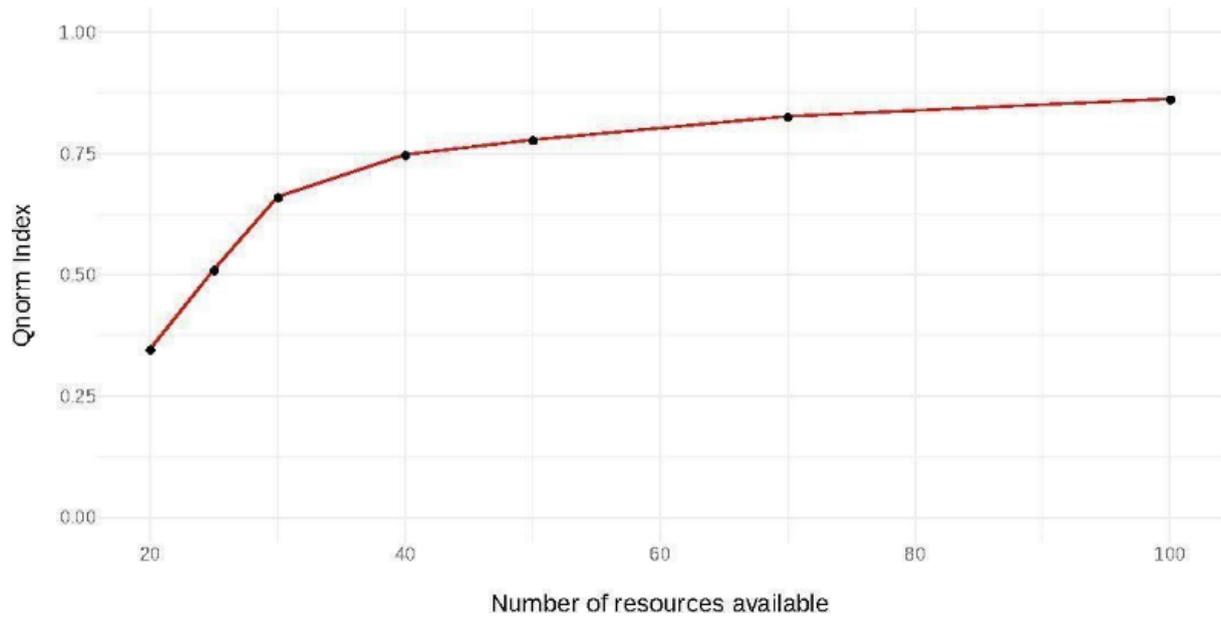


Fig A. Evaluation of the mean final Q_{norm} index (after 100 foraging bouts) as a function of increase resources availability. The model run has the positive reinforcement factor set at 1.5, and the negative reinforcement factor set at 0.75 (Model 3[+|-]) with five bees foraging in environments of one patch.

S5 Text. Supplementary information on the similarity index.

We used a route similarity index between two consecutive visitation sequences in order to assess how similar they were. We designed an index that would account for both the similarity of transitions used, but also the number of transitions used. For the computation of this index, visitation sequences are decomposed into smaller sequences, whose length is set by a parameter of the function. In our study, we set this value arbitrarily to 3. These smaller sequences are made by sliding a window of the specified length through the visitation sequence, moving the window 1 visit further each time (see example below). The compared sequences, a and b , are thus decomposed into small sequences of the defined length after excluding the next position. The number of similar small sequences used in both sequences are stored in an object called s_{ab} . All uses of these common small sequences in the sequences a and b are then highlighted, and all flower visits highlighted as such are counted, and stored in an object called s_{ab} . The longest sequence between a and b has its number of visits stored in a second object called l_{ab} . The similarity index is then calculated using the formula:

$$SI_{ab} = \frac{s_{ab}}{2l_{ab}}$$

Which represents the number of visits part of common small sequences (s_{ab}) divided by the total number of visits in both sequences ($2l_{ab}$). This multiplication by 2 in the denominator allows for accounting in length differences between the two compared sequences.

Example:

We retrieved two visitation sequences a and b from successive bouts:

Sequence a : N 5 3 4 N

Sequence b : N 5 3 4 2 5 3 4 N

First, the visits to the nest are removed, giving the following sequences:

Sequence *a*: 5 3 4

Sequence *b*: 5 3 4 2 5 3 4

Then, the small sequences used in both sequences are identified:

Small sequences	Used in seq. <i>a</i> ?	Used in seq. <i>b</i> ?
5 → 3 → 4	Yes	Yes
3 → 4 → 2	No	Yes
4 → 2 → 5	No	Yes
2 → 5 → 3	No	Yes

The two sequences show a common small sequence: 5-3-4. The uses of this triplet in the sequences *a* and *b* is highlighted (here in bold):

Sequence *a*: **5 3 4**

Sequence *b*: **5 3 4** 2 **5 3 4**

In this case, 9 total visits are part of repeated sequences. The longest sequence, *b*, has a length of 7 visits. Thus, the computation of our index is:

$$SI_{ab} = \frac{s_{ab}}{2l_{ab}} = \frac{9}{2*7} = 0.643$$

S6 Text. Details on the movement probability matrix.

In our model, the agent bees rely on a movement probability matrix to navigate from one flower to the other. This design was kept from the initial model developed in Reynolds et al. (Reynolds et al., 2013). To obtain such a matrix, the coordinates of all flowers and nest are used to compute the distance between each pair of entities. From there, the probability P to go from a flower i to a flower j is determined by the following formula:

$$P(i \rightarrow j) = \frac{\frac{1}{d_{ij}^n}}{\sum_j \frac{1}{d_{ij}^n}}$$

where d_{ij} is the distance between the flower i and j , and n an exponent arbitrarily inserted to change the way distance would affect probabilities. This design was chosen because it approximated closely the probabilities to find the different flowers when using a simple random walk.

To present their similarities, we compared the probabilities obtained by both our probability matrix and a lattice-based random walk. We used the regular pentagon used for Lihoreau et al. (Lihoreau et al., 2012) as an example for this comparison, with all flower positions rounded to the nearest integer for simplicity. We simulated 10000 bees leaving the nest, with the goal to find any flower in 5000 steps. In each step, an agent had 4 choices, to go up or down on either the x or y abscisses, with the same probability. The simulation stopped if the bee got at a distance of 5 metres or less than a flower, thus mimicking a perception range. The results of this comparison are presented in Fig A.

The results in Fig A show that as the exponent n increases, the probabilities to do the shortest movements (towards flowers 1 and 5) increases while those of longer movements (towards flowers 2, 3 and 4) decreases. In this specific environment, an exponent of 3 would replicate most accurately the initial probabilities of encountering each flower using a random

walk. In our study, however, we did set the value of this exponent to 2 throughout all simulations, as it was the value used in the previous paper using this method (Reynolds et al., 2013).

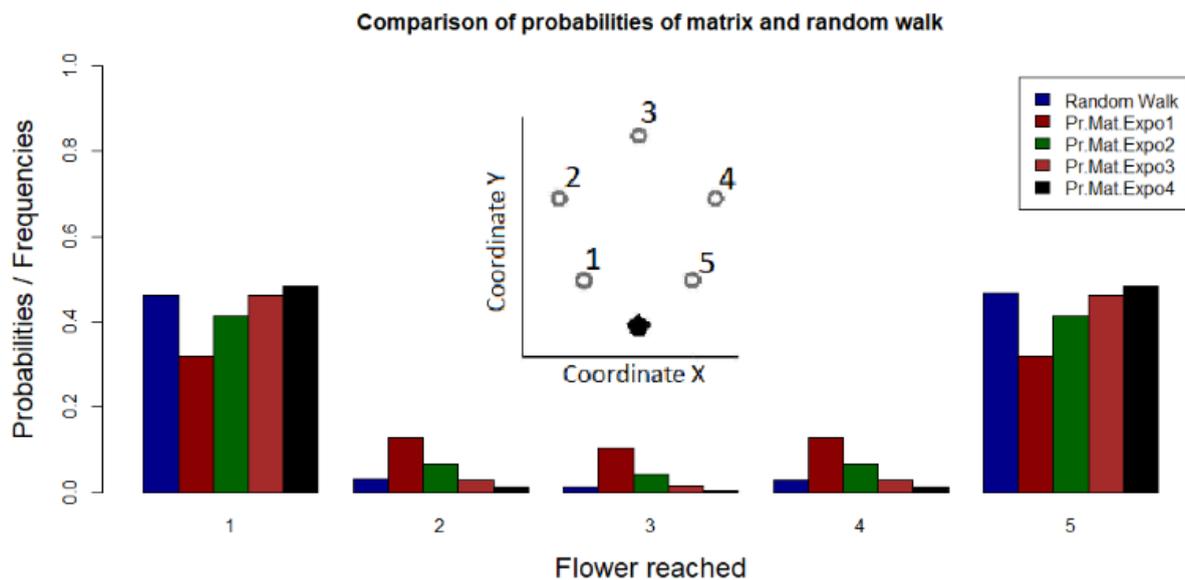


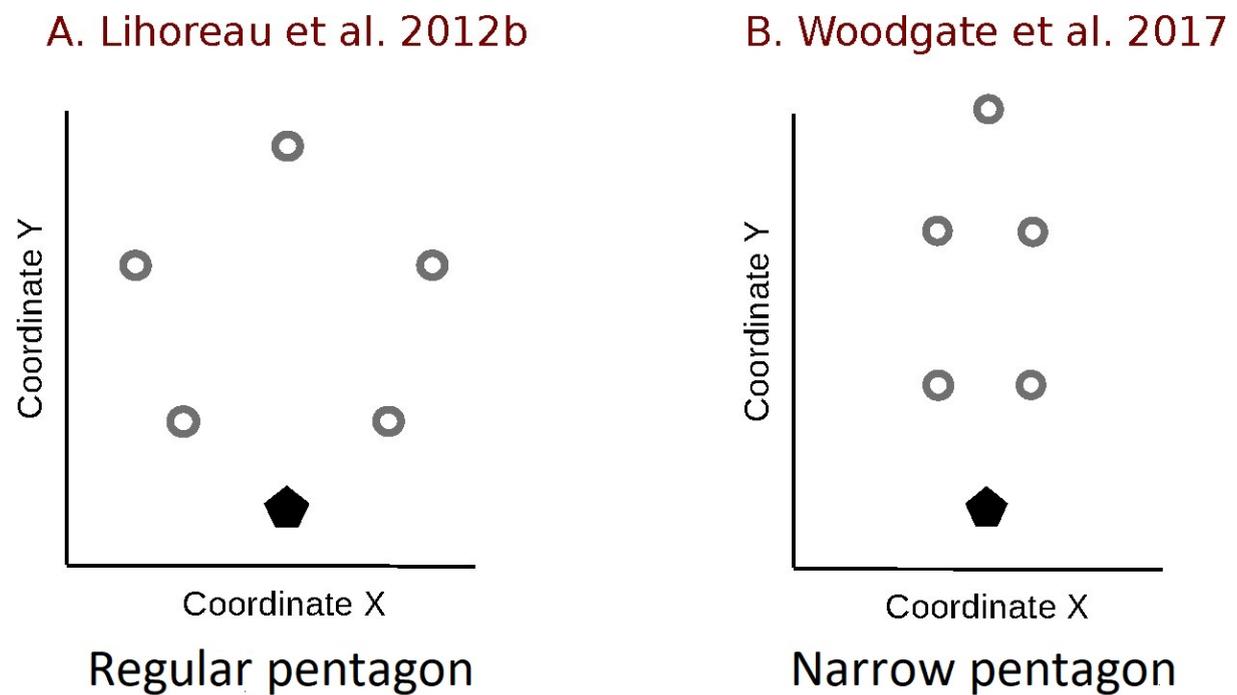
Fig A. Comparison of probabilities of reaching any flower (numbered circles) from the nest (black pentagon) using either a random walk or the probability matrix used in the models. For the probability matrix, 4 distinct parameter values were tested for the exponent found in the equation used to compute the transition probabilities.

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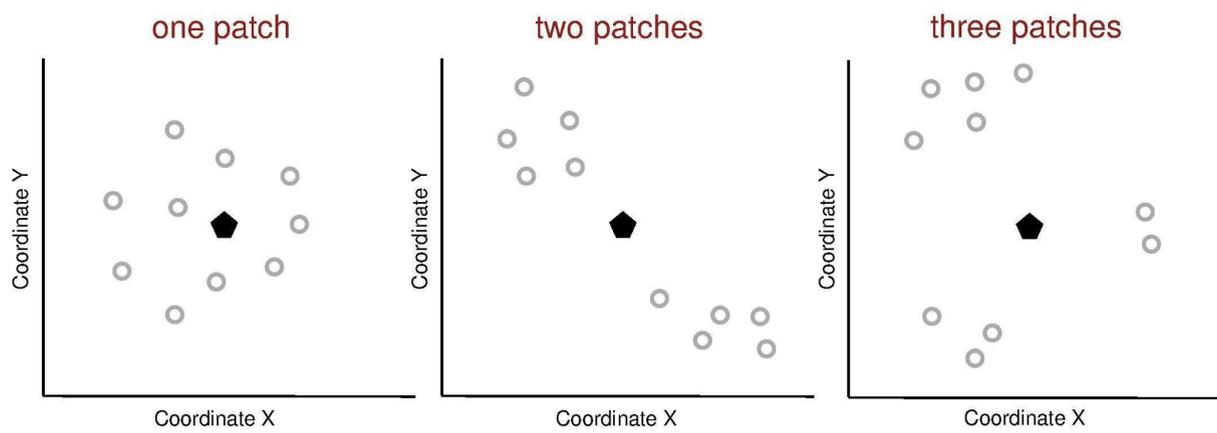
S1 Fig. Experimental Flower Arrays.

Arrays of artificial flowers (grey circles) and the colony nest (black pentagons) used to obtain the experimental datasets. A. Regular pentagon, modified from Lihoreau *et al.* (Lihoreau *et al.*, 2012b). B. Narrow pentagon, modified from Woodgate *et al.* (Woodgate *et al.*, 2017).



S2 Fig. Simulated Flower Arrays.

Examples of simulated environments. Spatial distribution of 10 flowers (grey circles) and a colony nest (black pentagon) in three types of environments defined by different levels of flower patchiness. A flower patch was characterised by: 1) a uniform distribution of flowers, 2) a lower distance between flowers within the patch than between all flowers from different patches (see details in methods).



S1 Video. Animation of a model simulation with 5 bees.

Example of simulation of five bees foraging in an environment with one patch of 50 flowers.

Both positive and negative reinforcement rules are implemented (Model 3[+|-]). Bees

performed 100 foraging bouts. (doi: doi.org/10.1371/journal.pcbi.1009260.s009)

Chapter IV.

**Partial partitioning of foraging resources by honey
bees at small spatial scale**

Abstract

Honey bees tend to optimise their foraging behaviour through the use of stable, repeated routes called traplines. But these traplines are seldom seen when bees forage at a small spatial scale, and little is known about how bees adapt their foraging strategies in competitive situations with nestmates. Competition is omnipresent in the foraging activity of bees. As such, if bees use strategies to improve their efficiency, it is reasonable to assume that they are not dissociable from the competition pressure bees face. Thus, in order to understand the foraging behaviour of bees, we must look at how they forage in competitive situations. We trained groups of 2 bees to forage in an arena on 6 artificial flowers, and recorded their visitation sequences throughout a period of observation of 3 hours. We analysed these visitation sequences to understand how bees foraged in this specific situation. Our results suggest that bees did not use traplines in this situation, but showed signs of partial partitioning, as each bee of the same pair often spent different amounts of time on different flowers in the arena, suggesting a reaction to the presence of a competitor. These observations support the idea that bees do not use traplines at small spatial scales, as well as providing new insights on the small scale foraging behaviour of bees.

1 - Introduction

The literature on foraging behaviours and partitioning of resources between animals is extensive, and how animals resolve foraging competition has received a lot of attention (e.g. African grazers, Kleynhaus et al., 2011; squirrels, Wauters et al., 2002; bats, Emrich et al., 2014; Goldshtein et al., 2020; bumblebees, Morse, 1977; Inouye, 1978; seabirds, Kappes et al., 2011). This subject has also been considered theoretically in terms of how competition can influence home ranges (Börger et al., 2008; Riotte-Lambert et al., 2015). However, these studies have focused on competitive exclusion between species (e.g. honey bees stopping visits to a patch foraged on by bumblebees, Balfour et al., 2015), and their consequent specialisation on different resources (e.g. sympatric species of boobies foraging on different prey, Kappes et al., 2011). Intraspecific competition, or even competition between genetically related individuals has received far less attention.

Bees are interesting models for the study of foraging strategies, since they easily learn to forage in artificial situations and they are numerous, allowing us to easily observe and quantify foraging behaviour (Inouye, 1978). Studies on the foraging strategies of bees in field conditions suggest they can establish stable repeated routes between sets of flowers (i.e. “traplines”; Ohashi & Thomson, 2009; Lihoreau et al., 2012b; Woodgate et al., 2017), and exclusive foraging ranges through competitive interactions (i.e. resource partitioning; Morse, 1977; Inouye, 1978; Nagamitsu & Inouye, 1997; Lihoreau et al., 2016; Pasquaretta et al., 2019). We still know very little about the decision-making processes behind these strategies. In Chapter II, we suggested that the foraging strategies of bees could emerge from spatial and temporal constraints of their environment and behaviour, with little to no cognitive load involved. In this chapter, we are interested in how the spatial scale of the foraging affects the foraging strategies of bees. A recent study suggested that bees were more likely to establish complete traplines when foraging at a large scale (Reynolds et al., 2013), a result coherent with the literature on traplining as bees in general establish traplines

more consistently and faster (lower number of foraging bouts needed) at larger spatial scales (Lihoreau et al., 2012b; Buatois & Lihoreau, 2016) than at lower scale (Ohashi et al., 2007; Lihoreau et al., 2012a). This is consistent with our proposal in this thesis; we hypothesised that learning routes between different resources was unlikely to happen at lower spatial scales as the cognitive load of finding the shortest path would be an inefficient use of the limited memorization capacity of bees (Ardin et al., 2016), as they can be brought to visit hundreds of flowers throughout multiple patches.

To address our poor understanding of how bee foraging strategies develop we previously developed an agent-based model to explore a hypothesis that resource partitioning can emerge as a consequence of basic rules of positive and negative reinforcement associated with the rewards (or lack of) found by bees in flowers (Chapter III). In our model, we assumed bees to perceive empty flowers as a punishment, in a context of optimisation of one's foraging intake. Predictions from the model showed that different resource distributions and abundances led to different influences of the positive and negative reinforcements on partitioning. Positive reinforcement had a greater impact on partitioning as the number of flowers available per bee increased and as flowers became more homogeneously distributed, whereas negative reinforcement had a greater impact on partitioning when flowers were heterogeneously distributed between patches.

The objective of this study was to compare predictions from this model with the foraging behaviour of honey bees competing over a floral array. The first goal was to obtain clear observations of multiple individually tracked honey bees foraging together in a controlled competitive foraging environment. The second objective was to provide an experimental test for the predictions of our model (Chapter III). Since the model predicted that bees would partition very easily in environments containing low numbers of flowers (≤ 10), when given around 40 successive foraging bouts, we replicated such a situation experimentally. We trained two bees to forage on 6 artificial flowers distributed inside a

closed arena, and tracked their visitation sequences to the different flowers. Most studies on partitioning in bees were done on bumble bees (Lihoreau et al., 2016; Pasquaretta et al., 2019). While this study did not intend to draw comparisons between the two species, looking at the foraging strategies in honey bees allowed to extend our knowledge on the phenomenon in a species with slightly different foraging habits, as honey bees forage socially and recruit nestmates.

2 - Methods

The experiment was conducted between April and May 2020 at Macquarie University (Sydney, Australia). A honeybee colony was placed at one end of a flight cage of dimensions 30x5m and 5m tall. The flight cage contained bees in an environment where their foraging experience could be controlled experimentally. In the flight cage the bees were provided with an *ad libitum* gravity feeder of 20% (w/w) sucrose solution located 2 metres in front of their colony, and 30 grams of pollen per day delivered on a flat dish placed next to the gravity feeder. We used for this experiment two colonies (Colony 1: April, Colony 2: May).

2.1 Foraging Arena

To control which bees were allowed to forage on the array of artificial flowers, we built an arena of dimensions 120x90x30cm (Fig 1a) using Corflute. The experimental arena was placed at the opposite end of the flight cage, at about 25m from the colony. A short platform was set at the entrance of the arena to allow bees to land. The top of the arena was covered with a removable mesh layer, which could be lifted to release bees at the end of their foraging bout. Five windows covered with removable mesh were also made in the sides of the arena to allow the observer to access and manipulate the objects inside the arena. The floor of the arena was covered with laminated paper covered in a red/white random pattern, to help the bees navigate inside the arena (Fig 1b).

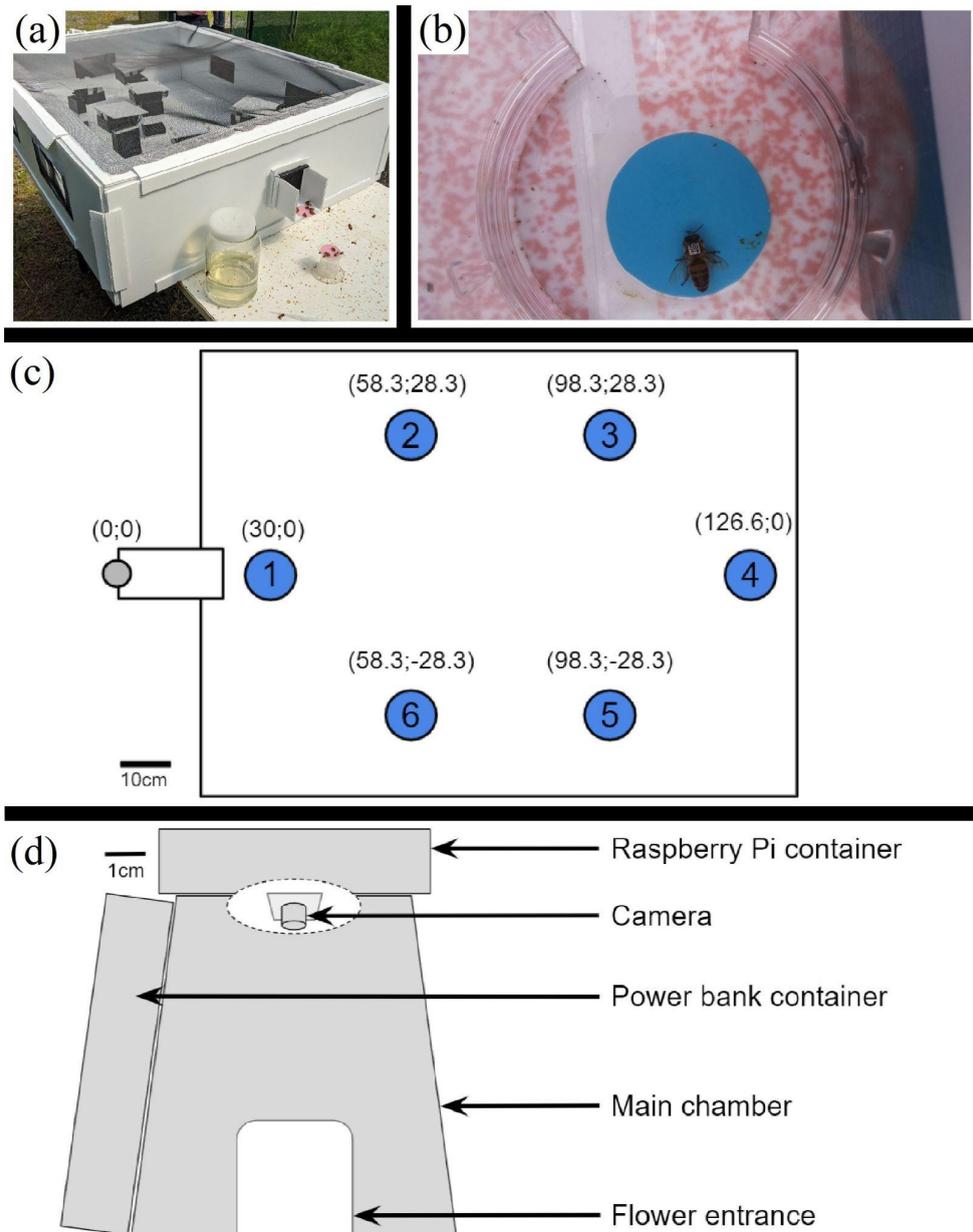


Fig 1: (a) Experimental foraging arena, of size 120x90x30cm. (b) Example of image taken by the artificial flowers every second when a bee is visiting, which were then used to reconstruct visitation sequences through identification of the QR codes on the bees. (c) The positions of flowers (blue circles) inside the arena. Coordinates are marked above each flower. The entrance to the arena is displayed on the left side (grey circle). An ID number was given to each flower (as seen in the circles) for identification purposes. (d) Schematic of an artificial flower as used in the experiment, with descriptive legend.

2.2 Tagging and training of bees

All the bees used for this experiment were first trained from the *ad libitum* gravity feeder, to a set of blue laminated papers in front of the arena. Bees foraging on 20% (w/w) sucrose at the gravity feeder were presented with a Q-tip soaked in 50% (w/w) sucrose solution that was touched to their antenna. Most bees then began to drink sucrose from the Q-tip. While doing so they could be taken to the front of the foraging arena where droplets of the same 50% (w/w) sucrose solution were dispensed *ad libitum* by the observer onto the laminated colored papers.

Any bee that arrived on its own at the experimental setup was paint marked using a dot of paint (thin-tipped POSCA® Markers) on the thorax. If the same bee visited the arena 3 more times in the following 15 minutes, it was selected to be tagged. We used paper tags on which were printed QR Codes (2.625x2.625 mm, developed by Gernat et al., 2018) and glued them to the bees thorax using super glue (UHU® Super Glue).

The tagging required the bees to be momentarily anaesthetised by placing them in a small plastic vial (50 cc), and put for one minute inside a styrofoam ice box. After that, the anaesthetised bee was taken out of the vial, onto a piece of tissue. A dot of super glue was applied to its thorax using a toothpick, and then the QR Code was gently and accurately placed on the middle of the thorax (Fig 1b). Once the tagging was done, the bee was put back on the laminated paper where it could feed on sucrose solution, and left to recover for as long as needed. Most bees flew away after < 15 minutes of rest. Then, we waited up to 1 hour for them to come back. If the tagged bee was not seen foraging on either the front of the arena or the *ab libitum* feeder at the entrance of its colony, she was considered lost. This tagging process was repeated until two bees were tagged and successfully returned to the foraging arena.

Once that occurred, any other bees that had been trained to the front of the arena were captured and released into an enclosed mesh box close to the experimental setup, in which an *ad libitum* 20% (w/w) sucrose solution was provided. They were kept in this box until the end of the day to prevent them from interfering with the foraging of the two tagged focal bees. The two tagged focal bees were trained from the front of the arena, to the platform at the entrance, through the entrance tunnel, and inside the arena, by moving the colored laminated paper they were feeding from progressively deeper into the arena in between their visits. Once inside the arena, 6 laminated papers of the same color were used instead of 1, placed around the position of flower 1 (see Fig 1c) in an hexagonal shape, and only 20 μ L of solution was dispensed on each. During this step, bees learned to move between locations within the arena to find food. Once the bees visited multiple locations to feed, we waited until the arena was empty of bees to set up the artificial flowers and start the experiment.

2.3 Artificial Flowers

For the purpose of the experiment, we built artificial flowers allowing us to track bees visiting them. The flowers were composed of a main cylindrical chamber of diameter 8cm, which could be accessed from its base on two opposite sides (Fig 1c). In the middle of this chamber we put a piece of laminated colored paper that the bees recognised as where they should go to get a reward. Above this chamber, a Raspberry Pi (Raspberry Pi 4 Model B 8GB) was set, equipped with a camera (Raspberry Pi Camera Module V2, 8 Megapixels) and plugged to a power bank (imuto® Portable Charger 10000mAH, 5V/2.1A). This device took time stamped pictures every second and stored them (Fig 1b) on an SD card of 32GB. All images were stored on a computer after the observation, and analysed to search for QR codes.

2.4 Experimental Protocol

The flowers were positioned in a hexagonal formation (Fig 1c), using an edge length of about 40cm. Sliding doors made of laminated paper in the entrance tunnel were used to exclude any untagged bees from entering the arena. The entrance was monitored manually and the doors closed if an untagged bee approached. Otherwise, these doors were kept opened for the whole experiment as the vast majority of visits were from the tagged bees. The observation lasted until a total of 80 cumulative foraging bouts were done by the two bees, or until one of the bees stopped foraging. A bee was considered missing if it stopped coming for an hour.

When a flower was depleted by a bee, the observer started a 3 minutes timer on a computer next to the arena, after which it was replenished with 20 μ L of 50% (w/w) sucrose solution. Each of the 6 flowers had its own independent timer. A bee that completed its foraging bout in the arena would fly upwards to the mesh roof of the arena, at which point we lifted the mesh roof so that the bee could return to the hive. For the duration of the observation, all visits to flowers were captured by the cameras.

2.5 Data Analysis

All images captured by cameras on each flower were retrieved from the Raspberry Pi to a computer, and from there, all images were analysed to identify any QR code, using a Python script (courtesy of Tim Gernat, available at <https://beemonitoring.igb.illinois.edu/>). From this analysis, we reconstructed the visitation sequences for both bees. The visitation sequences were divided into foraging bouts (i.e. foraging trips from nest departure to nest return) by assuming any gap in detection longer than a minute indicating that the bee had left the arena. This assumption proved to be very robust when testing the experimental protocol beforehand.

We first analysed the extent of traplining of bees in this situation using our similarity index (see Chapter III) measuring how similar two flower visitation sequences are. To record this similarity index between two sequences, we first identified in each sequence all the transitions between flowers (e.g. for a sequence of flowers identified by numbers, 1 - 2 - 3, the transitions recorded would be 1-2 and 2-3). Then, these transitions were compared to identify which are used in both sequences. In the following sequences *a* and *b*:

Sequence *a*: **1 - 2 - 3** - 4 - 5

Sequence *b*: **1 - 2 - 3** - 5

The transitions 1-2 and 2-3 are found in both sequences. The number of flowers included in these two repeated patterns (highlighted in bold in the sequences above; the flower “2”, which is part of the two different patterns, is only counted once) form a similarity score, s_{ab} . Finally, this score is divided by two times the length of the longest sequence between *a* and *b*, l_{ab} (with in this example $l_{ab} = 5$), giving the following equation:

$$SI_{ab} = \frac{s_{ab}}{2l_{ab}}$$

Where SI_{ab} is the similarity index between the sequences *a* and *b*. The goal of the division here is to obtain an index between 0 and 1. As for the value of the denominator, $2l_{ab}$, it is to take into account differences in sequence lengths and not just differences in patterns. This means that a similarity index of 1 can only be obtained if the two sequences present identical patterns and are of the same length. All experimental data for this index were fitted to a binomial distribution using a binomial GLM of formula *similarityIndex* ~ *bout*. This fit was made to summarise the general trend of the model's predictions in a concise way.

We also analysed the extent of resource partitioning by the bees using the Q_{norm} measurement (Becket, 2016; Pasquaretta & Jeanson, 2018), derived from the modularity index Q (Dormann & Strauss, 2014). To compute this index we used the *DIRT_LPA_wb_plus* function from the R library “bipartite”, and the *optim_matrix_bip* function (Pasquaretta & Jeanson, 2018) normalised the index (giving Q_{norm}). Because of the asynchronous visits of both bees to the array, it was not possible to use the foraging bouts of the bees as a comparison unit. Instead, we extracted the mean time between two successive arrivals of a bee (i.e. the time for a complete foraging bout), and computed the Q_{norm} index in successive windows of twice this time, to try and have at least one foraging bout per bee in each window. The mean time to complete a bout was 265 seconds (mean = 264.9927, n=817 foraging bouts), hence each window was 530 seconds. As with the similarity index, we fitted the data to a binomial distribution using a binomial GLMM of formula *partitioningIndex* ~ *time*. This fit was made to summarise the general trend of the model’s predictions in a concise way.

We also looked into a more direct and precise measurement of partitioning of bees, with a measurement of time spent on each flower by each bee. We compared the time spent on each flower by each bee of a pair throughout the whole experiment to test if a given flower was more prone to be partitioned between the bees based on its spatial location. For each pair of bees we measured the relative visit time difference, using:

$$D_f = \frac{|t_{a,f} - t_{b,f}|}{(t_{a,f} + t_{b,f})}$$

Where the relative visit time difference D_f of a flower f was equal to the absolute difference of time spent t by the bees a and b on flower f , normalised by the sum of these times. The result is an index between 0 and 1, indicating how unbalanced the time spent on a flower is

(0 meaning the two bees spent exactly the same time on a flower, and 1 meaning that only one bee spent time on this flower). This normalisation was necessary to combine the different pairs of bees together for statistical analysis, as each pair of bees did not forage the same amount of time on all flowers. The data obtained through this method were compared between flowers to assess any statistical difference. Because of the non-normality of the residuals obtained using a parametric approach with a one-way ANOVA (Shapiro-Wilk test of normality: $W = 0.94707$, $p = 0.003$), we used a non-parametric Kruskal-Wallis rank sum test to assess differences between the different flowers. We also wanted to control that any partitioning observed was not a result of one bee foraging more than the other. If a bee's foraging time accounted for 70% of the combined foraging time of the pair, this more active bee could have spent more time on all flowers by default. To control for this, we searched for a correlation between our index of relative visit time difference and the absolute difference in total time spent foraging by both bees.

2.6 Comparison to model simulations

In Chapter III we developed an agent-based model to explore how the spatial foraging strategies such as traplining and partitioning could emerge from typical competitive situations. Simulations in a large variety of environments showed that simple rules of positive and negative reinforcements influencing the probabilities to visit different flowers, were able to explain the emergence of these strategies (Chapter III). Our model suggests that given a simple environment (low number of flowers, homogeneously distributed), bees would constantly be able to partition between different flowers and establish traplines. For a more thorough description of the model, see S1 Text of Chapter III.

For the sake of testing how each of the reinforcement rules influenced the foraging strategies of bees, the model was divided into 4 submodels. The first model only used positive reinforcement (Model 1 [+]), the second only used negative reinforcement (Model

2[-]), the third used both reinforcements (Model 3[+|-]), and the fourth, a control, used no reinforcements (Model 4[Null]).

To generate the predictions of this model, we ran 100 simulations per submodel in a generated environment replicating that of our experiment. We expected for such a spatial configuration of flowers that positive reinforcement would be the main driver of partitioning, but with still a certain degree of influence from negative reinforcement as the low number of flowers would encourage competitive interactions (Chapter III). To compare these predictions to our experimental data, the same similarity and partitioning indices were computed for all simulations, and the data fitted to a binomial distribution using a GLM with the formulas *similarityIndex* ~ *bout* (for the similarity index) or *partitioningIndex* ~ *time* (for the partitioning index). As our model did not use time during the simulation, we converted the foraging bouts in time, using the same mean value from our experimental results (i.e. 1 bout takes 265 seconds).

For the analysis on time spent on flowers (i.e. handling time), the model did not include such a feature. However, using the experimental data of this study, we were able to add such a measurement by attributing to rewarding and non-rewarding visits to flowers a mean amount of time, based on how long the bees stayed landed on a flower in both these situations during the experiment. In our experiment rewarding flower mean handling time was 26s; non-rewarding flower mean handling time was 5s). We applied these times to each visit of the bees in the model and used it to do the same analyses as the experimental data on differences in time spent on a flower.

For all analyses, we were especially interested in the predictions of the control model (Model 4[Null]), as it provided a control on what degree of traplining and partitioning was expected if bees did not learn anything. The model's implementation of bee navigation

(abstracted as probabilities to go from one flower to the other) proved to be a very good proxy of what bees did in experimental situations (Chapter III).

3 - Results

A total of 13 independent pairs of bees were observed for times ranging between 4800 seconds (~1h20) and 12500 seconds (~3h30).

3.1 Route Similarity

For this analysis, the pairs of bees were combined to fit a statistical model following a binomial distribution, and compared to the output of the 4 different models.

The models using positive reinforcement (Model 1[+] and Model 3[+/-]) predicted both an increase in similarity index throughout the experiment, while Model 2[-] predicted a slight decrease in similarity (Fig 2a). The null model (Model 4[Null]) predicted a route similarity index of approximately 0.25, which remained stable.

The experimental data showed a non-null degree of route similarity higher than that of the Model 4[Null], but no significant increase of this index with successive foraging bouts. The bees thus did not seem to develop traplines throughout the experiment, and yet showed a route similarity higher than what was expected under a null hypothesis. None of the other models seemed to fit this experimental data either.

3.2 Partitioning

For this analysis, the pairs of bees were combined to fit a statistical model following a binomial distribution, and compared to the output of the 4 different models.

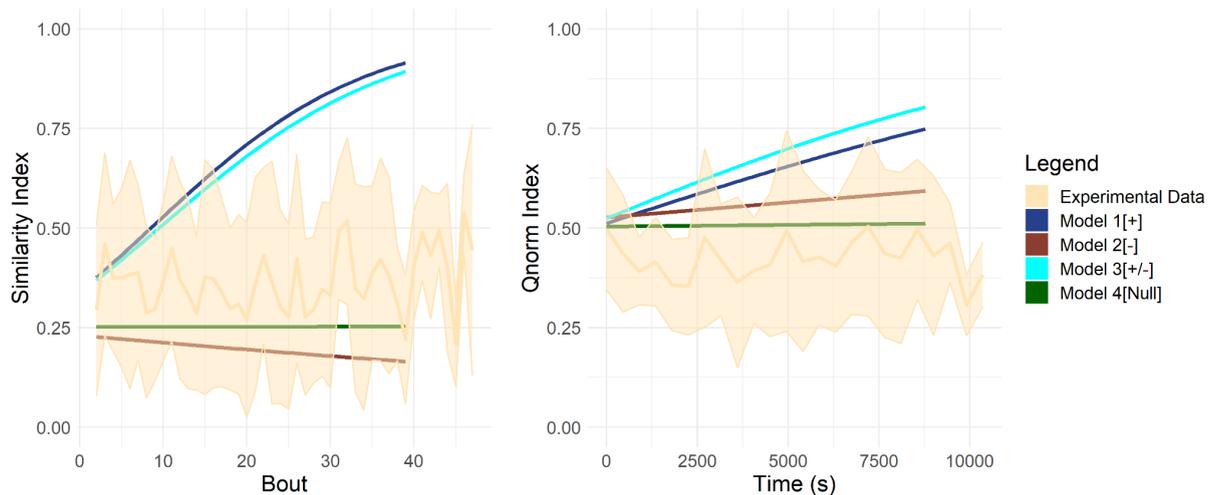


Fig 2: (a) Similarity Index throughout the successive foraging bouts. For each model, a Binomial GLM fit is represented. Experimental data are represented by mean \pm sd (n=26). (b) Partitioning Index Q_{norm} throughout time spent foraging. For each model, a Binomial GLM fit is represented. Experimental data are represented by mean \pm sd (n=13). Confidence intervals of models in both (a) and (b) were omitted to improve visibility.

Models 1[+], 2[-] and 3[+/-] predicted an increase in partitioning throughout the experiment, with a higher predicted partitioning in the model 3[+/-] (Fig 2b). Model 4[Null], predicted a null threshold value of the partitioning index of about 0.50. Experimental data, however, showed a lower partitioning than the null model's prediction, as well as no increase of this partitioning index with time. While the predictions of the models were in accordance with the general conclusions drawn in Chapter III regarding the effect of each type of reinforcement on the partitioning (i.e. positive reinforcement has a greater impact than negative reinforcement in this situation), none of them seemed to predict correctly the behaviour observed experimentally.

3.3 Time spent on flowers

Using the detections of bees every second on each flower, we were able to accurately assess the time spent on each flower for each observed bee. In most pairs of bees, at least

one flower was visited significantly more often by one bee than the other (Fig 3). While the total time spent foraging was never exactly the same for both bees of a pair (Fig 4), no clear correlation could be established between the different times spent foraging by each bee and how partitioned the flowers were (Linear Regression Model: Estimate = $4.731e-05 \pm 1.318e-04$, $p = 0.727$; Shapiro-Wilk Normality test for normality of residuals: $W = 0.906$, $p = 0.159$; See Fig 5), suggesting that any difference observed in the visitation times on each flower were not a result of different total times spent foraging.

When looking at the relative difference in visit times of the experimental data (Fig 6a), no statistical difference was found when comparing the mean absolute difference in time spent on each flower by all pairs (Kruskal-Wallis rank sum test, chi-squared = 8.3629, $df = 5$, p -value = 0.1373), meaning that all flowers showed similar differences in the visit rates of bees. The results from the models (Fig 6b-e) varied greatly. Models using the positive reinforcement (Model 1[+] and Model 3[+/-]; Fig 6b & 6d) showed greater partitioning on flowers 2, 3, 5 and 6, while flowers 1 and 4 were visited in a much more balanced way. This result was possibly obtained because of how flowers were positioned and how the bees in the models move; both bees had very high probabilities of visiting the flower 1 as they entered the arena (91.7%) making it difficult to exclude their competitor, while the other flowers were part of symmetrical paths (1-2-3 or 1-6-5) that each bee could use, presenting a simple solution for partitioning. Models 2[-] and Model 4[Null] (Fig 6c & 6e) both showed a lower time difference on all flowers than the experimental data, although both models previously showed a high partitioning when looking at the Q_{norm} modularity index. This last result shows that for these two models the partitioning observed, while true, was not a sign of some flowers being consistently partitioned, but rather different flowers every bout being partitioned. In each successive foraging bout bees visited flowers that the other bee did not visit, but these flowers changed at every bout.

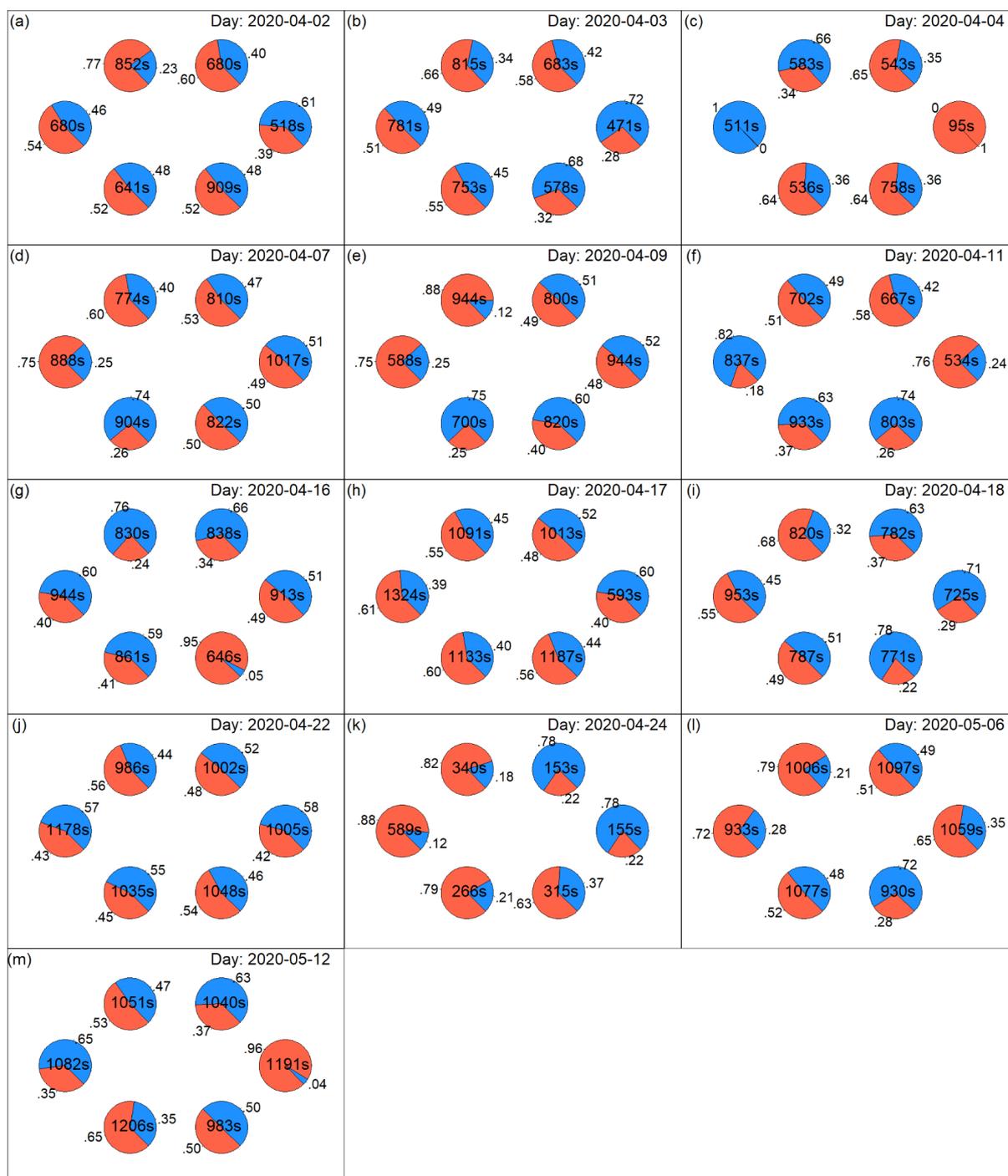


Fig 3: Time spent (in seconds) on each flower for each bee (colors blue and red) of each pair of each day (a-m). Flower positions are as displayed in Fig 1c. Inside each pie chart, the number indicates the total number of seconds of activity recorded by the pair of bees. The percentages represented by the pies are specified around the pie.

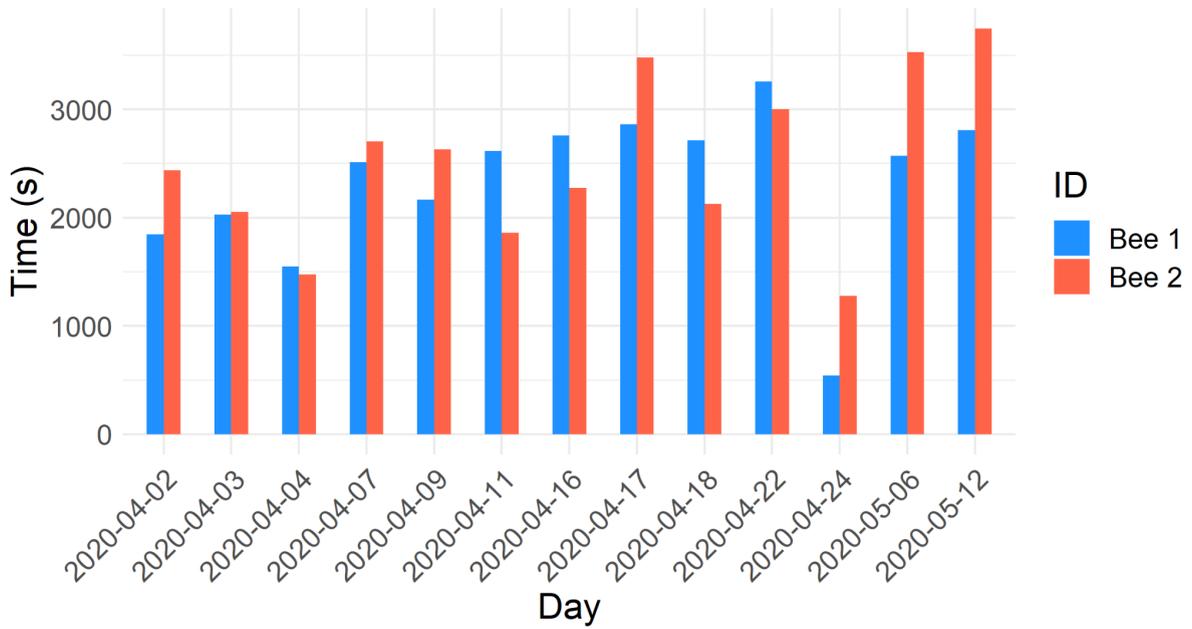


Fig 4: Time spent (in seconds) foraging on all flowers for each bee (“Bee 1” and “Bee 2”) in each pair (“Day”).

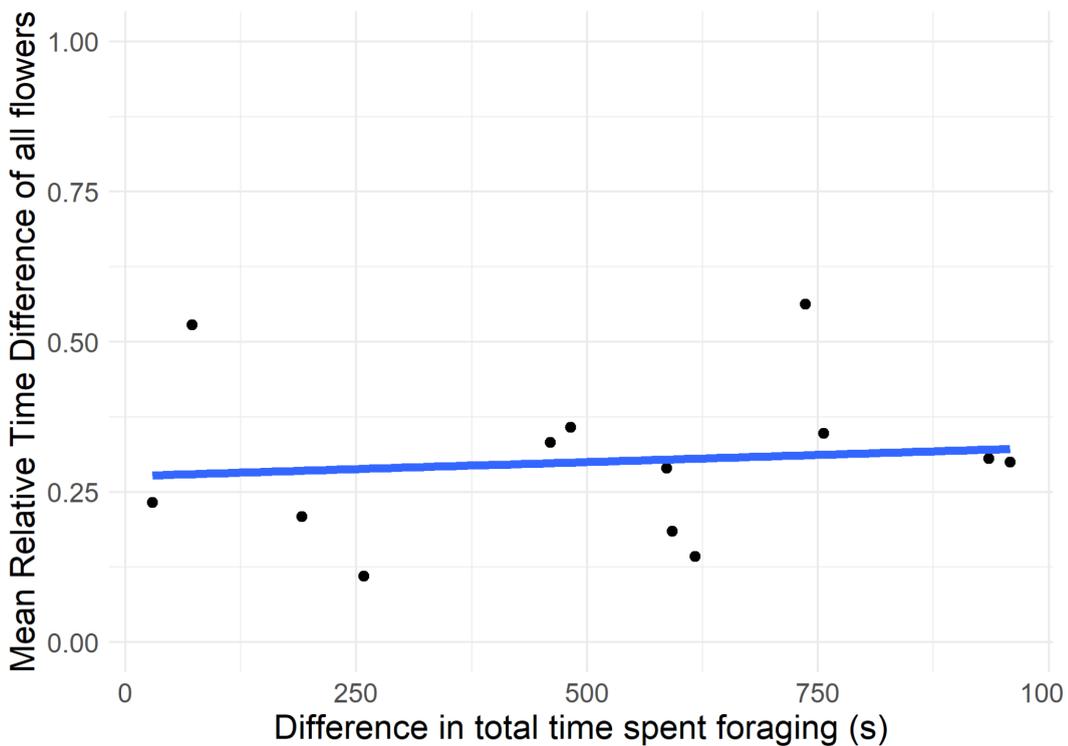


Fig 5: Representation of a linear model between the mean Relative Time Difference index of all flowers and the difference in total foraging time of a pair of bees, along with the data points (n=13).

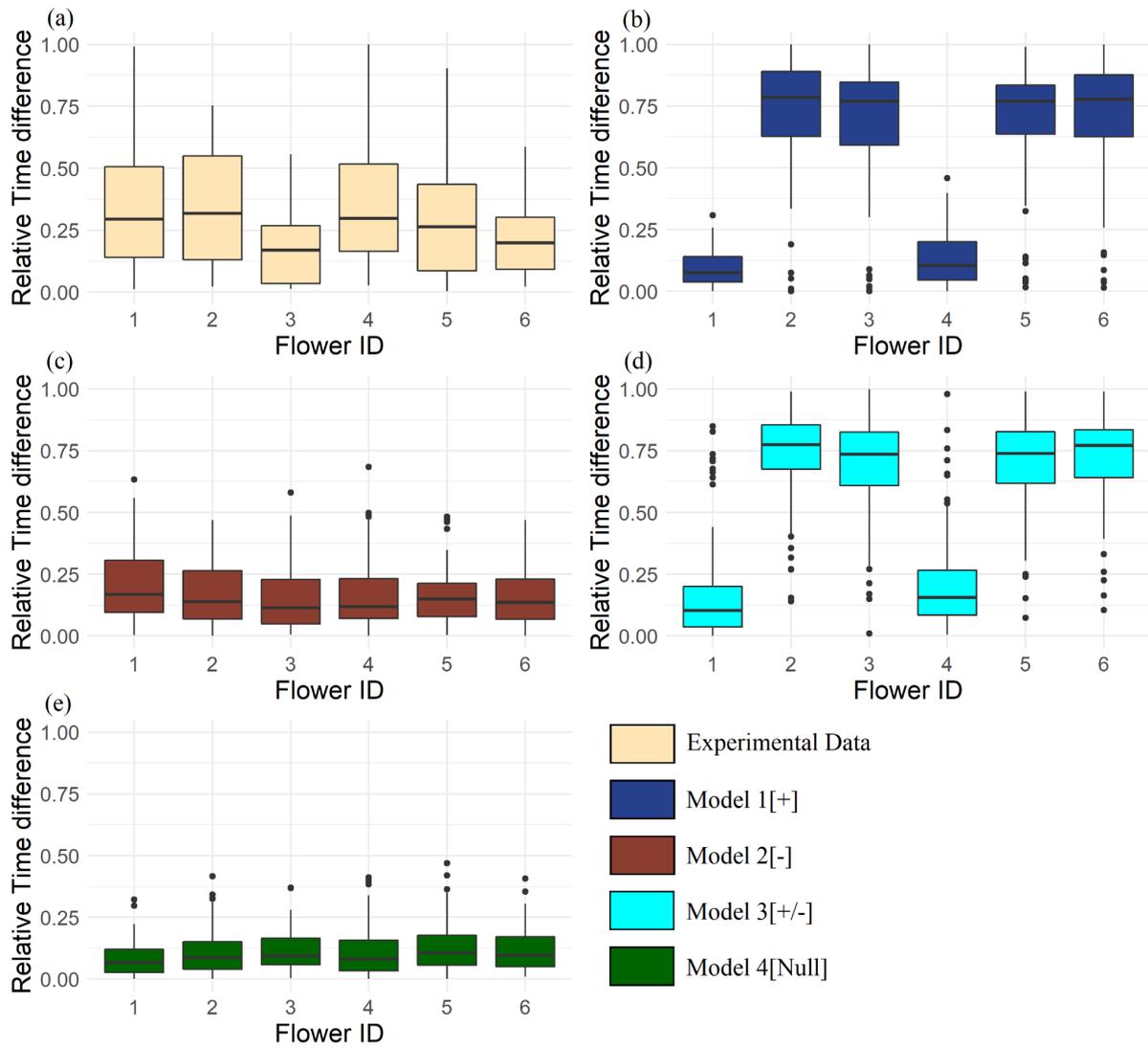


Fig 6: Boxplot of the relative difference in time spent on each of the 6 flowers by all pairs of bees. Experimental data (a) and the different models (b-e) are represented independently.

Flower ID numbers (x axis) refer to ID as represented in Fig 1c.

4 - Discussion

Studies on bumble bees have found that multiple individuals foraging on the same patch of flowers were able to partition resources efficiently to avoid competition (Lihoreau et al., 2016; Pasquaretta et al., 2019). In this study, we set out to understand if honey bees were able to partition between different resources in a competitive situation at a low spatial scale (i.e. at the scale of a patch of flowers). To achieve this, we tracked 13 pairs of honey bees foraging for multiple hours on a set of spatially close artificial flowers to get an idea of their ability to partition. We built an agent-based model to explore how the establishment of foraging strategies could be explained by simple learning mechanisms (Chapter III). This model generated predictions as to the properties of emerging traplines and partitioning behaviour assuming the hypothesised learning mechanism proposed in the model. We here compared our experimental results to the model's predictions.

In our experiment, bees only showed a low, stable level of traplining yet higher than the one expected from our null model (Model 4[Null]), suggesting their route similarities were not just the result of distance-based movements between flowers, as is the case in our models. The low traplining observed in our experiments most likely means bees did not rely on any learning as they kept the same level of traplining throughout the observation period (Fig 2a). These results are in accordance with previous observations at a similar scale (bumblebees on artificial flowers: Saleh & Chittka, 2007; honey bees on natural flower patches: Williams, 1997), while differing from results found in similarly complex artificial flower arrays but at larger scales (honey bees: Buatois & Lihoreau, 2016; bumblebees: Ohashi et al., 2007, Lihoreau et al., 2012a, Lihoreau et al., 2012b). This suggests that scale is an important factor to consider when considering the formation of traplines.

We further hypothesise that directionality of movements could explain the difference between our results and the null model's predictions. Studies on bumblebees have shown

that a forager's choice of flower to visit depends on the distance (i.e. they prefer short distances) but also the angular difference between their previous movement and the next (Ohashi et al., 2007; Woodgate et al., 2017). This persistence in angular direction could further drive the bees towards a stable repeated route without relying on any learning. Our null model ignored directionality and could thus have been the reason why it predicted a lower traplining.

We assessed the ability of honey bees to partition between resources using two complementary measurements, the modularity index Q_{norm} and the relative visit time difference. The Q_{norm} showed bees had a low, stable degree of partitioning, fitting none of the model's predictions, as even the null model (Model 4[Null]) showed higher partitioning. Using the relative visit time difference, however, we showed that the bees did not spend the same amount of time on each flower, with on rare occasions flowers exclusively visited by one bee. The partitioning in this experiment took the form of 1 or 2 flowers being unequally visited by the pair of bees, but the identity of the partitioned flowers was different between pairs of bees, implying their position was irrelevant. Moreover, the lack of increase in partitioning throughout the observation period could mean that the bees did not progressively learn from experience to avoid certain flowers. We can hypothesise then that the partitioning observed could have emerged from cues available to bees from their first visit to the array, such as olfactory cues in the form of scent marks, or visual cues of competitor presence.

Honey bees produce a footprint chemical, called a scent mark, which they leave on plants as they visit them (Giurfa & Núñez, 1992, Giurfa, 1993). Multiple studies have shown how bees would use these chemical cues during subsequent visits to avoid visiting flowers visited recently, and thus more likely to be devoid of rewards (Giurfa & Núñez, 1992; Goulson et al., 2001). More recent studies on bumblebees showed in more detail how

individual behaviours were affected by the presence of these footprints (Pearce et al., 2017). This study in particular showed how the presence of scent marks reduces drastically the probability of landing and drinking attempts, but not the hovering, meaning that this chemical cue does not save the bee from navigating to empty flowers. This chemical cue should be efficient in situations of high density of flowers (a patch of flowers) where the energy and time cost of travelling between each flower is low, and the largest gain in energy and time for the bee is to know whether to land and probe a flower or not. However, as flowers get further apart, the cost in energy and time of travelling to an empty flower increases, making the use of memories of the rewarding flower's position much more energetically efficient. Therefore, it appears to be much more efficient and parsimonious to rely on scent-marks in situations where a bee visits large numbers of spatially close flowers, instead of trying to memorise the rewarding flowers' positions as our model does. This could also explain why it becomes more efficient to rely on memories of flowers and trajectories as the flowers, or patches of flowers, get further apart.

Honey bees have long been studied for their visual abilities (review in Avarguès-Weber et al., 2012), and are able to accurately discriminate different visual cues (Hempel de Ibarra et al., 2002). Thus, we cannot exclude that they could see the competitors as they forage and use this information to avoid competition. Bees have often been observed adapting their behaviour in the presence of competitors, reducing their foraging area as competitors arrive, and expanding this same area as competitors are removed (bumblebees: Thomson et al., 1987; Makino & Sakai, 2005; Ohashi et al., 2013; honey bees: Williams, 1997).

Although our model has provided interesting comparisons with this experiment's results, they have also highlighted some of its limitations, some of which appear in hindsight to be important in the study of foraging strategies in competitive situations. Namely, in the model bees are always leaving the nest synchronously, and so always have immediate and

direct foraging competition in the environment. However, in an experimental situation, it was not conceivable to replicate such behaviour, as bees forage at different speeds and with different motivations, often leading to differences in activity and inter-trip interval. One of the proven advantages of traplines being the regularity of visits to flowers by a same bee (Ohashi & Thomson, 2005), it is important to consider the motivation of a bee as it greatly affects the time between foraging bouts. Another important discrepancy of the model is the lack of flower manipulation time. At a low spatial scale, travelling between flowers represents only a small portion of time compared to the time bees are landed on a flower looking for nectar and retrieving it. In a situation such as our experiment, this lack of time restraint allows a bee to potentially compete on multiple successive flowers and potentially win all these interactions, drastically changing the dynamic of nectar retrieval in the pair of bees and as a consequence the outcome of the simulation. These new results and observations therefore provide important feedback on the conception of our model (further developed in Chapter V), which will be used to further refine our predictions.

This new study has given us some first insights as to the establishment of foraging strategies of honey bees in intra-patch foraging conditions. As the behaviour observed during our experiment differed significantly from the predictions of our model based on learning and memorising the trajectories between flowers, we hypothesise that at this spatial scale, learning and memorising spatial information about numerous flowers is inefficient, when compared with other cues available to bees, such as scent-marks and direct visual cues of competitors. Our results further support the idea that traplines get harder to develop at low spatial scale, but also suggest that the partitioning strategy could show the same constraint. Our experimental situation, however, only presented a very limited number of flowers, each rewarding amounts of sucrose solution exceedingly high when compared to what bees usually experience in natural conditions. A future step would be to design a controlled “intra-patch” foraging situation more closely related to their natural counterpart.

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Chapter V.

**Foraging strategies of bees in a competitive
intra-patch situation**

Abstract

The Optimal Foraging Theory proposes that animals such as bees could optimise, along a given currency, their foraging behaviour. Yet, we have little evidence on how bees improve their foraging efficiency at the scale of a single patch. We argue that understanding the foraging behaviour of bees at small spatial scale is key to grasp how foraging strategies can emerge in their natural context, and what the underlying mechanisms are. We observed bees foraging in both natural and controlled conditions and used these observations to assess whether and how bees were optimising their foraging behaviour. We compared our experimental results to the predictions of our model to gain some insights on the potential mechanisms responsible for the development of foraging strategies. We found that bees did not exploit resources randomly, and globally improved their foraging efficiency when possible. Their strategies appeared much more adaptable to the context of their foraging than previously thought, and these differences can be reasonably related to variations in the spatial and temporal constraints of their environments, suggesting these observed strategies could be emerging from simple behavioural responses of bees to these constraints, and not from cognitively intensive processes.

1 - Introduction

When exploiting patches of flowers, generalist bees (such as honey bees and bumblebees) have been observed establishing “traplines” in a way that often minimises the overall distance between the different flower patches. While traplines are commonly used in various situations, they are seldom seen when bees forage at the scale of a single patch of flowers, where nearest-neighbour movements are preeminent (bumblebee: Burns & Thomson, 2006; Saleh & Chittka, 2007; honey bee: Williams, 1997; Buatois & Lihoreau, 2016). In Chapter II, we explored the reasons why traplines could be irrelevant at such spatial scales, yet, the absence of traplines should not imply an absence of foraging optimisation. Here, we ask the following question: do bees learn to improve their foraging performance at the scale of a patch? If so, how?

For forager bees that travel several kilometres every day finding the shortest route between flowers clustered in a patch would be expected to have a high cognitive load (i.e. many flowers to learn and remember and different routes to compare with high precision) for a likely minimal benefit, in terms of saved travel distance (Chapter II). Yet, this does not mean that bees should not try to improve their foraging efficiency in these conditions. This could be done by simply learning to avoid empty flowers, avoiding revisiting a flower too soon, or avoiding any other behaviour that may have a cost without any apparent benefit. In fact, we argue that traplines might just be one of many different ways bees can improve their foraging efficiency, but that they could all be different outcomes of a general foraging behaviour applied to different situations.

To explore the nature of traplines at various spatial scales we developed an agent-based model to predict how reliable foraging routes might be formed by bees, based simply on positive and negative reinforcement rules (Chapter III). We conducted experiments during which we tracked multiple individual bees competing for forage on a plant (*Borago*

officinalis), and also in a controlled array of artificial flowers. We then compared our experimental results to the predictions of our model in order to understand the kinds of foraging strategies observed in a small scale flower patch. In both experiments, we tried to identify if the bees were improving their foraging efficiency through changes in behaviour. Furthermore, the comparison between our model and our experimental results allowed us to compare the behaviour of bees predicted by our model that used only simple learning rules with the behaviour of real bees in small scale patches.

2 - Methods

2.1 - Natural Experiment

Experimental protocol

We designed an experiment, inspired by Williams, 1997, to observe the behaviour of bees in a natural flower patch. This was then used as a comparison with observations of bees foraging in controlled environments on a small patch of artificial flowers. This experiment was conducted in October 2020 at the Macquarie University (Sydney, Australia). For this experiment, two borage plants (*Borago officinalis*) were planted 1 metre apart from each other inside the bee yard of the university (Fig 1a), at about 50m from the 20 hives reared in this area. The choice of the borage plants for this experiment was following their use in Williams, 1997. Around these plants were other potted plants and naturally growing plants, giving ample foraging opportunities for bees. Because the borage plants used were located within a garden with multiple other plants, their maintenance process was not specifically recorded, as they were maintained along the other plants of the garden (i.e. watered everyday). Foragers were not trained to forage in this area, but rather allowed to freely discover, forage and recruit to the borage plants as to not artificially impact the visitation rates of the plants. Branches of the borage plant were held in place using stakes, to avoid them from falling over. As flowers opened, we attributed to each an ID number, written on a piece of yellow-colored tape and stuck to the base of the flower. While the colour of the tag could

have influenced the bees, all flowers were similarly tagged so no flower should have been treated differently. Using an array of three rulers, each perpendicular to the others, we assessed the 3D position of each flower as they opened as cartesian coordinates to 1cm accuracy. Using these measurements, we were able to reconstruct the 3D distribution of flowers within the patch of borage.

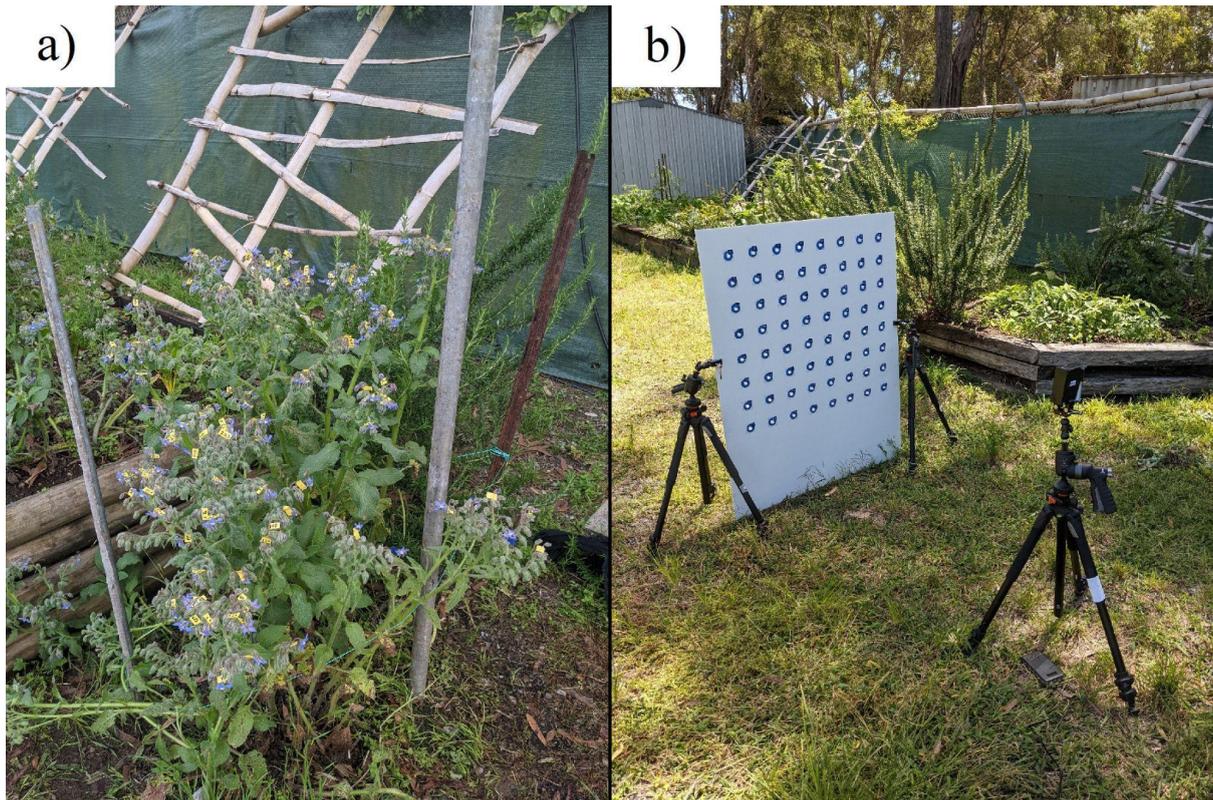


Fig 1: a) Picture of one of the *Borago officinalis* plants used for the natural experiment. b) Experimental setup for the controlled experiment.

On the days the experiment was run, any new flowers were marked and their position reported. Then, 30 minutes were spent marking any bee visiting the plants with dots of paint (thin-tipped POSCA® Markers) of different colours on the bees' backs, to identify them individually. Once the bees visiting the patch were identified, we started recording the sequence of flowers visited by bees as they foraged on the borage plant. Because only one observer was present on the experiment, only one bee could be followed at a time. Thus, the

first bee to arrive to the patch was followed, the IDs of the visited flowers reported, while ignoring other bees arriving until the focal bee left. A focal bee's visit to the patch was considered over if it left the patch for one minute. A total of 4 days of experiment were conducted, following bees visiting the patch until visits to the plant stopped.

Data Analysis

We were first interested in quantifying how often bees followed nearest neighbour movements. When foraging at the scale of a single patch (i.e. intra-patch foraging), bees are not expected to memorise rewarding flowers and develop stable routes (Chapter II; Burns & Thomson, 2006). In fact, naive bees were most often observed using nearest neighbour rules instead. To confirm this, the visitation sequences of bees were extracted from audio recordings taken during the observations. Then, we quantified the distances travelled between each two successive flowers visited (hereafter "the interflower distance"), looked at the proportions at which each distance was observed and compared it to the distribution of distances between each pair of flowers of the plant.

Secondly, we focused on the ability of bees to establish "repertoires" of flowers (coined in Williams, 1997): groups of food sources that are memorised and regularly revisited, although in no specific order. How many flowers are visited in a patch depends on many factors, such as the rewards from each flower visited (i.e. a low volume on a flower increases the probability of leaving the patch; Pyke, 1982a), the presence of competitors or their scent-marks (Stout & Goulson, 2001). While traplining is not expected at this spatial scale, we still expected to find at least partial partitioning through the development of different repertoires of flowers (Chapter II). However, because only one bee's sequence was recorded at a time, and the visits of bees to the array were sometimes sparse and spread in time, the use of common partitioning measurements such as the modularity index Q_{norm} (Chapters III and IV) were irrelevant. Instead, we used a measurement called Mean Location

Estimates (hereafter MLE; from Williams, 1997), a bootstrapped estimation of the centre of gravity of all flowers visited. For this index, we grouped all the flowers visited during all bouts of a same bee, and for 1000 bootstraps we sampled as many flowers as the number of flowers visited during the bee's longest bout, and computed the mean position of these flowers. The result, a scatter of 1000 points per individual bee, was displayed in a 3D graph. Because of the low sample size in this experiment (2 to 5 bees followed each day, for 4 days, visiting the patch for 1 to 21 foraging bouts), no statistical analysis was conducted on these data.

2.2 - Controlled experiments

Setup and training

We then planned two controlled experiments as a follow-up on our first natural experiment. Their goal was to replicate as faithfully as possible the conditions of the natural patch of flowers, while also providing the control we need to properly quantify the behaviours of bees and manipulate nectar availability. The two experiments were conducted between February and May 2021 at Macquarie University (Sydney, Australia). Bees from the nearby hives were given an *ad libitum* feeder of 20% sucrose solution (w/w) 10 metres away from the experimental site. From there, individual bees were taken indiscriminately to be part of the experiment by presenting a Q-tip imbued with a 50% (w/w) sucrose solution, and brought to the experimental array.

We built an artificial array of 64 flowers (Fig 1b). The array consisted of a Corflute plastic board (120x90 cm) reinforced with a wood frame on the edges. The flowers were set in a square of 80x80 cm (i.e. 8 rows and 8 columns of 8 flowers spaced by 10cm each). Each flower consisted of a 1.5ml Eppendorf tube with its cap removed, placed through drilled holes in the board and hot glued. Around the tube, a 4x4cm paper colored circle was glued to help the bees locate the positions of the flowers. Circles of blue or yellow colors were

used during different days. Throughout the experiments the array was held vertically, from the sides by tripods equipped with clamps. All movements on the array were recorded by a camera. A 20x20cm array with 4 flowers (hereafter the “training array”) was also made to train the bees before the experiment and avoid any bias of previous knowledge of the array.

Through these two experiments, we focused on the competition aspect of foraging behaviour, trying to understand if the consequence of the competition pressure (i.e. empty flowers) was enough to drive bees to trapline and/or partition and improve their foraging efficiency, or if other cues (scent-marks, visual cues) were necessary. To test this, in the first experiment, we trained one bee to forage on our artificial array, where 12 flowers out of 64 (20% of flowers) were rewarded with sucrose solution, mimicking the natural variability in rewards from various factors (i.e. competition or plant physiology or strategy) through the empty flowers. In the second experiment, 5 bees were trained to come to the artificial array, with all flowers rewarding sucrose solution.

One bee experiment

The goal of the first experiment was to observe how a single bee would forage on the array with stable resources throughout the day. A single bee was brought to the training array from the *ad libitum* feeder, by presenting a Q-tip soaked in 50% (w/w) sucrose solution to the antenna of a bee present at the 20% sucrose gravity feeder until it started feeding on and climbed onto the Q-tip. The bee was transferred from the Q-tip to one of the flowers of the training array, which were at first filled with ~15-20 μ L of 50% (w/w) sucrose solution. The bee was left to forage freely until its crop was full and left the array. If the bee came back on its own to the training array, it was tagged using a dot of paint (thin-tipped POSCA® Markers) on the thorax, and was left to forage again. If the tagged bee came back a third time to the training array, the training was considered successful and the experiment started.

The main array was set in place where the training array was initially set, and the camera started recording before the bee came back. For this experiment, 12 flowers were chosen pseudorandomly every day to be rewarding. The experiment went on until either the bee completed 40 foraging bouts on the array (i.e. a foraging trip, with return to the nest before the next one) or stopped coming to the array. For the duration of the experiment, the flowers emptied by the bee would be refilled (with a variable volume, see below in “Estimation of reward volumes dispensed”) between two successive visits to the array. The sequences of flower visits on the array were then extracted manually through video analysis. Each flower visit was categorised as either “hover” or “landing”, reflecting the probing behaviour of the bee. A hover was considered when the bee would momentarily perform a stationary flight in front of a flower. When looking at visitation sequences for statistical analyses, both hovers and landings were counted. The number of landings per foraging bout were used to assess the amount of resources gathered by the bee (i.e. a resource was considered taken when the bee landed on a rewarded flower).

Five bees experiment

The second experiment’s goal was to test the foraging ability of bees in a competitive situation. The training was similar to the first experiment, albeit with 5 bees. The bees would be tagged with a unique colour if they came back on their own (without needing to pick them with a Q-tip from the main feeder again) to the training array a first time, and then the experiment started when all 5 tagged bees came back once more. For this experiment, all 64 flowers were rewarded at the beginning of the experiment, then every 5 minutes the left or right sides of the array were replenished, alternatively. As bees could be present on the array at the time of replenishing, only the depleted flowers on which there were no bees were replenished. The bees were then observed for 3 hours, which was enough for the fastest ones to do about 40 foraging bouts.

Through video analysis we manually extracted information about the foraging behaviour. We recorded the times of arrival and departure from the array, and for each flower visited by each bee, the ID of the flower, as well as the type of visit (hovering or landing). Because the resolution of the video was too low to confirm whether bees were actually feeding when they landed on flowers, all bees landing and staying static on a flower were considered as feeding, unless the flower was already depleted from a previous visit of a bee.

Estimation of reward volumes dispensed

In both experiments, a 50% (w/w) sucrose solution was dispensed in flowers of the array using a manual plastic transfer pipette. The pipettes used had a thin tip, and were often cleaned with water during the experiment to avoid crystallisation of sugar. The experimenter dispensed droplets of the solution at the entrance of the flowers, the volume of which could vary between droplets (thus mimicking the natural variability of nectar in flowers). We assessed the amount of solution delivered in a droplet by estimating the volume in a large sample (n=108) of droplets made using the same process. The volume was estimated using the area of the droplet as a proxy. The relation between volume and area of droplets was first quantified by making reference droplets with an electronic micropipette for volumes between 3 μ L and 10 μ L. The measurement of the areas of the reference droplets, done using the imageJ program, indicated that area was a good predictor of volume using a linear regression (Adjusted R-squared = 0.8845). The volumes of the sample droplets were then estimated using the following formula:

$$Volume = - 2.38 + 1.125 * Area$$

where the volume is expressed in μ L and the area in mm². The correlation is displayed graphically in Fig 2, and the distribution of the sample droplets in Fig 3.

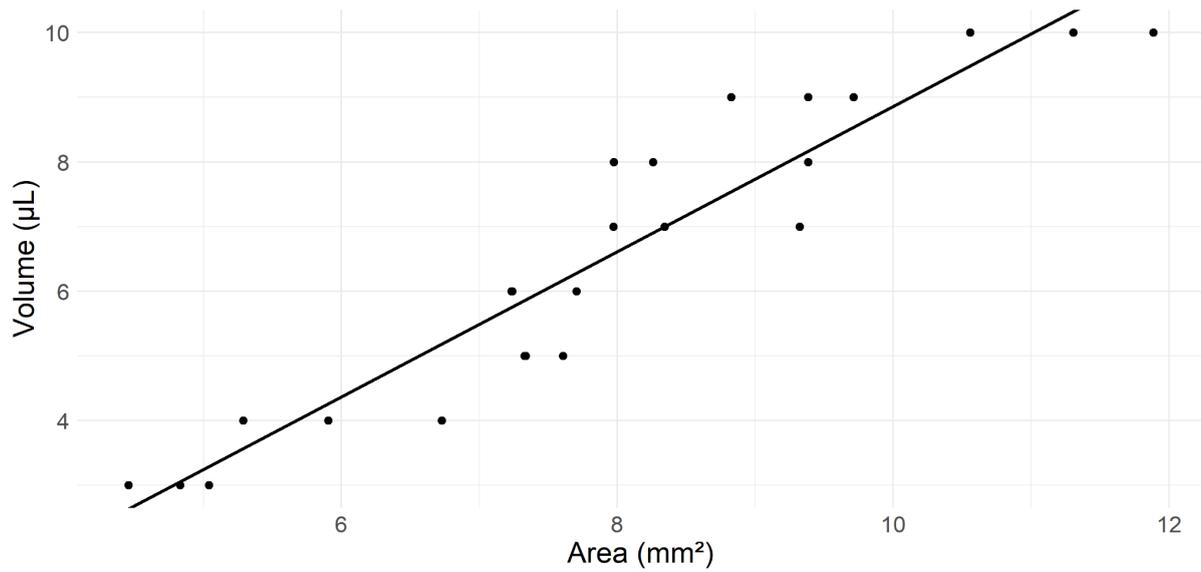


Fig 2: Linear regression showing correlation between area and volume of sucrose solution droplets, used to assess the volumes typically dispensed by our thin-tip micropipette. The regression had an adjusted R-squared = 0.8845.

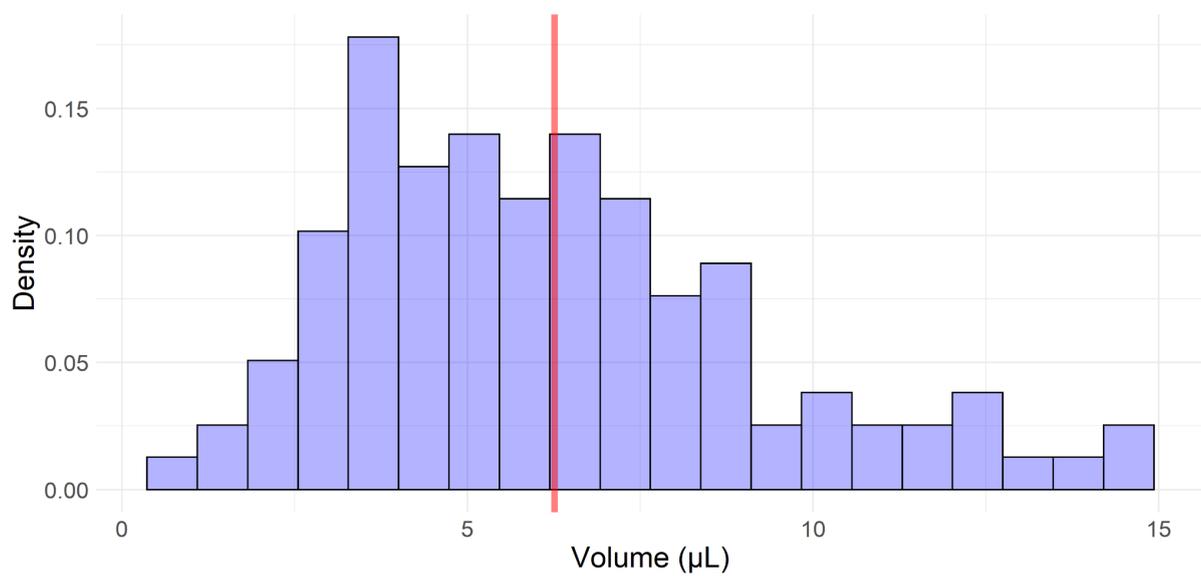


Fig 3: Distribution of sucrose solution volumes dispensed by our thin-tip micropipette by the experimenter. Vertical red bar represents the mean value of 6.26µL.

Data Analysis

Firstly, in both experiments we looked at the bees' ability to form traplines, using our route similarity index (defined in Chapter III & IV), with the expectation that no traplines would be formed at this spatial scale. Secondly, we focused on their ability to partition in the 5 bees experiment, using the modularity index Q_{norm} (defined in Chapters III & IV). For these two indices, we computed statistical models with mixed effects (Binomial GLMMs) looking at the effect of successive bouts on the indices, while adding a random effect for the identity of the bees.

Thirdly, we also looked at diverse criteria obtained by analysing the visitation sequences, such as the number of unrewarded and rewarded visits per bout and the interflower distance. These criteria provide supplementary details about potential ways bees could improve their foraging efficiency without needing traplines. They also support considerably the information given by our indices, which do not always capture completely the complexity of these behaviours (Chapter IV). For the unrewarded and rewarded visits, we computed GLMMs to quantify how their numbers varied with each successive foraging bout. Finally, the interflower distances used by each bee were statistically compared to that of the distribution of interflower distances in the array.

All analyses were done using R version 4.0.5 (R Core Team, 2021).

2.3 - Modelling

In Chapter III, we designed an agent-based model to attempt to explain the decision processes behind the establishment of foraging strategies, using simple positive and negative reinforcements. One of the goals of these experiments was to provide a new dataset to challenge the model's assumptions at small spatial scales. After comparing this model to the results of our first experiment, it appeared that some of the limits of our model

were preventing us from accurately predicting the bees' behaviours (Chapter IV). The model was therefore improved to overcome these shortcomings. We provide here a brief overview of this model's algorithm and highlight the changes made to it.

In the model, the main goal of the bees was to fill their crop by visiting feeding sites (hereafter called "flowers") which provided resources (sucrose solution). The bees navigated between the different flowers using a transition probability matrix, which gave for any pair of flowers a probability of navigating between them. For every movement the bee did within the environment, the matrix was consulted and the next destination chosen probabilistically. This system was inspired from a previous model (Reynolds et al., 2013), and we showed that these distance-based probabilities were accurately mimicking the probabilities of an agent to find a point in space while navigating according to the rules of a random walk (S6 Text of Chapter III). These probabilities were then altered throughout the simulation, increasing probabilities of certain movements if they led to a reward, and decreasing them if they did not. These two simple reinforcement rules, based on the abilities of bees to learn through appetitive learning (Goulson, 2010) and to avoid visiting flowers recently visited or on which they had been previously displaced by a competitor (Pasquaretta et al., 2019). The bees would thus navigate between different flowers and alter the probabilities of future movements based on the outcomes of their visits. In each trip to the array (i.e. foraging bout), each transition vector between 2 flowers was only altered on its first use. Once the bee reached its crop capacity, it would leave the array and go back to its nest.

The first limit of our model was the forced synchrony of foraging bouts for all bees. Indeed, in the initial version of the model (Chapter III) all bees were made to leave the nest together to force them to forage interactively, but also to simplify how the different flowers were replenished, which was right before all the bees would start a new bout. However, our various experiments showed that it was impractical to try and keep the bees synchronised, and that doing so impacted heavily on the dynamics of the foraging activity (Chapter IV). At

the scale of a single bush with 50 to 100 flowers, how often flowers are depleted by bees depends on their visitation rates. These vary greatly between individuals may it be from the distance between this plant and their nest, or their motivation to forage. Moreover, when multiple bees visit the same patch together, the time required to execute short tasks such as moving between two flowers or retrieving the nectar from a flower becomes relevant. In our experiments, bees visiting our array typically stayed for 90 to 120 seconds. During this period, assessing the status of a flower (unrewarding or rewarding) and travelling between neighbouring flowers generally took 1 second, while feeding on a flower ranged between 6 and 30 seconds. It is reasonable to assume that these costs in time, as well as the interindividual variations in revisitation rates, could shape how multiple bees forage in a patch.

To implement these new constraints in the model, we changed how time was considered. The previous model directed that at every “time” step, each bee would visit a new flower or get back to their nest. In our updated model, each step is a second, and we attributed to each action an amount of time. From our available experimental data (n=35 bees, each doing 10 to 46 foraging bouts), we calculated the average time between a bee’s departure from the array and the moment it returned (i.e. the “inter-bout time”) and used this data to implement the same timings in our model. Each modelled individual was given at random a value from this experimental distribution. For the duration of an unrewarding visit and the time to travel between flowers, their average duration were not easily assessed as they often took no more than a second, and would have required a much more detailed analysis (frame-by-frame) of the video to get a precise assessment. For simplicity, these durations were approximated to 1 second. For the time taken to consume the reward of a flower, our recordings were not able to discriminate between a bee feeding and a bee landed but not feeding. Moreover, as the amounts dispensed in each flower varied, it was not possible to estimate correctly this value. Thus, it was attributed an arbitrary value of 0.5 μ L/second.

In the former model flowers gave one arbitrary unit of resource and the bees needed 5 of these arbitrary resources to fill their crop. In order to fit the variation in rewards introduced in our sucrose solution dispensing process, we changed how resources were attributed to each flower. In our new model, the reward of each flower was sampled from the distribution we established from 108 samples of sucrose solution droplets (Fig 3). Along with this change, modelled bees' crop capacity was changed from the arbitrary 5 units to a value of 40 μ L, based on the result of the one bee experiment where bees visited in average 6.5 rewarding flowers per trip, each containing an average of 6.2 μ L ($6.5 \times 6.2 = 40.3$).

While foraging, the bees interacted just as they did in the previous model (Chapter III). If they encountered a competitor on a flower, each bee had an equal chance of winning the interaction. The only change to these competitive interactions was that they now could happen at any time during the presence of a bee feeding on a flower, and the winner of an interaction would only be able to take whatever reward was left.

The learning rules of the model remained nearly unchanged. They were slightly adapted as a consequence of the changes in competitive interactions. In this new version of the model, a bee positively reinforced a transition vector only if it emptied the flower. Any bee dislodged from a flower before its content was completely emptied negatively reinforced the associated transition vector. Each transition between pairs of flowers was still only affected on their first execution each bout, preventing a transition from being affected multiple times per foraging bout. This change to the learning process reflects the idea that bees would reduce how often they visit a flower when their previous nectar expectations are not met anymore (Greggers & Mauerlshagen, 1997; Gil et al., 2007; Gil, 2010).

2.4 - Comparisons between observations and simulations

This updated model was used to predict the outcomes of the controlled experiments. As each experiment presented specific ways to replenish the flowers during its duration, the model was adapted to replicate these specificities. To explore the effect of each type of reinforcement, 4 variants of the models were derived from the main algorithm, based on the use of each reinforcement. A model using the positive reinforcement (Model 1[+]), a model using only the negative reinforcement (Model 2[-]), another using both reinforcements (Model 3[+/-]) or finally none (Model 4[Null]).

For the one bee experiment, each of the 4 models was run for each day, as rewarding flowers changed between days. For each day of the experiment, each model was run for 100 simulations, each lasting 10800 seconds (i.e. 3 hours). For the five bees experiment, the 4 models were run only once for all the experimental days as there were no constraints on the rewarding flowers. Each model was run for 100 simulations of 10800 seconds. For each model we looked at the same statistics and criteria as the experimental data (when applicable) with similar statistical models, but only compared the experimental and model data graphically. To assess if our variables were significantly affected by experience (successive foraging bouts), we observed the slope values of the GLMMs and considered them to be statistically different from 0 if their 95% confidence interval did not include 0. The lower and upper confidence interval limits were calculated and reported in Table 1 and Table 2 along with their associated variable.

3 - Results

3.1 - Natural experiment

We monitored the visits of bees coming to a patch of *Borago officinalis* on which we marked each open flower and reported their 3D positions. A total of 8 bees were tagged throughout 4 days of observations. First we pooled all the visits made to flowers of the patch by each bee

during a day, and used them to compute a Mean Location Estimate, a cloud of points representing their average foraging area. All individuals seemed to use similar areas, as MLEs appear to overlap (Fig 4). For the few individuals that were followed multiple days in a row, the MLEs of different days differed, likely occurring as previously opened flowers wilted and new flowers emerged, forcing foragers to change their exploitation area. This lack of partitioning could be simply explained by a general lack of rewards. The labelled flowers rewarded very low amounts of nectar (volumes could not be reliably assessed as they were lower than $0.1\mu\text{L}$), which could explain why bees would have to visit most flowers. The amount of nectar rewards per flower was lower than what would be typically expected from a borage plant (Descamps et al., 2018; 2021), which could be explained by various abiotic factors such as temperature, humidity or soil quality. We verified that the bees observed during the experiment were not pollen foragers, by the lack of accumulated pollen in the baskets.

Then, we looked at the distribution of trips between flowers, and compared them to the distribution of inter-flower distances in the patch. If the bees navigated between any flower at random, we would expect the two distributions to match. Because there were opened flowers on both borage plants during the first three days the distributions appeared to be bimodal (Fig 5a-c). Bees displayed a non-negligible amount of long distance movements, representing in part the movements bees made between the two borage plants. Throughout the four days where these “inter-plant movements” appeared, they represented 26.81% of all movements. Bees transitioned between the two plants typically 0 to 3 times per foraging bout, but rarely they did so up to 30 times per foraging bout (Fig 6). Bees very rarely displayed movements between nearest-neighbours. Bees travelled higher distances between successive visited flowers ($40.88 \pm 26.29\text{cm}$ - excluding inter-plant movements) than what they would have if using nearest-neighbour movements ($3.84 \pm 2.82\text{cm}$). Throughout the 4 days of observations nearest-neighbour movements represented a very small minority of movements (0.38% of movements).

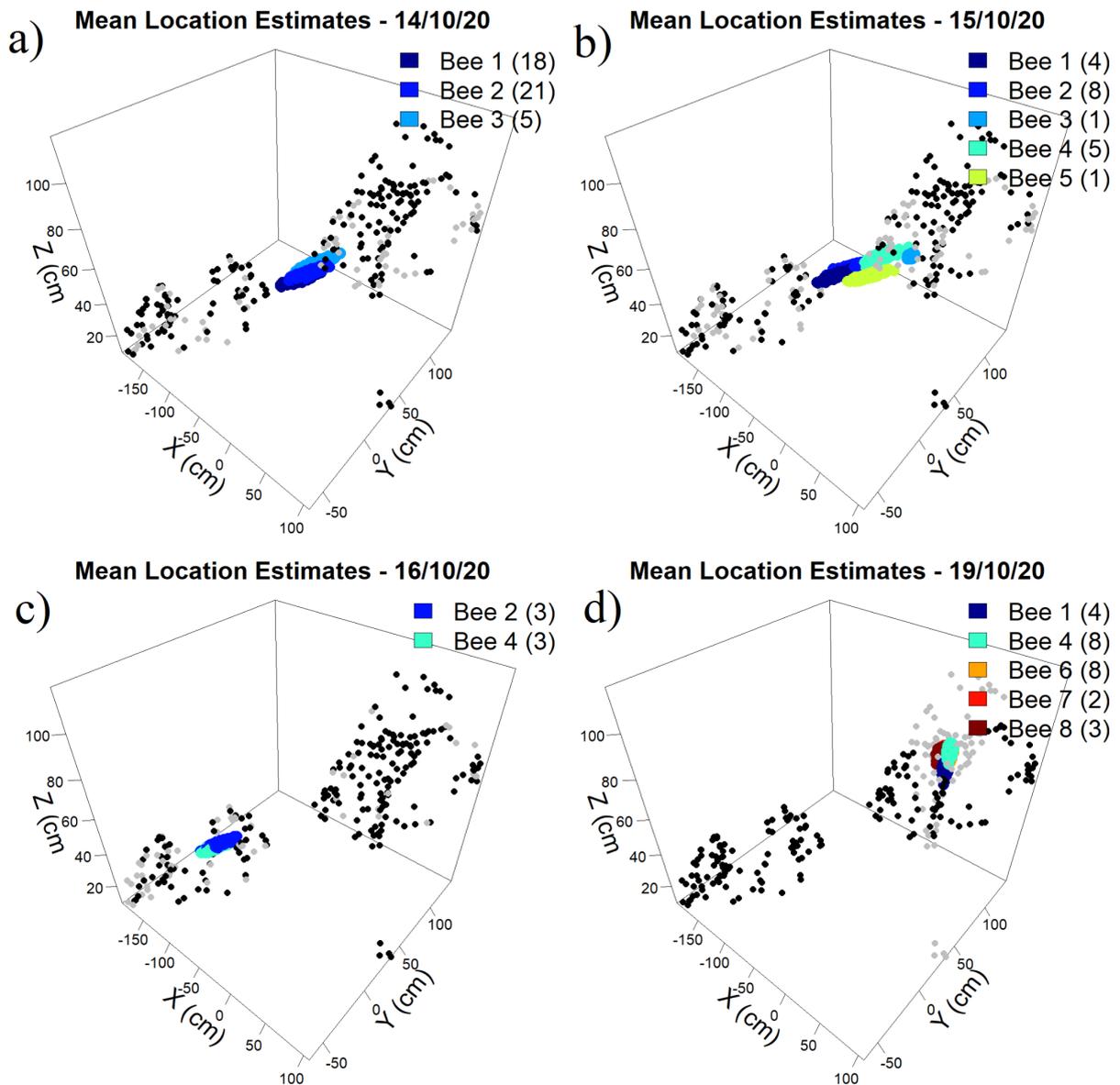


Fig 4: Mean Location Estimates for each day of observation (a-d). Legend for each day shows which individual foraged and in parentheses the number of visits to the plant. Black dots represent unvisited flowers (either open but not visited, or closed), while grey dots represent visited flowers.

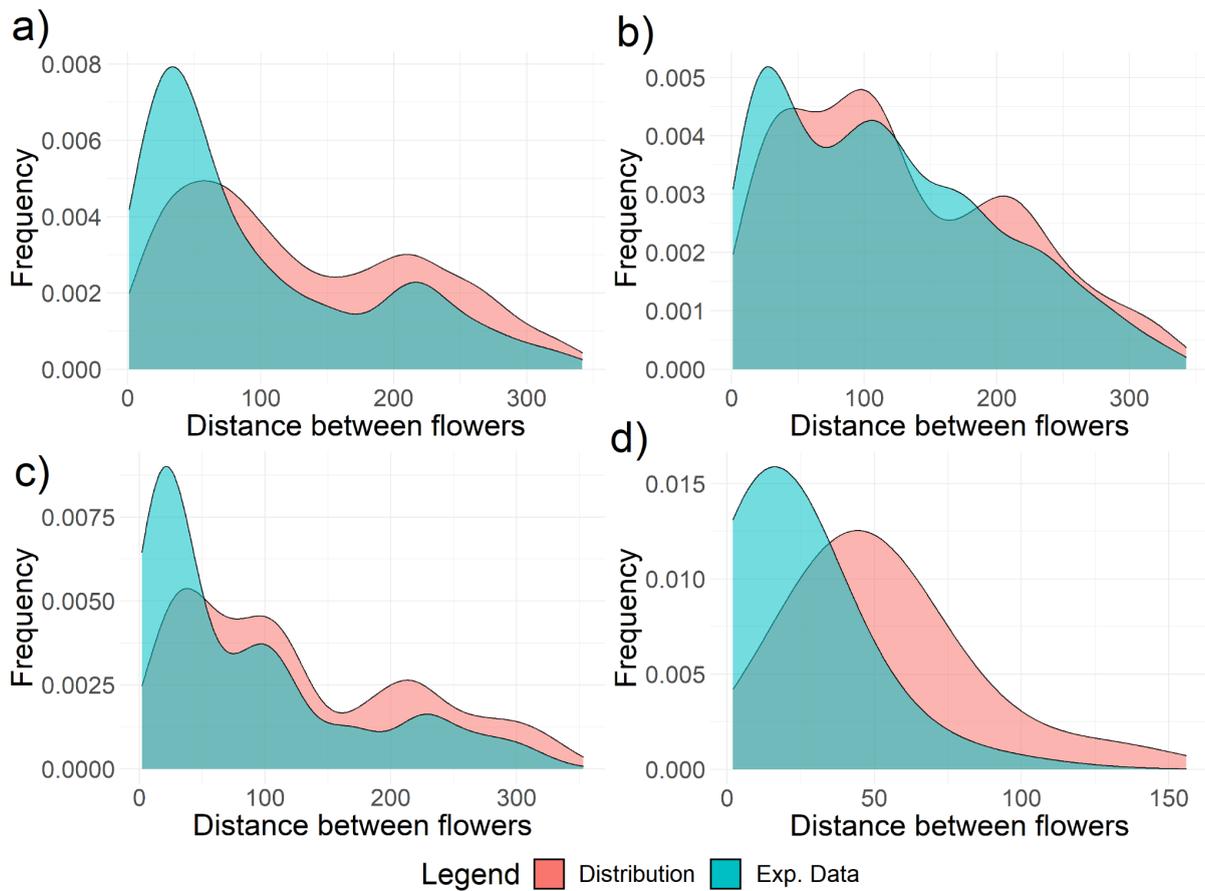


Fig 5: Inter-flower travel frequencies for each observation day (a-d). Each day, the data of all observed bees were pooled together (blue) and compared to the distribution of distance between all flowers of the patch (red).

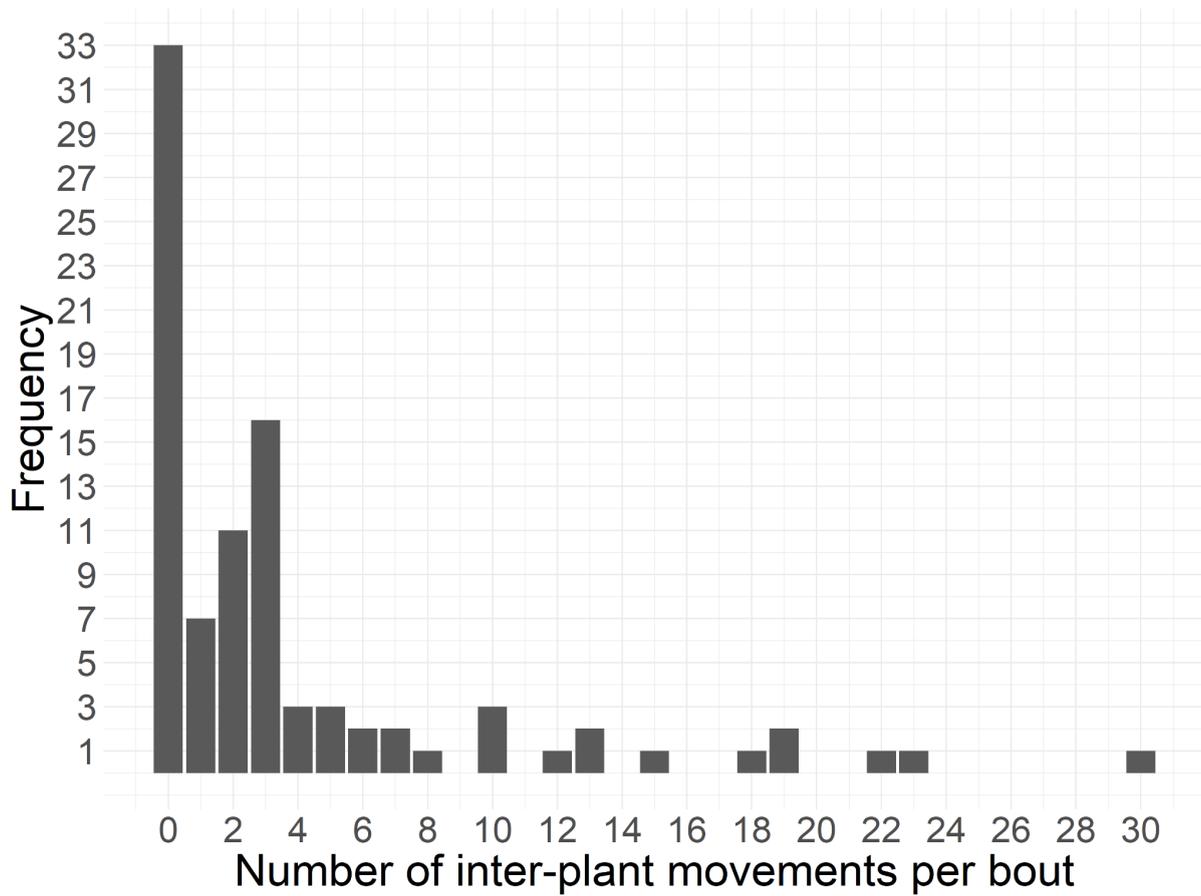


Fig 6: Number of transitions between the two monitored plants of borage by bees per foraging bout. All 4 days of observations are pooled together.

3.2 - Controlled experiment: One bee

We next ran an experiment with a set of artificial flowers to see if and how a single bee in a stable, competition-free, small scale environment would improve its foraging efficiency. In this experiment, we trained a single bee to forage on an artificial array of 64 flowers, of which 12 were constantly rewarding throughout the period of observation. All flowers that were depleted during a foraging bout of the focal bee were replenished before its next visit. We also compared the results of this experiment with our models. For all the criteria observed, the experimental data of the 4 days were pooled together for analysis, although there is no certainty that the different rewarding flowers chosen pseudorandomly during each day did not affect the experience of the foragers. All the statistical results are shown in Table 1.

We first examined the ability of bees to form traplines through our similarity index. The index did not significantly increase with successive foraging bouts (Table 1), suggesting bees did not form traplines (Fig 7a). In comparison, the models predicted slightly lower values of this index. All models showed no significant variation of the similarity index. Besides the slight underestimation in route similarity, the models predicted correctly the lack of development of traplines in this experiment.

Bees significantly reduced their visits to empty flowers with each successive visit to the array (Table 1 and Fig 7b). This suggests that although they did not establish traplines, bees still seemingly learned to avoid empty flowers. All the models showed a significant decrease in these visits to empty flowers with visibly different effect strengths. Given the much lower slope estimate for Model 4[Null] (100 times smaller than other models; Table 1), we still conclude that the decrease in number of visits to empty flowers is a result of the learning rules of the model, but we cannot identify clear different effects of the different learning rules, or their combinations.

Table 1: Statistical output for the experiments and model simulations in the one bee experiment. Comparisons of (i) route similarity index, (ii) number of unrewarded visits, (iii) distance travelled and (iv) number of rewarded visits through GLMMs using bee identity as a random effect. The results presented are the slope estimate \pm Standard Error of the Mean, along with a 95% confidence interval of the mean (Mean \pm 1.96*SEM) and the significance. The variable observed is counted as having a significant variation from 0 if the 95% confidence interval of the mean slope does not intersect the value of 0.

Variable	Data	Slope Est. \pm SEM	Low. 95% CI	Upp. 95% CI	Significance
Similarity Index	Exp. Data	3.59e-02 \pm 3.75e-02	-3.76e-02	1.09e-01	No
	Model 1[+]	1.98e-02 \pm 3.92e-02	-5.71e-02	9.67e-02	No
	Model 2[-]	-2.33e-02 \pm 1.18e-01	-2.54e-01	2.08e-01	No
	Model 3[+/-]	1.39e-01 \pm 1.05e-01	-6.65e-02	3.45e-01	No
	Model 4[Null]	7.84e-02 \pm 6.61e-02	-5.12e-02	2.08e-01	No
Unrewarded Visits	Exp. Data	-8.31e-03 \pm 1.20e-03	-1.07e-02	-5.96e-03	Yes
	Model 1[+]	-1.53e-02 \pm 2.24e-04	-1.58e-02	-1.49e-02	Yes
	Model 2[-]	-1.00e-02 \pm 2.25e-04	-1.05e-02	-9.60e-03	Yes
	Model 3[+/-]	-2.05e-02 \pm 2.25e-04	-2.09e-02	-2.00e-02	Yes
	Model 4[Null]	-5.16e-04 \pm 2.28e-04	-9.63e-04	-6.89e-05	Yes
Distance Travelled	Exp. Data	-5.759 \pm 3.214	-12.06	0.541	No
	Model 1[+]	-10.211 \pm 0.623	-11.43	-8.990	Yes
	Model 2[-]	-3.236 \pm 0.635	-4.48	-1.992	Yes
	Model 3[+/-]	-9.691 \pm 0.591	-10.85	-8.532	Yes
	Model 4[Null]	-0.693 \pm 0.695	-2.06	0.668	No
Rewarded Visits	Exp. Data	1.61e-03 \pm 2.98e-03	-4.24e-03	7.46e-03	No
	Model 1[+]	-9.04e-05 \pm 5.73e-04	-1.21e-03	1.03e-03	No
	Model 2[-]	-4.70e-05 \pm 5.85e-04	-1.19e-03	1.10e-03	No
	Model 3[+/-]	-2.79e-04 \pm 5.45e-04	-1.35e-03	7.89e-04	No
	Model 4[Null]	8.16e-05 \pm 6.41e-04	-1.17e-03	1.34e-03	No

While non-significant, bees tended to reduce the total distance travelled with experience (Fig 7c, Table 1). Our models with learning rules (Model 1[+], Model 2[-] and Model 3[+/-]) reduced significantly their total travelled distance with experience, while the null model (Model 4[Null]) did not. Overall, the models' predictions still appear to be quite faithful to the experimental data, suggesting that bees could have learned and reinforced their visits even at this small spatial scale. Nonetheless, it appears bees learn at a slower pace than our models, a suggesting in accordance with earlier models on the ontogeny of traplines (Reynolds et al., 2013).

Finally, neither the bees in our experiment or the models showed any variation in number of rewarding flowers visited, indicating that the the bees kept foraging until their crop was full during each foraging bout throughout the observation period, and that they did not require any learning period to find the different rewarding flowers of the array (Fig 7d, Table 1). Bees used nearest-neighbour movements significantly more often than what would be expected if they navigated randomly between flowers of the array (Fig 8a; NN: 73.29% of all movements). All the models (here pooled together as they gave very similar results) provided similar results (Model 1[+]: 69.67%; Model 2[-]: 61.29%; Model 3[+/-]: 63.14%; Model 4[Null]: 68.06%), suggesting this behaviour would be independent from any learning and more a consequence of the natural nearest-neighbour preference of bees that we reproduced in our models through the distance probability matrix.

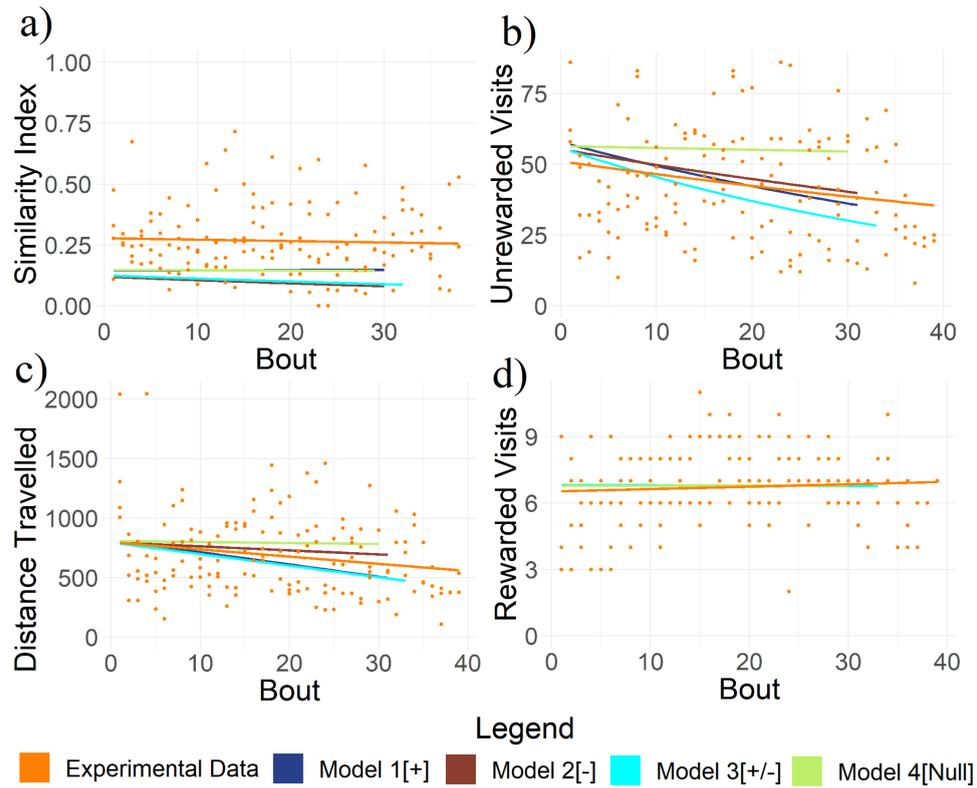


Fig 7: Graphical representations of (a) the similarity index, (b) the number of visits to unrewarded flowers, (c) the total distance travelled per bout (in cm) and (d) the number of rewarded flowers visited, for the experimental data of the 1 bee experiment and the 4 models (GLMs of the appropriate families: (a) binomial, (b,d) poisson and (c) gaussian).

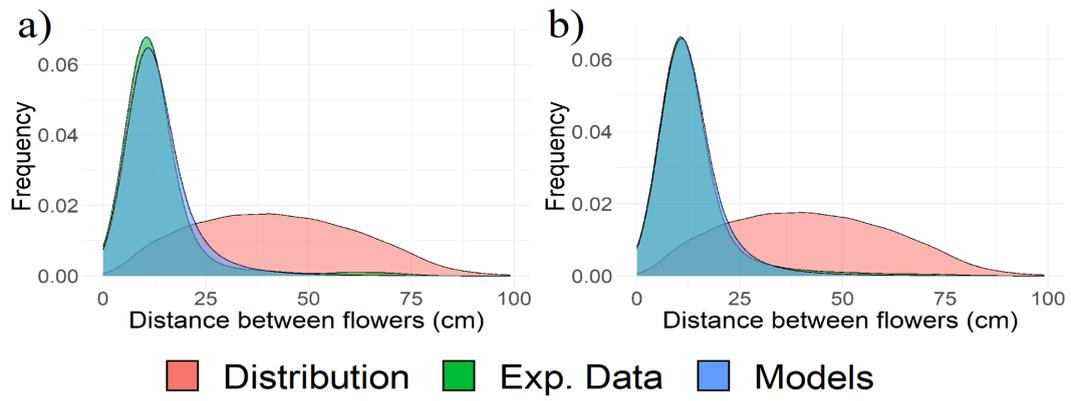


Fig 8: Inter-flower travel frequencies for (a) the 1 Bee experiment and (b) the 5 Bees experiment. Distributions for bees in our experiment (green; (a) $n=4$; (b) $n=35$), our models (blue; pooled together; 100 simulations of each of the 4 models) and the distribution of inter-flower distances between all 64 flowers of the artificial array (red).

3.3 Controlled experiment: Five bees

In this experiment, we were interested to see how 5 honey bees, naive to a new environment, would be able to improve their foraging efficiency throughout an observation period of 3 hours. We also compared these experimental results to our updated model predictions to see if simple learning mechanisms were able to explain the observed behaviour. All the statistical results are condensed in Table 2.

The bees presented no sign of repeated stable routes, as the route similarity index remained low throughout the whole observation period (Fig 9a), with no statistical difference with the null model (Table 2). All models concurred with this result, suggesting bees did not develop stable routes. The level of resource partitioning was constantly high in our experiment, but also in our models (Fig 9b), and the experimental data showed a slight, non-significant increase of this partitioning index during the observation period (Table 2). All models converged in their predictions (including the null model without any reinforcement), suggesting partitioning was not a consequence of the learning process but rather from how easy it became to partition between resources as their number increased, for the simple reason that even if bees navigated at random between flowers, the probability to encounter another bee or a flower already emptied by another bee diminishes with the number of flowers available.

Table 2: Statistical output for the experiments and model simulations in the five bee experiment. Comparisons of (i) route similarity index, (ii) resource partitioning index, (iii) number of unrewarded visits, (iv) distance travelled and (v) number of rewarded visits through GLMMs using bee identity as a random effect. The results presented are the slope estimate \pm Standard Error of the Mean, along with a 95% confidence interval of the mean (Mean \pm 1.96*SEM) and the significance. The variable observed is counted as having a significant variation from 0 if the 95% confidence interval of the mean slope does not intersect the value of 0.

Variable	Data	Slope Est. \pm SEM	Low. 95% CI	Upp. 95% CI	Significance
Similarity Index	Exp. Data	-7.09e-02 \pm 5.00e-02	-1.69e-01	2.71e-02	No
	Model 1[+]	4.23e-02 \pm 4.02e-02	-3.64e-02	1.21e-01	No
	Model 2[-]	1.75e-01 \pm 1.57e-01	-1.33e-01	4.83e-01	No
	Model 3[+/-]	7.88e-02 \pm 7.70e-02	-7.21e-02	2.30e-01	No
	Model 4[Null]	-1.49e01 \pm 8.84e-02	-3.22e-01	2.42e-02	No
Partitioning Index	Exp. Data	5.99e-02 \pm 4.32e-02	-2.48e-02	1.45e-01	No
	Model 1[+]	-9.94e-04 \pm 7.62e-03	-1.59e-02	1.39e-02	No
	Model 2[-]	-3.41e-03 \pm 6.94e-03	-1.70e-02	1.02e-02	No
	Model 3[+/-]	-7.11e-03 \pm 7.44e-03	-2.17e-02	7.46e-03	No
	Model 4[Null]	1.12e-03 \pm 7.66e-03	-1.39e-02	1.61e-02	No
Unrewarded Visits	Exp. Data	-1.06e-02 \pm 1.29e-03	-1.31e-02	-8.07e-03	Yes
	Model 1[+]	2.73e-04 \pm 3.18e-04	-3.51e-04	8.97e-04	No
	Model 2[-]	2.49e-04 \pm 3.17e-04	-3.72e-04	8.70e-04	No
	Model 3[+/-]	2.12e-04 \pm 3.24e-04	-4.23e-04	8.46e-04	No
	Model 4[Null]	2.76e-04 \pm 3.17e-04	-3.46e-04	8.97e-04	No
Distance Travelled	Exp. Data	-1.406 \pm 0.439	-2.267	-0.546	Yes
	Model 1[+]	1.199 \pm 0.125	0.953	1.445	Yes
	Model 2[-]	1.286 \pm 0.125	1.042	1.531	Yes
	Model 3[+/-]	1.279 \pm 0.128	1.029	1.529	Yes
	Model 4[Null]	0.766 \pm 0.125	0.521	1.011	Yes
Rewarded Visits	Exp. Data	-5.41e-03 \pm 1.42e-03	-8.18e-03	-2.63e-03	Yes
	Model 1[+]	9.08e-03 \pm 2.51e-04	8.59e-03	9.57e-03	Yes
	Model 2[-]	6.27e-03 \pm 2.54e-04	5.77e-03	6.77e-03	Yes
	Model 3[+/-]	8.02e-03 \pm 2.59e-04	7.51e-03	8.53e-03	Yes
	Model 4[Null]	5.04e-03 \pm 2.56e-04	4.54e-03	5.55e-03	Yes

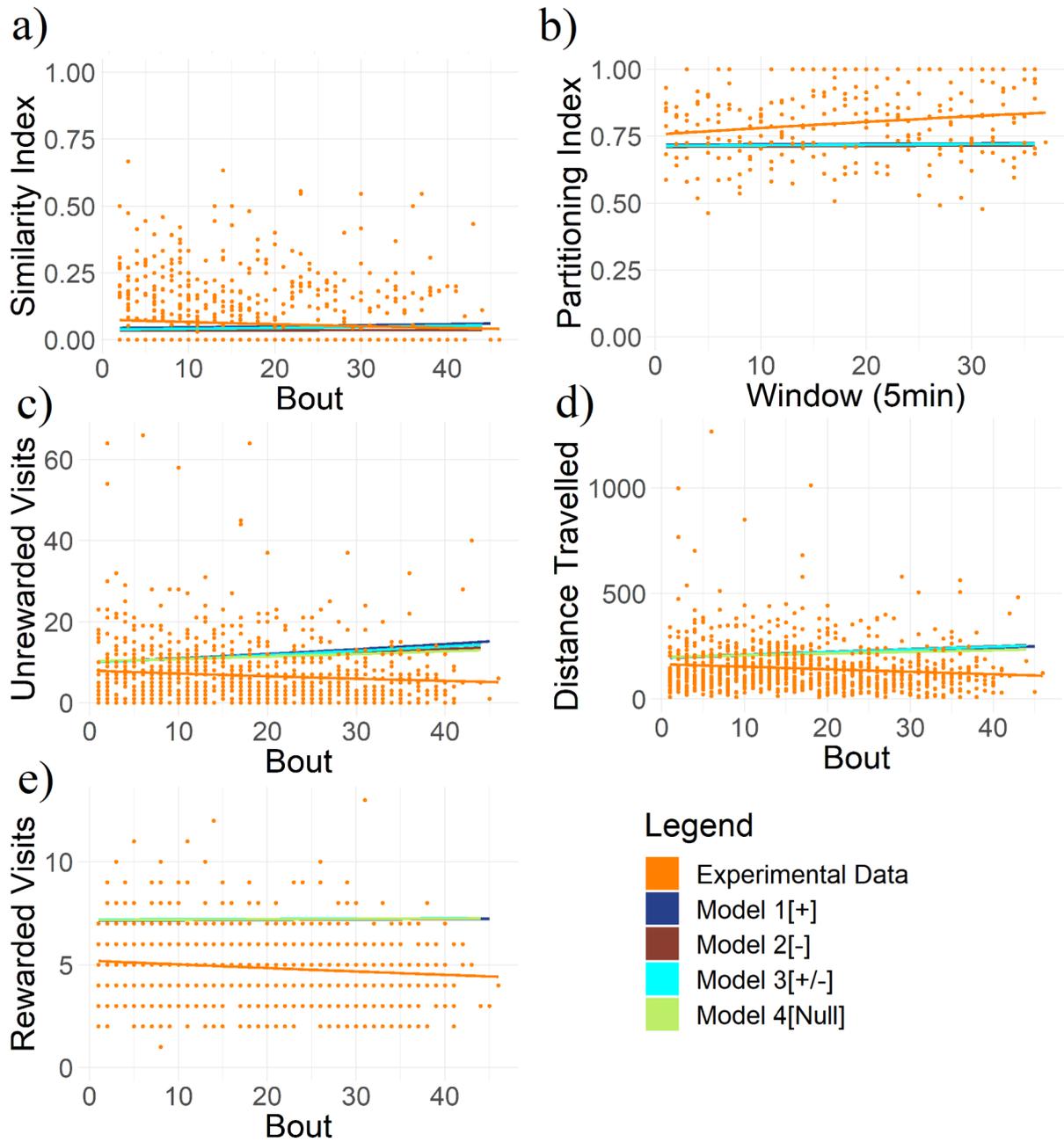


Fig 9: Graphical representations of (a) the similarity index, (b) the partitioning index Q_{norm} , (c) the number of visits to unrewarded flowers, (d) the total distance travelled per bout (in centimetres) and (e) the number of rewarded flowers visited, for the experimental data and the 4 models (GLMs of the appropriate family: (a,b) binomial, (c,e) poisson and (d) gaussian). All points of experimental data are displayed.

Similarly to the previous experiment with one bee, the five bees also significantly reduced the number of visits to empty flowers with experience (Fig 9c, Table 2), while all models presented a slight, non-significant increase. This interesting disparity between experimental data and model predictions points out potential mechanisms that our model could be missing. The convergence of all models indicates that learning is not responsible for the trend observed.

This decrease in visits to empty flowers correlated with a decrease in the total distance travelled by bees in our experiment with each successive foraging bout (Fig 9d, Table 2), which makes sense as the total distance travelled is directly linked to the total number of flowers visited. The models, which visited more empty flowers with each foraging bout, increased their total distance travelled, showing the same correlation between visits to empty flowers and distance travelled.

Bees reduced the number of rewarded flowers visited with experience (Fig 9e, Table 2), while all models slightly increased theirs. Although significant, all effect strengths were low, suggesting the variation in number of rewards acquired was minimal.

Finally, bees used nearest-neighbour movements much more often than expected if they were navigating between any 2 flowers of the array (Fig 8b), in both the experiment (NN: 72.00% of all movements) and all the models (Model 1[+]: 70.28%; Model 2[-]: 65.82%; Model 3[+/-]: 67.81%; Model 4[Null]: 68.34%).

4 - Discussion

One of the major components of foraging time for bees is time spent visiting flowers in a patch. In Chapter II, we argued that the foraging behaviour of bees was more likely to not include cognitively intensive mechanisms, and that foraging optimisation could be better

explained by simple behavioural rules and constraints of the environment and of the bees' biology. In these experiments, we set out to observe bees foraging on artificial and natural arrays of flowers, and to quantify their foraging behaviour to see if and how they would optimise their foraging efficiency. We compared part of our experimental results to our behavioural model's predictions to see if simple positive and negative reinforcement rules would be sufficient to explain their foraging optimisation.

4.1 - An absence of traplines

In our experimental setups, bees did not show any sign of traplining in any of our experiments; a result in accordance with other experiments in similar setups (bumblebees: Burns & Thomson, 2006; Saleh & Chittka, 2007; honey bees: Buatois & Lihoreau, 2016). Our models shared this prediction, which suggests that even if bees were to try to memorise the rewarding flowers they visited (which we showed leads to the establishment of traplines in many situations; Chapter III), no trapline would emerge. The lack of traplining here can be explained by the large number of spatially close flowers. Memorising a spatial location likely is a high cognitive load for the bee, and in the case of patches of flowers, bees can visit dozens of flowers before leaving for another patch. In this situation, the cognitive load of remembering a large number of flowers is likely to be inefficient, as the gain from finding the shortest path here would only save a negligible distance compared to what bees usually travel. It also becomes more difficult for bees, who may rely on storing views of locations with distinctive landmarks to navigate spatially (Dyer et al., 2008; Menzel et al., 2019), to discriminate flowers that are spatially aggregated.

Bees in our experiment mostly resorted to short movements, but not necessarily the nearest-neighbour movements. In the patch of borage, bees did not seem to bother using the nearest flower, and travelled approximately 10 times the nearest-neighbour distance between each flower. However, nearest-neighbour movements were the majority of

movements in both our controlled experiments, as well as the predictions of the models in these environments. A potential reason for this difference is in the interaction between how bees navigate and how the flowers were distributed in both types of environments. On a borage plant, flowers are distributed in 3 dimensions and can be partially hidden behind leaves of branches, making it much harder to gather information compared to our 2 dimensions array, where bees could easily see many if not all flowers by doing left/right movements. For the same reasons the shortest path appeared to be too cognitively intensive to find at the scale of a patch, so could be the “true” nearest-neighbour path. Instead, we should assume that bees would visit flowers opportunistically, based on which flower is first seen as the bee departs its current flower. This could also converge with records of bees retaining their flight direction between successive visits (Levin et al., 1971; Pyke & Cartar, 1992; Cresswell et al., 1995), as most often the first flower they would notice would be ahead of their current flower, in their line of sight. In addition, rewards per flower on the borage plant were minimal ($<0.1\mu\text{L}$), which could have led bees towards more exploratory behaviour and not as much exploitation of resources.

4.2 - Bees improved their foraging efficiency

Even without any displays of traplines, the honey bees in our experiments seemingly improved their foraging efficiency throughout the observation period. They displayed a high level of resource partitioning, an increased accuracy in avoiding empty flowers, and a lowered total distance travelled with each successive foraging bout, suggesting the bees did get more selective of the flowers they visited.

These results highlight how social interactions affected the development of foraging strategies of bees at the scale of a patch. Our natural experiment demonstrated that bees used nearest-neighbour movements preferentially, but that no partitioning between bees was possible, likely because of too low rewards. This specific result is difficult to interpret as no

control was exerted on the bees or the environment. Through the other two experiments, we designed an experiment addressing the lack of control of rewards we encountered in natural conditions, while trying to apply minimal control (bees coming at will) over the bees. In these new experiments, bees were able to improve their foraging efficiency. Yet our results suggest that the improvement in efficiency observed in single bees and groups of bees relied on different mechanisms.

Single bees presented initially much higher levels of visits to empty flowers and of distance travelled compared to the groups of bees, suggesting that being in a competitive environment greatly improved the bees' ability to forage efficiently. The models give potential insights as to why this difference was observed. While both the single bees and groups of bees reduced their visits to empty flowers and distance travelled (non-significant for single bees), the models only predicted correctly the behaviour of the single bee. For the 5 bees, the models predicted an increase of both these criterias. This difference would suggest that the 5 bees likely did not rely on reinforcement rules to improve their foraging efficiency. Instead we propose they used social cues like scent-marks (Giurfa & Nunez, 1992; Giurfa, 1993) or direct avoidance of competitors.

While scent-marks seem to be a passive deposit and not an active pheromone signal (Wilms & Eltz, 2008), bees have learned to use them to their advantage, meaning that naive foragers have seemingly no hard-wired response to their presence (Leadbeater & Chittka, 2011). Bees will usually learn to associate the presence of scent-marks with empty flowers (Giurfa & Nunez, 1992; Goulson et al., 2001) and appear to be able to discriminate between their own scent-marks and that of conspecifics or other species (Pearce et al., 2017). At the scale of a single patch of flowers, we do not expect bees to be able to gauge the presence of scent-marks on each single flower before it reaches the flower. The effect of scent-marks in this situation is mostly to deter the bee from landing and probing a flower (Williams, 1998), which in itself saves a substantial amount of time, as bees visit hundreds of flowers per

foraging bout. This could explain why our models, which did not include these behaviours, did not predict correctly.

This discrepancy in our models' predictions leads us to question how the algorithm could be improved through the addition of the social cues. The direct avoidance of competitors could be implemented through the adjustment of the transition probability matrix by artificially decreasing the probability of executing a movement towards a competitor (or increasing this probability in case of social learning). Scent-marks could also be implemented as a cue reducing the necessary time for a bee to gauge whether to land on a flower, ultimately allowing it to visit more flowers in the same amount of time.

Through these experiments, we learned that nearest neighbour movements, just like traplines, likely are specific outcomes of a simple foraging behaviour. We suggest that bees navigate within a patch by choosing the next flower to visit based on their perception and other olfactive and social cues, which will most of the time lead to the nearest flower as they theoretically represent the most striking visual stimulus. Groups of bees seem to forage more efficiently than single individuals, suggesting they are able to use social cues to their advantage. In general, bees are able to improve their foraging efficiency within a patch by reducing their number of visits to empty flowers, but, as expected, showed no sign of traplining.

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Chapter VI.

General Discussion

1 - Summary of results

Here we studied the foraging strategies of honey bees, and argued that bees likely only relied on very simple behaviours to form complex spatial foraging patterns. Throughout our modelling and experimental approaches, we suggested how bees manage to improve their foraging efficiency through simple positive and negative reinforcement rules applied to an environment with heavy constraints on resource distribution, both at the scale of a patch of flowers (intra-patch foraging) and between patches of flowers (inter-patch foraging).

No traplines were observed at the scale of a single patch (Chapters IV and V). At this spatial scale, bees were observed using nearest-neighbour movements, i.e. visiting flowers by moving from one flower to the next closest unvisited flower. This was in accordance with previous observations in the field and in the lab (Burns & Thomson, 2006; Saleh & Chittka, 2007). While we found this behaviour in our controlled experiments (when foraging on artificial flowers), bees nearly never used this strategy in natural conditions (when foraging on real flowers; Chapter V). We argued that if bees rely on their vision to find flowers within a patch, we should indeed not expect the next flower visited to always be the nearest-neighbour, but rather be chosen opportunistically. It is also possible that in this case as the flowers offered very low amounts of nectar ($<0.1\mu\text{L}$), bees might have adopted a near-far search pattern (Burns & Thomson, 2006) leading them towards longer distances travelled.

At a small spatial scale, bees were nevertheless able to show various degrees of partitioning, depending on the number of flowers available and their distribution (Chapters IV and V). Bees did not increase or decrease their degree of partitioning throughout the observation periods, suggesting the degree of partitioning was not affected by their experience in the patch and their history of rewards.

Where we could quantify their foraging efficiency (controlled experiments; Chapter V), bees displayed an improvement in their foraging efficiency. Groups of 5 bees were consistently more efficient than single bees in comparable setups. Both single and groups of bees showed a reduced number of visits to empty flowers, which was often linked to a decrease in total distance travelled. As our models failed to predict the improvements observed in groups of bees, we argued that they could originate from mechanisms unrelated to any positive or negative reinforcements, such as social cues (scent-marks, visual cues of competitors). Bees might have considered the positions of competitors currently on the array when choosing their next visit to a flower, thus avoiding areas of the array that were already exploited.

2 - Traplins as “paths of least resistance”

In Chapter II we introduced the idea that in some cases, natural selection would favour animals displaying more efficient foraging strategies, as they affect the fitness of these individuals. This theory was coined the Optimal Foraging Theory (hereafter “OFT”, Emlen, 1966; MacArthur & Pianka, 1966), and has since received a lot of attention, including in bees (Pyke, 1978; Waddington & Holden, 1979; Zimmerman, 1979;1982). Models of OFT have received substantial criticism regarding their underlying hypotheses (Pierce & Ollason, 1987), mostly because it is impossible to do anything but speculate on the currency of the optimisation, and whether this currency is applicable to any observation of bees during the foraging activity. However, the usefulness of the OFT is that it allows us to speculate on “null” optimal models of behaviour, under the assumption of a given currency being optimised. Yet, the different studies on the foraging behaviour of bees show that they are perhaps not optimising a single currency, but rather multiple currencies (e.g. nutrient intake rate, Lihoreau et al., 2011; distance travelled, Lihoreau et al., 2012a;2012b; nutrient balance, Kraus et al., 2019; risk avoidance, Ings & Chittka, 2008). These different currencies can also interact. Such interactions will lead to complex behaviours that are not optimal anymore for

any single given individual currency, but likely could reflect the best compromise or best estimate between the different currencies being considered. Presently we do not have a good theoretical framework for modelling optimality when multiple currencies are being considered.

This concept is relevant to the case of traplines at large spatial scales when bees forage on distance patches of flowers. The “perfect” traplines, as seen in controlled experiments (Ohashi et al., 2007; Lihoreau et al., 2012a; Lihoreau et al., 2012b), can be the result of the optimisation of a single currency (e.g. distance travelled, energy return) as all the other potential currencies are easily optimised in this narrowed context (e.g. no other flowers to explore, no variations in reward, no predation risk, limited competition, stable hive conditions). Meanwhile, the more complex behaviours observed in other situations (bumblebee: Saleh & Chittka, 2007; Woodgate et al., 2017; honey bees: Williams, 1997; Chapters IV & V) might reflect a compromise between these same currencies. How bees in this context compromise between the different currencies of foraging being considered will be very strongly influenced by the behavioural and environmental constraints we discussed in Chapter II.

Pierce & Ollason (Pierce & Ollason 1987) have argued that evolved strategies were not designed to solve problems; that independent of their function, their execution was neither premeditated or purposeful. In this thesis, we argued that traplines and the true nearest-neighbour movements were good examples of this concept. As such, we could benefit from revising our approach to these strategies to see them for what they are: paths of least resistance to the constraints of the foraging environment, only as much formed through a process of elimination of other options as a process of selection of certain options, but in no way through complex calculations and planning.

3 - Principle of parsimony and cognitive load

For any given currency that bees would want to optimise, it is important to consider that how they optimise this currency will also be limited by what is cognitively feasible for the animal. While there are many mechanisms that could be responsible for the emergence of strategies such as traplines (e.g. cognitive map: Menzel et al., 2005; multi-leg routes comparison: Reynolds et al., 2013; view-based navigation: Collett & Collett, 2002; Cruse & Wehner, 2011; Ardin et al., 2016), they seem to rely on processes that are more or less cognitively intensive. The idea that bees use a cognitive map, for example, is still debated (Cruse & Wehner, 2011) as it implies the existence of concepts that may not exist in bees (see Cheeseman et al., 2014 and ensuing debate from Cheung et al., 2014). When multiple explanations could explain the same behaviour, choosing the more parsimonious explanation has been suggested the best approach.

Principles of parsimony have for a long time been the norm in academic fields where multiple theories can explain the same phenomenon, such as Ockham's Razor (Sober, 2015). For the psychological and behavioural sciences Lloyd Morgan adapted the principle of parsimony in what has become known as Morgan's Canon (Morgan, 1903). This theory presented the idea that if "lower psychological faculties" could explain an action, it should be favoured against "higher psychological faculties" (Morgan, 1903), assuming a "psychological scale" to discriminate these notions of lower and higher. An enduring problem with this proposition has been that Morgan did not elucidate his psychological scale. More recent studies attempted to correct and improve it (Fitzpatrick, 2008), suggesting this scale was one of "sophistication" of cognitive processes. In our study, we made use of the concept of cognitive load to represent how computationally intensive different mechanisms are expected to be. An example of a cognitive load could be the requirement for visual learning relative to the estimated limit of the storage capacity of the mushroom bodies for visual memories, as has been assessed in ants in Ardin et al., (Ardin et al., 2016). They estimated

the number of visual snapshots an ant could store to around 375 before any new snapshot would be too likely to interfere with previously stored ones. Under the assumption that the computational capacity of bees is similarly limited, it becomes possible to assess through computational models what the cognitive load of a process might be.

In the new model we presented (Chapter III, and update in Chapter V), most of the path is established by behavioural and environmental constraints, which could translate into a low cognitive load for the bees, as the only information required for the bee would be the association between vectors and their estimated rewards. Such requirements appear low when compared to those used in the model of Reynolds et al. (Reynolds et al., 2013) which requires comparisons of 2 routes composed of multiple vectors each, which have to be summed and then compared, with the underlying assumption that both have equal rewards. The reliance of the model on these well-established constraints supports its ecological and cognitive feasibility, as well as a good complement to existing navigation models. Our approach to the traplining phenomenon also generates predictions on whether we should or should not see traplining appear for specific environmental conditions, and to what degree traplines would be expected to form. This suggests how we might further test our hypothesis. It would be possible to design semi-natural and controlled experiments in which we would and would not expect traplines and in which we would expect imperfect traplines. Examining behaviour of bees in these conditions would allow us to understand in greater details how these behavioural and environmental constraints affect the establishment of traplines.

4 - Models, a tool to answer “What If...” questions

Models are built using relevant knowledge of a system translated into a mathematical language as tools to explore the unknowns in the same system,. As such, they are not intended to prove or disprove hypotheses, as they only explore virtual simulations of what we think a phenomenon is, and not the phenomenon itself. Yet what appears to be their

weakness is actually their strength. Models allow to explore hypotheses based on what we might expect a system to operate. Models give us a tool to test any mechanism or specific scenario of interest without any of the logistic problems caused by experiments. They allow us to generate formal predictions based on an assumption of how the system operates that we can then compare to data collected from the real world. In the case of conflicting hypotheses to explain a same phenomenon, models can direct future experiments by suggesting an efficient way to discriminate the two hypotheses through an experiment.

Mathematical and computational modelling have diversified over the years, with the expansion of our knowledge and of computational power of computers. Many types of model are used to study different aspects of the biology and cognition of animals, such as differential equation models for the study of population dynamics (Khoury et al., 2013; Perry et al., 2015), machine learning and neural networks models allowing us to tackle complex problems by training a model to recognise and discriminate patterns (Valletta et al., 2017), and agent-based models relying on the high computation power of computers to simulate a large number of possible outcomes to a problem (Railsback, 2001; DeAngelis & Mooij, 2005). These models have in common their relation between the number of parameters and the specificity of their predictions. The more parameters there are in a model, the harder it is to find an analytical solution, forcing us to rely on numerical approaches. The fields of ethology and neuroscience are particularly fit for numerical approaches because of the high variability in behaviours between individuals, and the complexity of the systems being considered (Klein et al., 2017). In these cases, agent-based models offer a very interesting approach, as they provide a large distribution of potential outcomes of independent computer simulations, thus covering the potential diversity of behaviours naturally occurring (DeAngelis & Mooij, 2005).

In the case of our model, the technological challenge of tracking multiple bees in a large space brought forward the need to articulate more specific hypotheses as to what were

the mechanisms responsible for the emergence of traplining. We could then design experiments to test for the existence of these mechanisms in ways that used technology currently available. Through this model (Chapter III), we learned that traplines and resource partitioning could emerge from much simpler mechanisms than what was suggested in previous models (Reynolds et al., 2013). While there is still no clear answer for which mechanisms are responsible for these foraging strategies, our model has allowed us to take a step forward and suggest an alternative hypothesis (Chapters III, V). Hopefully in the future this will lead to more specific tests between competing hypotheses.

While future steps could be, as suggested from our results (Chapter V), to add new consideration for other mechanisms such as social cues to our models, it is important to keep in mind the necessary balance of models between precisely replicating the observed system by adding more features that are believed to impact the general outcome of the focal phenomenon, and the ability of a model to be generalised to a broader context. Indeed, a model with too much information might generate predictions so specific that their range may not affect any other conspecific for which the parameters would have varied. Moreover, with every added concept and parameter to the model comes underlying assumptions about how they affect the rest of the system and how that parameter should be quantified and mathematically expressed. There is a risk that as every more parameters are added to a model, the model drifts further and further from the real world. Thus, we must be exercising caution in adding new concepts to a model. On the other hand, a model with too few parameters might not be able to predict correctly the behaviour of the animals, because it is too simple to capture a complex reality. Because traplines are seemingly widespread throughout many taxa (Berger-Tal & Bar-David, 2015), it is more likely that they are the result of an evolutionary convergence, and as such could potentially be explained through mechanisms that are not specific to one species, or that at least have functional equivalence in other species.

5 - Conclusion

We have proposed a new way to look at the emergence of trapline foraging strategies of bees, one that is not bound to cognitively intensive mechanisms, but rather simple rules of foraging behaviour and constraints of the environment. We present phenomena such as traplines, or resource partitioning as consequences of environmental and behavioural constraints, a path of least resistance to the foraging activity of bees. We expect this new approach will bring forward more works on these simpler behaviours to identify traplines in a bottom-up approach by observing how all these constraints drive bees towards these strategies, rather than the current top-down approach of trying to define traplines in the only currently available conditions we have to observe them (i.e. controlled environments), which has potentially led us into thinking about them as this extraordinary ability of bees to solve this complex mathematical problem of the Traveling Salesman Problem. Realising that most of the complexity in the formation of traplines is resolved by the environmental and behavioural constraints will allow us to reframe the role of cognition in traplining.

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