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Carolyn A. Ayers Date

BEE FORAGING PATTERNS: NEW STATISTICAL METHODS AND THE EFFECT OF SPECIES RICHNESS AND SUB-LETHAL PESTICIDE EXPOSURE

BY

CAROLYN ANNE AYERS

B.S., THE COLLEGE OF WILLIAM AND MARY, 2011

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AN ABSTRACT OF
A DISSERTATION SUBMITTED TO THE FACULTY OF THE
JAMES T. LANEY SCHOOL OF GRADUATE STUDIES OF EMORY UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
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2016

ABSTRACT

BEE FORAGING PATTERNS: NEW STATISTICAL METHODS AND THE EFFECT OF SPECIES RICHNESS AND SUB-LETHAL PESTICIDE EXPOSURE

By Carolyn Anne Ayers

My dissertation consists of two distinct components, each represented by two chapters. In the first component, I develop new statistical techniques to quantify and test the significance of trapline foraging, a behavior in which foragers repeatedly visit spatially fixed resources in a predictable sequence. Though traplining is taxonomically widespread, the few metrics and null models that exist to statistically test traplining have substantial drawbacks. In my first chapter, I present a modified version of determinism from recurrence quantification analysis as a standard metric for quantifying traplines. Using empirical data to compare metrics, I find that determinism offers an improvement over other metrics since it does not depend on the arrangement of resources or experimental design, which allows for comparisons between differing environments. In my second chapter, I present a spatially explicit, individual-based null model designed to test whether resource layouts and realistic forager movements alone can account for suspected traplines. Using empirical data, I find that my null model is less prone to Type I or II statistical error relative to existing models.

In the second component, I use a foraging enclosure with artificial flowers to examine the effects of bee species richness and sub-lethal neonicotinoid pesticide exposure on functionally important bee foraging behaviors. Pollinator diversity is declining worldwide, yet it is relatively unknown how species losses will affect plant pollination services. In my third chapter, I examine how bee species richness drives patterns of bee specialization, which is important for conspecific pollen transfer. I find that species-level specialization and complementarity increase with bee species richness. The focus of my fourth chapter is exposure to neonicotinoid pesticides, which have been implicated as a potential driver of bee declines and have been shown to affect bee foraging behaviors at low concentrations. I examine how field-realistic neonicotinoid exposure interacts with lost bee diversity to affect bee behaviors important for bee fitness. I find that neonicotinoid exposure decreases total flower visits and bee energy gains in a multiple species context. These findings indicate that neonicotinoid exposure and bee species losses may negatively affect bee and plant fitness more greatly than previously anticipated.

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INTRODUCTION

Bees and other pollinators are important for pollination in agriculture and natural ecosystems. Pollinators are critically important for global food production, with approximately 2/3 of our food crops and 1/3 of calories depending on pollinators (Klein et al. 2007). The widespread loss of managed and wild pollinators is therefore a great challenge for global food security and the conservation of natural ecosystems (Biesmeijer et al. 2012, Potts et al. 2010). My dissertation is focused on factors affecting bee foraging patterns which can have direct implications for plant pollination.

My dissertation consists of two distinct components, in which I examine bee foraging patterns using a statistical and an experimental approach. In the first component of my dissertation (Chapters 1-2), I develop new statistical techniques to quantify and test the significance of suspected traplines, which will be discussed in greater detail in the next section. In the second component of my dissertation (Chapters 3-4), I use an experimental approach to examine the effects of bee species richness and sub-lethal neonicotinoid pesticide exposure on bee foraging patterns important for both plant and bee fitness.

TRAPLINING

Trapline foraging is a behavior where animals repeatedly visit spatially fixed, replenishable resources in a predictable order (Thomson et al. 1997). Traplining is a taxonomically widespread foraging strategy which has been observed in a variety of bee taxa (Ackerman et al. 1982); hummingbirds (Gill 1988); vultures (Deygout et al. 2009); bats (Woodsworth et al. 1981); and other mammals including rats, opossums, and

primates (Garber 1988). The widespread use of traplining may be in part because it increases foraging efficiency by allowing foragers to minimize travel and search times between resources (Ohashi et al. 2007, Lihoreau et al. 2011, Saleh and Chittka 2007). Increased foraging efficiency due to traplining has been shown to offer a competitive advantage to traplining foragers (Ohashi et al. 2008).

Due to the prevalence of the traplining in pollinators including bees and hummingbirds, traplining is frequently studied in terms of its functional implications for plant pollination. For example, traplines incorporating conspecific flowers may enhance conspecific pollen transfer (Ohashi and Thompson 2009). Traplining is also commonly used as a system for studying the role of spatial memory in solving complex routing problems (Lihoreau et al. 2011, Lihoreau et al. 2012), since complex cognitive processes, including spatial reference memory and iterative learning heuristics, are proposed mechanisms of trapline foraging behavior (Saleh and Chittka 2007, Reynolds et al. 2013).

Despite its importance, the difficulty of quantifying imperfect or highly variable traplines has generally prevented comparisons between traplining studies. Existing metrics have many shortcomings which make it difficult to compare traplining across multiple environments, including sensitivity to the number and spatial arrangement of resources. An appropriate metric for traplining is needed to quantify and compare the level of predictability in traplining sequences regardless of the resource layout or experimental design.

There are also very few null models for testing the significance of suspected traplines, which has led many traplining studies (e.g. Thomson et al. 1997) to only compare different foragers (i.e. is one forager traplining more than another?) rather than

asking the question of interest: is a particular forager traplining? A null model, or a pattern-generating models based on randomization of data or random sampling from a specified distribution, can be used to answer this question by deliberately excluding the mechanism of interest (Gotelli and Graves 1996). In the case of traplining, null models that exclude the use of spatial memory may be used to test the statistical significance of memory-driven traplining behavior observed in empirical data. An appropriate null model for traplining will be especially important for explicitly testing the role of individual learning and decision-making versus the role of non-cognitive factors (e.g. the spatial geometry of resources) or cognitive processes of lesser interest (e.g. the type of random walk used by the forager).

BIODIVERSITY ECOSYSTEM FUNCTIONING RELATIONSHIPS

One of the key findings in ecology over the past 25 years, is the discovery that greater biodiversity leads to greater ecosystem functioning and stability. Biodiversity ecosystem functioning (BEF) relationships are also important for people since ecosystems provide an invaluable suite of services, including cleaning air and water, cycling nutrients, preventing floods, and pollinating crops (Egoh et al. 2007). With bee species diversity declining worldwide, understanding BEF relationships will be vitally important for predicting how bee species losses will affect plant pollination services.

One of the primary mechanisms that drive BEF relationships is complementarity (i.e. niche partitioning), where different species specialize on different resources (Loreau et al. 2001). Complementarity provides ecosystem stability by promoting greater overall resource use in a community (Hooper et al. 2005). Traditional studies of BEF have

focused on fixed traits in plants (Tilman et al. 1997) and assume that the fundamental niche (the total breadth of resources a species can use) always equals the realized niche (the breadth of resources actually used by a species).

However, this assumption is not necessarily valid in plant pollinator systems, since pollinators can rapidly change their foraging patterns in response to competition over ecological timescales (Pimm 1985, Rosenzweig et al. 1991, Bolnick et al. 2010). In other words, when phenotypic plasticity is present, as is the case with pollinators, the realized niche does not always equal the fundamental niche. These dynamic (i.e. plastic) behavioral traits, while little studied in a BEF framework, can have direct consequences for plant pollination and reproduction.

Previous studies have shown that interspecific and intraspecific competition have opposing effects on specialization, where interspecific competition increases niche breadth while intraspecific competition decreases niche breadth (e.g. Rosenzweig 1991). For example, bees become more specialized in the presence of interspecific competition (Inouye 1978, Fontaine et al. 2005), whereas they become less specialized following release from interspecific competition (Brosi & Briggs 2013).

It is not well understood, however, how phenotypic plasticity (whether morphological or behavioral) can change BEF relationships. If species tend to specialize on resources they are most efficient at utilizing in the presence of competition, I predict that phenotypic plasticity will lead to greater ecosystem productivity than previous predicted by studies examining fixed traits.

POLLINATORS AS A STUDY SYSTEM FOR BEF

Pollinators are an excellent study system for examining BEF relationships mediated by phenotypic plasticity. Pollinators have been shown to rapidly change their behavior in response to competition (Inouye 1978, Fontaine et al. 2005, Brosi & Briggs 2013), and their behaviors have direct functional impacts on plant pollination.

Pollinator behaviors are important for plant pollination at individual, species, and community levels (Brosi 2016). At the individual level, specialization and floral fidelity are both important for transferring conspecific pollen (Morales & Traveset 2008, Flanagan et al. 2009, Waser 1986, Chittka et al. 1999, Brosi & Briggs 2013). Conspecific pollen transfer, or the transfer of pollen between flowers of the same species, is important for plant reproductive success, while heterospecific pollen, or pollen transferred between flowers of different species, does not benefit plant reproduction. The term "specialization" refers to a foraging pattern where bees visit a high proportion of one flower type, whereas floral fidelity refers to when bees regularly transition between flowers of the same species. Floral fidelity in particular leads to greater plant reproductive success, as bees visit flowers in an order that allows for great conspecific pollen transfer (Morales & Traveset 2008, Flanagan et al. 2009, Waser 1986, Chittka et al. 1999, Brosi & Briggs 2013).

Specialization at the species level, where individuals of a particular bee species tend to specialize on the same plant (Brosi 2016), has also been shown to aid in conspecific pollen transfer and seed set (Fründ et al. 2013). Species-level specialization can be decoupled from individual-level specialization (Bolnick et al. 2010). For instance,

species-level specialization increases as niche overlap between individuals decreases, even if the average niche width of individuals remains constant (Brosi 2016). Finally, pollinator complementarity (i.e. niche differentiation at the community level), where different bee species specialize on different plant species (Brosi 2016), is associated with greater recruitment of diverse plant communities (Fontaine et al. 2005). Both species-level specialization and community-level complementarity could enhance functioning if particular pollinator species specialize on the plant species for which they have a relatively high pollination efficiency.

There is a small but growing BEF literature using pollinators (Fontaine et al. 2005, Greenleaf & Kremen 2006, Brittain et al. 2013, Brosi & Briggs 2013). One common finding is that the identities of the plant species have a large effect on the degree of complementarity, particularly because pollinator species vary in their ability to interact with different pollination syndromes (Fenster et al. 2004). For example, pollinators have been shown to prefer and be more efficient at utilizing flowers with a similar corolla length as their proboscis (Inouye 1980). One remaining challenge in the field is to determine the role of species richness per se in driving bee foraging patterns independent from the confounding effects of bee and plant functional groups. Additionally, most previous studies are also focused on a single hierarchical level, whereas there are bee behaviors important for pollination across the individual, species, and community level.

NEONICOTINOID PESTICIDES

Neonicotinoid pesticides have been implicated as one possible cause for bee species declines (Godfray et al. 2014). Neonicotinoids were introduced in the mid-1990's

and have become the most widely-used class of insecticides worldwide (Goulson 2013; Van der Sluijs et al. 2013). Neonicotinoids are systemic and are incorporated into plant tissues, including plant pollen and nectar which are consumed by bees. Sub-lethal neonicotinoid exposure at field-realistic concentrations has been shown to have detrimental effects on bee cognitive processes (Mommaerts et al. 2010). Low doses of pesticides have been shown to impair foraging behaviors including navigation (Henry et al. 2012), the ability to learn how to handle flowers efficiently (Stanley and Raine 2016), and the ability to collect pollen (Feltham 2014). Changes in pollen foraging due to sub-lethal neonicotinoid exposure has also been shown to negatively affect plant reproduction (Stanley et al. 2015).

Though it is well established that field-realistic exposure to neonicotinoid pesticides alters bee behavior, there is very little understanding of how sub-lethal exposure affects bee behaviors important for bee and plant fitness in a multiple versus single species context. Previous laboratory studies have primarily examined the effect of neonicotinoid exposure in single-species contexts (Morandin and Winston 2003, Schneider et al. 2012). In field studies, which likely contained multiple species, no one has specifically tested for the role of species diversity. Since environmental complexity (e.g. species richness) may increase the difficulty of learning and processing environmental cues (Dukas and Real 1993, Laverty 1994, Naug and Arathi 2007), neonicotinoid exposure will likely differentially affect foraging in single versus multiple species contexts. This could have important implications for how bees respond to pesticide exposure in complex natural environments where multiple species are present.

OVERVIEW OF DISSERTATION

My dissertation is focused on factors influencing bee foraging patterns important for bee or plant fitness. Bee foraging behavior is of great interest for conservation, since many bee behaviors play a vital role in promoting plant pollination and reproduction. Bee foraging is also an important study system in the behavioral and cognitive sciences, since bees are well-known for their resource optimization strategies and for solving complex routing problems. In this dissertation, I make new contributions to our knowledge of bee foraging by (1) developing new methods for statistically testing traplining, a common bee foraging strategy; and (2) examining how two key conservation threats for bees (bee species losses and sub-lethal neonicotinoid exposure) can alter bee foraging behaviors important for both plant and bee fitness.

My dissertation consists of two distinct components, in which I examine bee foraging patterns using a statistical and an experimental approach. In the first component of my dissertation (Chapters 1-2), I develop new statistical techniques to quantify and test the significance of trapline foraging, a taxonomically widespread behavior in which foragers visit spatially fixed resources in a repeated sequence. In the second component of my dissertation (Chapters 3-4), I use a foraging enclosure with artificial flowers and four total bee species to examine the effects of bee species richness and sub-lethal neonicotinoid pesticide exposure on bee foraging patterns important for both plant and bee fitness.

Each of my four chapters also serves as an independent unit. Chapter 1 is published in a peer-reviewed journal, Chapter 2 is in review, and Chapters 3-4 are in preparation for submission to peer-reviewed journals. I am the lead author on all four

papers, and I have also worked with collaborators who offered invaluable input and support for each chapter.

In my first chapter, I present a standard metric to quantify traplining using a modified version of determinism (DET) from recurrence quantification analysis. I find that DET offers an advancement over other metrics for sequential behaviors, including traplining, since it allows for comparisons between differing environments in a range of ecologically important contexts. Determinism can also be used to analyze other sequential behaviors, including bird mating dances or insect grooming sequences. I co-authored this paper with Paul Armsworth and Berry Brosi. Brosi, Armsworth, and I together conceived and designed the study. I executed the study and wrote the manuscript with input from Brosi and Armsworth. The chapter is now published in *Behavioral Ecology and Sociobiology* (69(8): 1395-1404, August, 2015; reprinted with permission of Springer).

In my second chapter, I present a spatially explicit, individual-based null model designed to test whether realistic forager movements and the spatial layout of resources alone can account for suspected traplines. Using two sources of empirical data, I compare my spatially explicit model with two existing models: a completely random model and a sample randomization model. I find that my null model is less prone to Type I error relative to a random null model, and less prone to Type II statistical error relative to a sample randomization model. This type of null model may be useful for many other spatially explicit and individual-based processes, which are currently at the forefront in the field of ecology. I co-authored this work with Paul Armsworth and Berry Brosi.

Similarly as in Chapter 1, Brosi, Armsworth, and I conceived and designed the study. I executed the study and wrote the manuscript with input from Brosi and Armsworth.

In my third dissertation chapter, I examine how bee species richness drives bee foraging patterns important for plant pollination at the individual, species, and community level. I find that increasing bee species richness leads to greater specialization at the species level and greater niche partitioning at the community level. I co-authored this work with Emily Dobbs, Anna Mayrand, and Berry Brosi. Brosi and I conceived of and designed the study. I took the lead role in writing the paper and analyzing the data, with input from Brosi. Dobbs, Mayrand, and I all collaborated on collecting trials. Dobbs was instrumental in obtaining and maintaining bee stocks in the lab. Dobbs and Mayrand maintained the foraging enclosure.

In my fourth chapter, I examine how sub-lethal neonicotinoid exposure interacts with species richness to affect bumble bee (*Bombus impatiens*) behaviors important for bee fitness. I found that neonicotinoid exposure increases the number of flower visits and bee energy gains in a single-species context. However, exposure decreases total flower visits and bee energy gains in a multiple species context, which is more representative of real-world bee communities. This finding indicates that neonicotinoid exposure may have a greater effect on bee fitness than previously predicted by single-species experiments. I co-authored this work with Emily Dobbs, Anna Mayrand, and Berry Brosi. Brosi and I conceived of the study. I collaborated with Brosi, Dobbs, and Mayrand to design the study and plan trials, and I collaborated with Dobbs and Mayrand to run the trials. I took the lead role in writing and analysis with input from Brosi.

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Chapter 1.

DETERMINISM AS A STATISTICAL METRIC FOR ECOLOGICALLY IMPORTANT RECURRENT BEHAVIORS WITH TRAPLINE FORAGING AS A CASE STUDY

Ayers, Carolyn A., Paul R. Armsworth, and Berry J. Brosi. *Behavioral Ecology and Sociobiology* 69, no. 8 (2015): 1395-1404. Reprinted with Permission from Singer.

ABSTRACT

Patterns of discrete behaviors tied together in specific sequences are essential for the formation of complex behavioral phenomena. Such behavioral sequences can be of critical ecological importance, for example relating to resource acquisition, predator evasion, and sexual selection. The role of sequential behaviors in ecology, however, is understudied, in substantial part due to the difficulty of quantifying complex sequences. Here we present a modified version of determinism (DET) from recurrence quantification analysis (RQA) as a standard metric for quantifying sequential behaviors. We focus on a case study of trapline foraging, a taxonomically widespread behavioral strategy in which animals repeatedly visit spatially fixed resources in a predictable order. Using a bumble bee movement dataset, we demonstrate how to calculate DET and create and interpret recurrence plots, which visually demonstrate patterns in foraging sequences. We show a new method for statistical comparisons of DET scores, and assess the sensitivity of DET to resource density using simulated foraging sequences. We find that DET complements and offers distinct advantages over previously available methods for many questions and datasets since it does not depend on any particular resource arrangement or experimental

setup and is relatively insensitive to resource density. These features make DET a powerful tool for comparing sequential behaviors between differing environments in a range of ecologically important contexts.

INTRODUCTION

Patterns of discrete sequential behaviors are essential for the formation of complex behavioral phenomena. Such sequential behaviors can be of key importance for ecological or evolutionary processes, including host-parasite and predator-prey interactions, optimal foraging, and sexual selection. For host-parasite and predator prey interactions, these behaviors may include sequential grooming behaviors (Fentress and Stilwell 1973; Berridge et al. 2005; Kristan 2014) and patterns of time allocation to foraging versus scanning for predators in birds and mice (Caraco 1982; Maubourguet et al. 2008). Sequential behaviors are also important for establishing daily foraging patterns (Champion et al. 1994), including foraging on different prey or resource types, for example, pollen- versus nectar-focused foraging in bumble bees (Vaudo et al. 2014). Sequential behaviors which drive sexual selection may include the establishment of social dominance (Chase 1982) and complex courtship dances (Barske et al. 2011). Innate sequential behaviors have long been studied in the context of fixed action patterns, where a series of behaviors are completed in response to a distinct stimulus. A classic example is the greylag goose, which uses a series of egg rolling motions to return an egg to the nest when an egg is displaced, and performs the entire behavioral sequence even when the egg is removed (Lorenz and Tinbergen 1970). Many sequential behaviors,

however, are learned plastic behaviors that form in response to environmental stimuli and may greatly vary between individuals.

While a substantial body of work is devoted to the examination of the neurological process of learning sequential behaviors (e.g. Melamed et al. 2004; Rhodes et al. 2004; Jin et al. 2014), their role in ecology is understudied. This is due in large part to the difficulty of quantifying ordered patterns from a time series of behaviors. Though sequential behavioral data have been recorded at set time intervals to examine time budgets, a method in use for decades (Wiens et al 1970), these data are nearly always analyzed as the proportion of time spent on each activity (Williams et al. 1997; Sabine et al. 2008), which may obscure important temporal patterns. Other studies examining repetitive behavioral sequences only measure the rate of sequence initiation, without quantifying the degree of variation between sequences (e.g. Berridge et al. 2005). From these analyses, it is impossible to statistically test whether a particular sequence order is important for the outcome of the behavior, as opposed to the presence of all the behavioral elements in a random order.

An ideal standard metric of temporal behavioral sequencing should have several features. It should be able to detect imperfect repeats in sequence data, such that the omission or addition of a particular sequence point would not entirely disguise an otherwise perfect sequence. It should be capable of detecting long sequence repeats, rather than only examining very short subsets of sequences, in order to distinguish between long and short sequential behaviors. An ideal standard metric should also be able to distinguish between forward and reverse-order sequence repeats, since in some cases behavioral sequences can be executed in either “forward” or “backward” directions.

Finally, an ideal metric should be broadly applicable to different types of behavioral sequences, beyond a single study system or experimental design. In order to facilitate comparisons across studies, it should be able to quantify sequence predictability without relying on a specific reference sequence. To our knowledge, no widely applied metric for behavioral sequences meets all of these criteria for an ideal standard metric. However, it is important to note that, while these features are important for a standard metric, they may not be desirable for all specific questions and datasets, in which case multiple metrics may be used.

Here we address the lack of standard metric by altering and assessing a new metric for behavioral sequencing. We propose determinism (DET), adapted from recurrence quantification analysis (RQA), as a new standard metric, and recurrence plots as a tool for sequence data visualization (Zbilut 1992; Trulla et al. 1996; Marwan et al. 2007). Determinism (DET) is a metric adapted from recurrence quantification analysis (RQA), which was originally developed to investigate nonlinear dynamical systems, and has been applied to many fields including engineering, chemistry, astrophysics, and climatology (Marwan et al. 2007).

Though determinism has many of the required characteristics of an ideal standard behavioral sequencing metric described above, to our knowledge it has not been previously applied to any animal behavior. DET is broadly applicable to many ecologically important sequential behaviors, and we assess DET using the case study of traplining, a foraging strategy where animals foraging on replenishing, spatially-fixed resources visit resources repeatedly in a predictable order (Thomson et al. 1997). Traplining is important ecologically since it allows foragers to minimize distance

travelled between resources, reduce search times, and improve overall foraging efficiency (Ohashi et al. 2007; Saleh and Chittka 2007; Ohashi et al. 2008; Lihoreau et al. 2011; Lihoreau et al. 2012a; Lihoreau et al. 2012b). Trapline foraging is also taxonomically widespread, occurring in a variety of bee taxa (Ackerman et al. 1982), as well as in butterflies (Gilbert and Singer 1975), hummingbirds (Gill 1988; Garrison 1999; Gass and Garrison 1999; Temeles et al. 2006; Tello-Ramos et al. 2015), vultures (Deygout et al. 2009) and many mammals including bats (Gould 1978; Woodsworth et al. 1981; Lemke 1989), opossums (Wooller 1999), rats and primates (Garber 1988). Traplining behavior is thought to be driven by complex cognitive processes, including spatial reference memory and iterative learning heuristics (Saleh and Chittka 2007, Reynolds et al. 2013).

Traplining represents an ideal case study for quantitative assessment of behavioral sequences because the sequence order is of critical importance, and is therefore often recorded in data collection efforts of traplining in contrast to many other behavioral sequence data. Because traplining is sequence dependent, researchers have also considered a number of different metrics for quantifying traplining behavior (Table 1), but as with other behavioral sequences, no metrics have been proposed that meet all the criteria we outline in the previous paragraph.

In this paper, we demonstrate how RQA can be adapted for studying traplining and other sequential behaviors in ecology. We use a publicly available dataset of bumble bee (Hymenoptera: Apidae: *Bombus*) foraging movements (Lihoreau et al. 2012a) to demonstrate how to calculate DET and create and interpret recurrence plots, which visually demonstrate foraging sequence patterns. Using simulated sequences, we

demonstrate a new method for statistical comparisons of DET scores, and we assess the sensitivity of DET to resource density.

METHODS

Case study system

Traplining, a foraging strategy where animals repeatedly visit replenishing, spatially-fixed resources in a predictable order, is particularly important in two areas of behavioral and ecological research. First, since traplining behavior is thought to be driven by complex cognitive processes, it is commonly used as a model system to study spatial memory and foraging decision-making (Saleh and Chitka 2007, Reynolds et al. 2013). Second, due to the prevalence of the behavior in pollinators such as bees and hummingbirds, traplining is often studied in the context of its functional implications for plant pollination because traplines incorporating flowers of the same species may enhance conspecific pollen transfer and plant reproduction (Ohashi and Thompson 2009).

As with other types of behavioral sequence data, few tools exist to quantitatively assess traplining. While existing traplining metrics (Table 1) can be used to detect basic patterns in foraging movements, none of these metrics satisfies all of the aforementioned criteria of an ideal standard metric. Several of these measures, such as the asymmetry index (Sokal 1991, Thomson et al. 1997), examine bias in the direction of transitions between pairs of flowers, which cannot be used to distinguish between long and short traplines, or to detect traplines that may occur in reverse order. Another measure, the variation in return cycle (Ackerman 1982, Gill 1988, Thomson et al. 1997), is based on variability in the time required for foragers to complete a trapline. This may be practical

to measure in field studies but does not contain any information on the sequence of resources visited. Thus, very different sequences with similar completion times would be indistinguishable. Other metrics, including sequence similarity indices (Thomson et al. 1997, Lihoreau et al. 2010), are designed to test the self-similarity of a foraging sequence, but are sensitive to which resource is selected as the start and end of the trapline. In terms of behavioral sequences more generally, it may be challenging to select a starting or ending behavior for a grooming sequence or mating dance. Similarity indices also require a previously specified sequence for comparison. One common approach is to compare the similarity of consecutive foraging bouts, but this does not allow for detection of repeated sequences between nonconsecutive bouts. Many metrics may only be used to compare foraging sequences from identical resource layouts, for instance, metrics which quantify the number of different routes taken. Finally, several metrics are specific to particular experimental designs, including the spatial geometry of routes (Thomson et al. 1997, Lihoreau et al. 2010). These often require resources not to replenish during a foraging bout or foragers to return to a home base at the start and end of each trapline. Many other traditional metrics of bee foraging, such as average rank (Ohashi et al. 2006), are not easily adapted to quantifying traplines since they do not depend on the identity of flowers visited in the sequence. Determinism (see Description and Calculation below), however, has all the aforementioned properties of an ideal standard metric for behavioral sequencing, and offers many distinct advantages over existing metrics.

Description and calculation of determinism and recurrence plots

Originally developed for non-linear systems, RQA also has many biological applications, including detection of physiological patterns in heart rate variability (Marwan et al. 2002; Zbilut et al. 2002b), respiratory data (Webber and Zbilut 1996), and brain electrical activity (Thomasson et al. 2001), as well as analyzing amino acid sequences (Zbilut et al. 1998; Zbilut et al. 2002a; Porrello et al. 2004; Yang et al. 2009). Despite its applications in many different fields, to our knowledge RQA has not been used to analyze animal behavior.

Determinism (DET) measures sequence predictability by quantifying the number and length of recurrences and series of recurrences. A *recurrence* refers to any time a system returns to an area of phase space which it has previously visited (Marwan et al. 2007). For behavioral sequencing applications, a recurrence occurs when a discrete behavioral action (e.g. a single step in a grooming or courtship sequence) is repeated anywhere in the behavioral sequence. In the case of trapline foraging, a recurrence occurs whenever a forager revisits a resource. A *recurrent series* occurs when sequence elements are repeated in the same order (in either forward or reverse directions) in different parts of the sequence. DET is based on the proportion of recurrences (i.e. revisited behavioral actions) that belong to a recurrent series of a minimum designated length. In the case of traplining, DET represents the proportion of revisited resources which were visited in the same continuous order in multiple parts of the visitation sequence.

Determinism may be best understood graphically using recurrence plots, which visually depict the behavior of a dynamical system (Eckmann et al. 1987; Marwan et al. 2007). To construct a recurrence plot for traplining, we first assign a unique identification number to each individual resource (e.g. a flower). A resource visitation sequence is then

constructed by recording the unique number of each resource in the order of visitation by the forager. We construct the plot by placing the resource visitation sequence on both the horizontal and vertical axes, such that x_i and y_i both represent the i^{th} resource visited (on the *x* and *y* axes, respectively). A point is placed each time $x_i = y_j$, where *i* and *j* are the i^{th} and j^{th} resources visited in the sequence. Since the same sequence is placed on both axes, points always appear on the main diagonal, where $i = j$, and the plots are necessarily symmetrical across this diagonal. Points not on the main diagonal represent a revisit to a resource at a different time in the visitation sequence.

Determinism may be calculated directly using a recurrence plot, as follows:

$$DET = \frac{number\ of\ points\ above\ the\ main\ diagonal\ belonging\ to\ a\ contiguous\ diagonal\ of\ length \geq\ l}{number\ of\ points\ above\ the\ main\ diagonal} \qquad \text{eqn 1}$$

Where *'l'* indicates the minimum length (measured in number of contiguous points) of a recurrent series in order to be included in the numerator of the DET formula. Since recurrence plots are symmetrical across the main diagonal, we propose calculating DET only for the top half of each recurrence plot. This restriction, which departs from traditional forms of DET, is important for constructing a conservative statistical test of DET using generalized linear models (GLMs) with binomial errors (*see "Statistical Analysis" section*).

For example, in Figure 1, the hypothetical forager visited resources numbered:

1 2 3 8 9 10 2 3 4 5 7 3 8 9 10 7 5 4 8

beginning with “1” and ending with “8”. Each repeated number represents a revisit to a particular resource (e.g. resource number ‘8’ was visited three times by the forager). We first construct the recurrence plot by placing this sequence on both the *x* and *y* axes. A point is placed each time the resource identification number on the *x* axis equals the number on the *y* axis. Again, since recurrence plots are symmetrical, points on or below the main diagonal are not considered in our calculation of DET (light gray points in Figure 1). Points above the main diagonal which belong to unbroken diagonals of length $\geq l$ are included in the numerator of the DET formula (blue points in Figure 1). The denominator of the formula equals the total number of points above (but not including) the main diagonal.

Increasing the minimum length of a recurrence (*l*) typically decreases DET as it excludes points belonging to shorter diagonals, as demonstrated in Figure 1. When $l = 3$ (Figure 1A) the numerator of the DET calculation includes one diagonal of consisting of four points and one diagonal of three points:

$$DET_{l=3} = \frac{4(1) + 3(1)}{12} = \frac{7}{12} = 0.58$$

DET is dependent on the minimum sequence length, however, and when $l = 4$ (Figure 1B), the numerator of the DET calculation only consists of only one diagonal of four points:

$$DET_{l=4} = \frac{4(1)}{12} = \frac{4}{12} = 0.33$$

The structure of lines in a recurrence plot indicates when and how often the system returns to the same phase space. Vertical or horizontal lines indicate the system remains

fixed in space, such as when a forager repeatedly visits the same resource. Vertical or horizontal lines may be especially important for grooming sequences or mating dances where animals repeat behaviors multiple times in a row before advancing to the next behavior. Diagonal lines indicate repeats in the trajectory of the system, such as when a forager visits resources in a predictable order (i.e. traplining). Perpendicular diagonals indicate a reversal in the order of resources visited in a trapline. Such reverse sequences may be biologically relevant in the formation of optimal traplines, since the optimal route may be used in either clockwise or counterclockwise directions. However, such reverse sequences may not be relevant to all recurrent behaviors, in which case DET may be calculated without including perpendicular diagonals in the numerator of the determinism calculation (see Appendix 1). Exclusion of perpendicular diagonals may lead to lower estimates of DET, so it is important for users of determinism to document their inclusion or exclusion of perpendicular diagonals in order to facilitate comparisons across studies. Here, perpendicular diagonals are included unless otherwise stated.

STATISTICAL ANALYSIS OF DETERMINISM

Analysis of determinism with GLMs

To statistically compare the degree of traplining between two or more resource visitation sequences using DET, we propose the use of generalized linear models (GLMs) with binomial errors. DET values are typically not normally distributed, but instead more closely follow a binomial distribution since each point on a recurrence plot is either a "success" (belonging to a recurrent sequence) or "failure" (not belonging to a recurrent sequence). The use of a GLM with binomial errors allows for modeling nonlinear

responses of DET to differing levels of traplining (Figure 2). Since the statistical power of a binomial GLM depends on the number of counts (or points on a recurrence plot), we have modified traditional DET to only include the top halves of recurrence plots, which are symmetrical across the main diagonal, in order to maintain a conservative statistical test. We should emphasize that the GLM approach is for comparing traplining between two or more samples. To test whether or not individuals are "significantly" traplining requires an appropriate null model (e.g. Ohashi et al. 2007), which is beyond the scope of this paper, though DET could be used were such a model available.

To better understand how DET responds to varying levels of traplining, we analyzed DET values calculated from 1,044 simulated foraging sequences (See Appendix 1) with varying levels of predictability (Figure 2). To generate simulated sequences, we set a fixed probability that a forager would repeat a past transition, using a short sequence as a reference. To repeat a previous transition, the forager would repeat its behavior from the reference sequence. If the forager failed to repeat the transition, another resource was chosen at random, excluding the current resource and the one which would have led to a repeat transition. As expected, we found a highly significant positive relationship between DET and sequence predictability (Table 2).

Sensitivity to resource abundance

One potential issue in the analysis of behavioral recurrence data is that determinism could be sensitive to the number of potential discrete behaviors in a sequence, such that it might be inappropriate to compare behavioral sequences of differing complexity. In the context of traplining, sensitivity of determinism to the

number of possible resources would likely prevent comparisons across environments with different resource densities. To explore this issue, we analyzed DET values for hypothetical sequences with varying resource abundance (Figure 2). Using five resources as our baseline, we found that that DET was able to clearly distinguish variation in traplining despite a four-fold increase in resource abundance. This range of resource densities is applicable to the majority of traplining studies, which typically occur in controlled laboratory settings with a small number of resources (e.g. 6 resource points as in Lihoreau et al. 2012a, and up to 16 resources points as in Ohashi et al. 2008). Comparisons between extremely large numbers of possible sequence elements (e.g. 50 to 500 resources) may significantly impact DET (See Appendix 1), however such large numbers of possible elements are not likely to be required for the majority of ecological or behavioral applications. In rare cases where DET is likely to be sensitive to resource abundance, comparisons across studies are still possible after performing a sensitivity analysis of the effect of resource abundance on DET (See Appendix 1).

WORKED EXAMPLES

Here, we demonstrate how determinism and recurrence plots may be used to compare the behavior of one individual before and after gaining experience on a foraging array, or the foraging behavior of a number of different individuals. Recurrence plots were generated using the 'fNonlinear' package in R (Wuertz et al. 2013; R core team 2014). Calculations of DET were also performed in R, and the corresponding R code is provided in Appendix 1.

Recurrence plots were constructed using publicly available data from Lihoreau et al. (2012a). This dataset includes foraging data from eight individual bumble bees (*Bombus terrestris*) foraging in an enclosure containing six artificial flowers. Nectar rewards did not replenish and were calibrated such that bees typically visited all six flowers once per foraging bout, and the process was repeated until each bee performed 80 foraging bouts. The foraging data were processed to remove immediate revisits by a bee to a particular flower.

Figure 3 shows recurrence plots of the foraging behavior of a bee (indiv. 1 from Lihoreau et al. 2012a) after first entering the foraging array, and after gaining experience on the array (following 360 floral visits or approximately 60 foraging bouts). The DET calculations for these foraging sequences, with l=5, are as follows:

$$DET_{inexperienced} = \frac{26}{777} = 0.03$$

$$DET_{experienced} = \frac{517}{784} = 0.66$$

Using a generalized linear mixed-effects model with binomial errors, we found that traplining significantly increases after bees gain experience on the foraging array ($P = 4.77 \times 10^{-6}$; see Appendix 1 for full analysis).

Recurrence plots are useful to visually compare qualitative differences in traplining. Longer diagonal lines indicate greater predictability in the foraging sequence, corresponding to more numerous and consistent traplines. For the inexperienced foraging trials, all diagonals in the recurrence plot are short, and very few points belong to a long diagonal (Figure 3A). However, after the forager gains experience, the number and lengths of diagonals in the recurrence plot increases, and most points belong to a long diagonal (Figure 3B).

In Figure 4, we use recurrence plots to compare the traplining behavior of four different bees (indiv. 2, 4, 5, and 6 from Lihoreau et al. 2012a) after gaining experience inside a foraging array. The first two foragers shown have very predictable foraging sequences and therefore have high DET values, while the last two foragers have much less predictable sequences and low DET values. Though the pairs of predictable and unpredictable foragers have similar DET values, the recurrence plots reveal qualitative differences in traplining patterns. The recurrence plot for forager three, for instance, has a higher prevalence of diagonals perpendicular to the main diagonal. The percent of points in a perpendicular diagonal (out of all points belonging to a diagonal of $l=5$) was 40% for forager three, and only approximately 2% for foragers one, two, and four. This pattern indicates forager three was more likely to reverse the direction of its traplines. If we do not classify perpendicular sequences as recurrent series, we find that DET is slightly reduced for individuals one, two, and four (a decrease of 0.02, 0.01, and 0.01 respectively), and greatly reduced for forager 3 (a decrease of 0.12).

FINAL REMARKS

In this paper, we developed a modified version of determinism (DET) from recurrence quantification analysis (RQA) as a standard metric for quantifying sequential behaviors. We compared DET with existing metrics, and analyzed the sensitivity of DET to resource density using simulated foraging sequences in a case study of trapline foraging.

We found that many of the properties of determinism make it a promising metric for comparing sequential behaviors between a range of study systems and experimental

designs, which would not have been possible with existing metrics. First, we found that DET is able to detect recurrent patterns over the entire length of a behavioral sequence without relying on comparisons of sequential pairs or other subsets of the behavioral sequence. Second, we found that determinism is relatively insensitive to the number of possible sequence elements, thus allowing for direct comparisons between studies with roughly similar numbers of possible behavioral elements. Third, determinism does not require a specific start or end point or a particular sequence of interest, as is often the case with similarity indices (Thomson et al. 1997, Lihoreau et al. 2010). Fourth, DET is able to detect recurrent sequences from incomplete sequence data, which are the norm in field settings. Determinism also offers additional advantages for particular types of recurrent behaviors, for instance, allowing for detection of sequence repeats in either forward or backward directions, and the ability to set the minimum length of a recurrent sequence. Recurrence quantification analysis (from which determinism is derived) also offers additional tools (Marwan et al. 2007) which may be useful for quantifying other properties of interest, such as the average length of recurrent behavioral sequences.

One potential shortcoming of determinism, which is also prevalent among existing metrics, is that it may underestimate sequence predictability if there are many imperfections in otherwise consistent behavioral sequences. In such cases, DET can be extended by using modified DNA sequence alignment techniques to minimize the impacts of inserted or deleted elements in behavioral sequences (Waterman 1989; Thomson et al. 1997), a detailed discussion of which is beyond the scope of this manuscript.

Determinism may also need to be complemented with other metrics depending on the question of interest. For example, while DET can be used to detect the overall level of sequence similarity, it would not be used to directly compare an observed sequence with a specific reference sequence. In the case of trapline foraging, DET is useful to detect traplines following many different routes, but in some specific cases only one or two particular routes are of interest (Lihoreau et al 2012). For these cases, DET may be combined well with sequence similarity indices, which are designed to detect sequence similarity to a particular route (Thomson et al. 1997; Lihoreau et al. 2010). When combined, the two metrics may be used to ask additional questions, such as the proportion of all strong trapliners which are following a particular route of interest.

The straightforward application of determinism and other RQA-derived techniques will promote interest in the role of sequential behaviors important for ecological and evolutionary processes, including sexual selection (Barske et al. 2011), parasite-host interactions (Fentress and Stilwell 1973; Berridge et al. 2005; Kristan 2014), and efficient resource gathering (Lihoreau et al. 2012a, Vaudo et al. 2014). Specifically, the wealth of techniques developed for RQA may be applied to quantifying grooming sequences, complex mating dances or social dominance displays, as well as sequential patterns of time allocation. The versatility of RQA will enhance the use of sequential behaviors in ecology since it allows for comparisons of sequential behavior between individuals and across multiple studies, taxonomic groups, and environments. For example, sequential behaviors may be used for comparing ecologically important factors, such as perceived predation risk (Caraco 1982) or foraging efficiency, across varying environments. One might also use RQA to test whether the order of sequential

behaviors is critically important for the outcome or efficacy of the behaviors, as may be the case for complex mating dances or trapline foraging. In contrast, other sequential behaviors may be equally effective as long as all elements appear in the sequence, as may be the case for sequential grooming patterns. In these cases, functionally similar behaviors may be packaged together in a particular sequence only as a memory tool to decrease the likelihood of forgetting any one behavior. Though sequential behaviors important for ecological process have been traditionally neglected due to the lack of an appropriate metric for many questions and datasets, the versatility of determinism and RQA will enhance their use and potentially lead to important innovations in behavioral ecology.

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TABLES

Existing metrics for trapline foraging				
Name	*Description*	*Advantage*	*Disadvantage*	*Citations*
Asymmetry index	Measures bias in direction of transitions between pairs of flowers (first developed to test migration)	Can detect basic patterns in bee movements between pairs of flowers	Pairwise comparisons only; would not be useful whenever foragers repeat a trapline in reverse order	Sokal 1991, Thomson et al. 1997
Skeleton diagrams	Graphical depiction of asymmetrical transitions between pairs of flowers	Good visual summary of foraging movements	Pairwise comparisons only (very short repeats would be considered traplines)	Thomson et al. 1997
Variation of return cycle	Measures the variance in time or number of flowers it takes for a bee to return to the same point	Most practical measure when sequence data is difficult to collect	No information on identity of flowers visited (two very different paths could be of similar lengths)	Ackerman 1982, Gill 1988, Thomson et al. 1997
Sequence similarity indices	Similarity of sequences starting and ending with a "terminal flower"	Examines subsets of data longer than pairs of resources	Targets individual pre-specified trapline routes, so difficult to apply when there are many possible routes of interest; May be sensitive to the selection of the terminal flower	Thomson et al. 1997, Lihoreau et al. 2010
Spatial geometry of routes	Total number of different routes used by a forager between a specified start and end point (e.g. nest entrance)	Simple to calculate if sequence data is known; does not rely on pairwise comparisons	May only be used if resources are not replenishing and their spatial arrangement is fixed; only examines whether sequences are completely identical, and cannot distinguish degree of similarity between sequences	Thomson et al. 1997, Lihoreau et al. 2010

Table 1. Summary of existing metrics for trapline foraging

Determinism in response to sequence predictability and abundance

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*
Intercept	-9.34	0.18	-51.234	**$< 2 \times 10^{-16}$**
abundance 10	-0.29	0.33	-0.88	0.38
abundance 20	0.5	0.41	1.23	0.22
abundance 25	1.25	0.42	2.98	**0.003**
percent	0.12	0.002	52.5	**$< 2 \times 10^{-16}$**
abundance 10:percent	0.001	0.004	0.19	0.85
abundance 20:percent	-0.008	0.005	-1.5	0.13
abundance 25:percent	-0.02	0.005	-3.28	**0.001**

Table 2. Statistical analysis of DET (l=5) in response to changes in the percent chance of repeating a past foraging transition (sequence predictability) and the level of resource abundance. We used a GLM with quasi-binomial errors, where the dispersion parameter was estimated as 29.9. Resource abundance was treated as a factor, and an abundance of five resources was set as the reference group. As expected, there was a strongly significant effect of predictability on DET. Compared to an abundance of five resources, DET was significantly greater for 25 resources but not significantly different for 10 or 20 resources. There was a significant interaction between abundance and predictability only for the 25 resource case

FIGURES

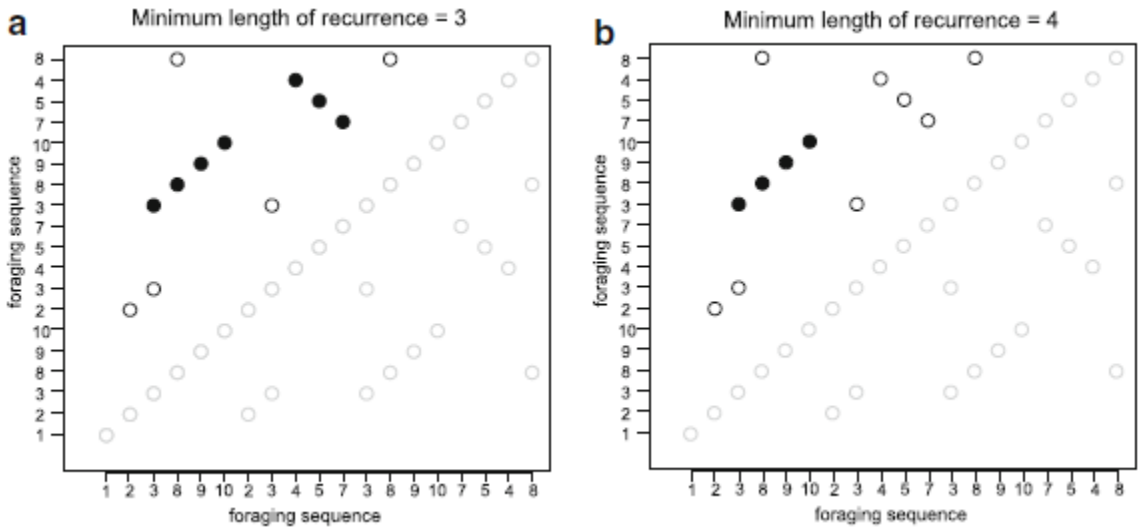


Figure 1. Recurrence plots for a hypothetical foraging sequence with a minimum recurrence length of A) 3 or B) 4 points. All points above (but not including) the main diagonal are included in the denominator of the determinism (DET) formula. Closed points, or those which belong to contiguous diagonals (recurrences) of at least the minimum length, are included in the numerator of the DET formula. All grey points are excluded from the calculation of DET, since the plot is symmetric across the main diagonal. DET= 7/12 = 0.58 and DET= 4/12 = 0.33 in parts A and B, respectively

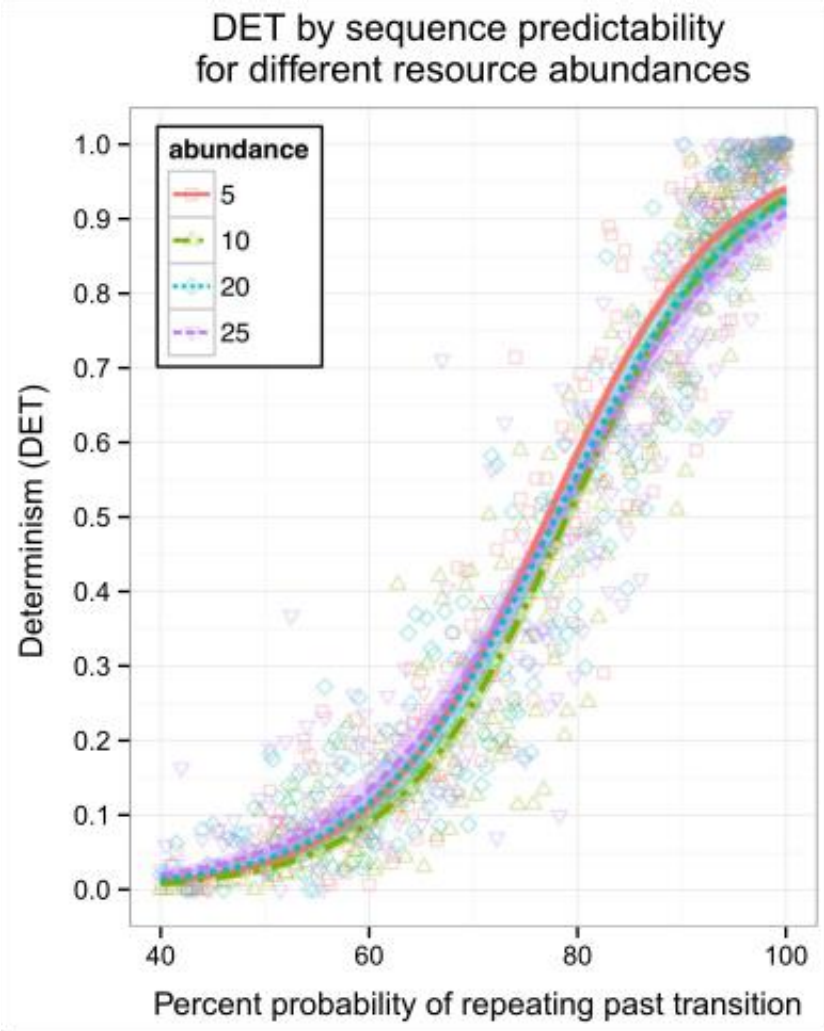


Figure 2. Determinism (DET) values (l=5) from hypothetical foraging sequences with varying predictability and resource abundance. We generated 1,044 sequences with 100 resource visits each while varying the probability of repeating a transition from an earlier stage of the foraging sequence. The lines of best fit and 95% CIs were calculated using GLMs with quasi-binomial errors to account for the nonlinear response of DET to sequence predictability. DET significantly increased with sequence predictability and was significantly higher for the case with 25 resources

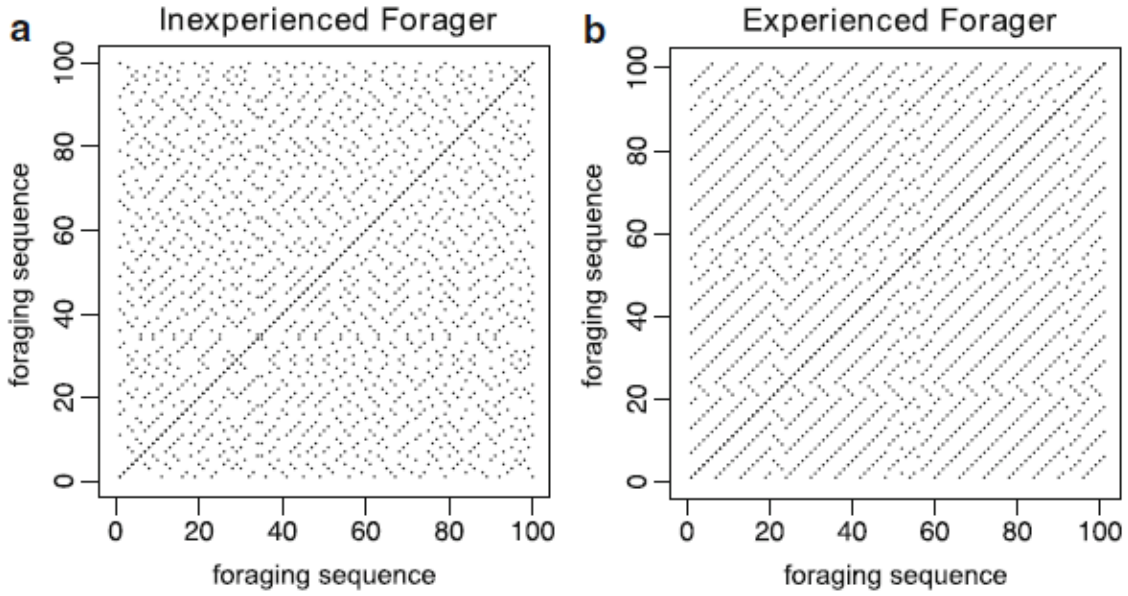
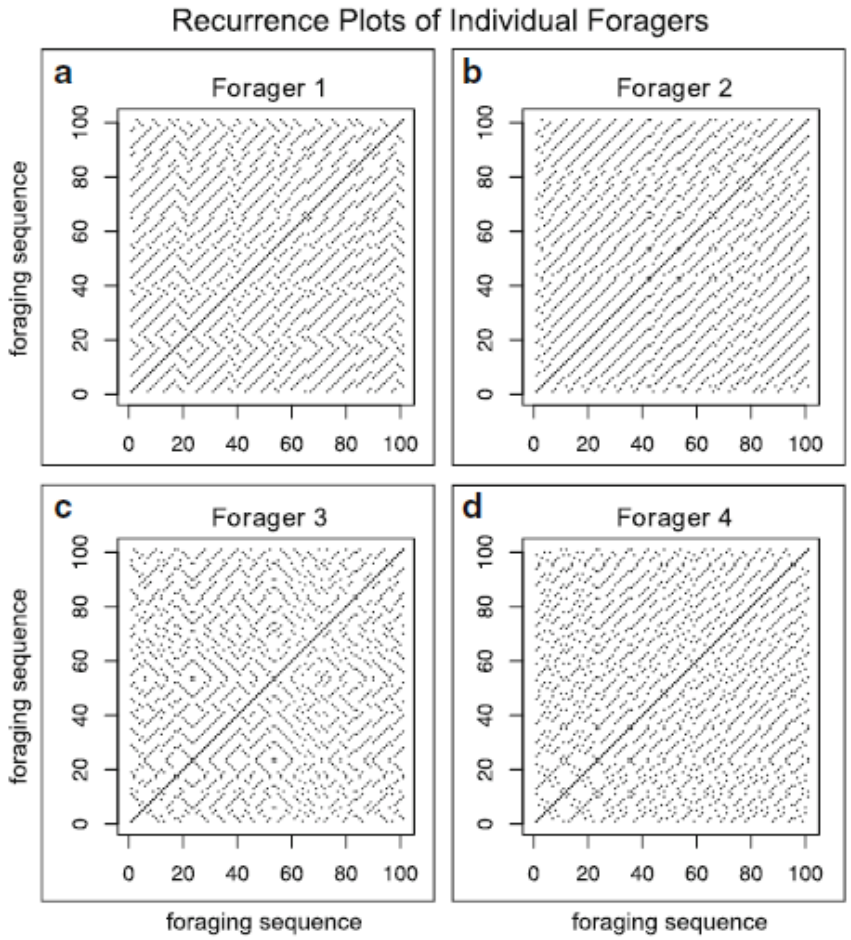


Figure 3. (A) Recurrence plot of the foraging behavior of a bee after first entering a foraging array. (B) Recurrence plot of the foraging behavior of the same bee after gaining experience on the array (following 360 floral visits). Longer diagonal lines indicate greater predictability in the foraging sequence, corresponding to more numerous and consistent traplines (Data from indiv. 1 in Lihoreau et al. 2012a)



Forager 1	Forager 2	Forager 3	Forager 4
$DET = \frac{469}{784} = 0.60$	$DET = \frac{455}{753} = 0.60$	$DET = \frac{203}{723} = 0.28$	$DET = \frac{160}{739} = 0.22$

Figure 4. Recurrence plots of four individual bee foragers and the corresponding DET values. The plots show the last 100 flower visits after the bumble bees gained experience on a foraging array (data from Lihoreau et al. 2012a). Foragers 1-4 correspond to indiv. 4, 2, 6, and 5 respectively from Lihoreau et al. 2012a. Longer diagonal lines indicate greater predictability in the foraging sequence and more consistent traplines (as in A and B). Many short diagonals or isolated points indicate less consistent traplines (as in C and D). Lines perpendicular to the main diagonal indicate a reverse in trapline direction (as is most prevalent in C). The corresponding DET values were calculated with l=5

Chapter 2.

STATISTICALLY TESTING THE ROLE OF INDIVIDUAL LEARNING AND DECISION-MAKING IN TRAPLINE FORAGING BEHAVIOR: A SPATIALLY EXPLICIT, INDIVIDUAL-BASED NULL MODEL APPROACH

Carolyn A. Ayers, Paul R. Armsworth, Berry J. Brosi

ABSTRACT

Trapline foraging, a behavior consisting of repeated visitation to spatially fixed resources in a predictable sequence, has been observed over diverse taxa and is important ecologically for efficient resource gathering. Despite this, few null models exist to test the significance of suspected traplines, particularly for studies interested in the role of individual decision-making in the formation of traplines versus the role of resource layouts and random movement patterns. Here we present a spatially explicit, individual-based null model, which may be used to test whether resource layout and realistic forager movement may account for sequence repeats in suspected traplines. We compare our model with two existing null models—a completely random model and a sample randomization model—using both simulated and empirical foraging data. We found that relative to our model, a random model is more prone to Type I statistical error while a sample randomization model is more prone to Type II error. The type of model presented here may be useful for other spatially explicit and individual-based processes, which are currently at the forefront in the field of ecology.

INTRODUCTION

Trapline foraging, where animals repeatedly visit spatially fixed, replenishable resources in a predictable order (Thomson et al. 1997) can increase foraging efficiency since it allows foragers to minimize travel and search times between resources (Ohashi et al. 2007, Lihoreau et al. 2011, Saleh and Chittka 2007). Such efficiency increases can confer an advantage to trapline foragers in competition with non-traplining foragers (Ohashi et al. 2008). These advantages are perhaps reflected in the taxonomically widespread adoption of traplining as a foraging strategy, in organisms including a variety of bee taxa (Ackerman et al. 1982); hummingbirds (Gill 1988), vultures (Deygout et al. 2009); bats (Woodsworth et al. 1981); and other mammals including rats, opossums, and primates (Garber 1988).

Complex cognitive processes, including spatial reference memory and iterative learning heuristics, are proposed drivers of trapline foraging behavior (Saleh and Chittka 2007, Reynolds et al. 2013), which makes traplining a common system for studying spatial memory and learning heuristics for complex routing problems (Lihoreau et al. 2011, Lihoreau et al. 2012). Due to the prevalence of the behavior in pollinators including bees and hummingbirds, traplining is also frequently studied in terms of its functional implications for plant pollination, since traplines incorporating conspecific flowers may enhance conspecific pollen transfer (Ohashi and Thompson 2009).

Few statistical tools, however, are available for testing the statistical significance of suspected traplines. An appropriate null model for traplining is especially important for explicitly testing the role of individual learning and decision-making versus the role

of non-cognitive factors (e.g. the spatial geometry of resources) or cognitive processes of lesser interest (e.g. the type of random walk used by the forager). Null models (i.e. pattern-generating models based on randomization of data or random sampling from a specified distribution) are used to test the statistical significance of biological processes by deliberately excluding the mechanism of interest (Gotelli and Graves 1996). In the case of traplining, null models that exclude the use of spatial memory may be used to test the statistical significance of memory-driven traplining behavior observed in empirical data. In the past, the difficulty of creating a relevant null model has led many studies to only compare different foragers (i.e. is one forager traplining more than another?) rather than making comparisons against a null model (i.e. is a particular forager traplining?)(e.g. Thomson et al. 1997).

The flexibility and specificity of pattern-generating null models can often make it difficult to select the most appropriate model (Harvey et al. 1983). Many null models contain hard-to-define parameters and can make model results susceptible to higher Type I (Wilson 1995) and Type II statistical errors (Grant and Abbot 1980). Type II error, or the failure to reject a false null hypothesis, may occur when the mechanism of interest is accidentally incorporated into the null model (Colwell and Winkler 1984, Gotelli 2001). Type I error, or the incorrect rejection of a true null hypothesis, may occur if alternate mechanisms other than the one being tested are not incorporated into the null model (Wilson 1995). An ideal null model for traplining should result in low Type I and Type II statistical errors when used to test the statistical significance of empirical data.

Two types of null models currently exist for traplining: a completely random null model and a sample randomization null model. We hypothesize a completely random null

model will often lead to high Type I statistical error when used to test the significance of empirical data because the model assumes an equal probability of transitioning between any pair of resource points (Figure 1A). Since predictable patterns may emerge due to the spatial structure of resources and limitations of forager movement (i.e., foragers cannot teleport) this type of null model will be too easy to reject. Sample randomization null models do not suffer from these problems since they generate null sequences by randomizing observed distances and turning angles between resources from empirical data (Ohashi et al. 2007). However, since distributions of angles and distances are heavily influenced by the level of traplining itself, we hypothesize that these tests may lead to high Type II error by accidentally incorporating the mechanism of interest (Colwell and Winkler 1984, Gotelli 2001). For instance, a forager with a high level of traplining could have very low variability in travel distances and turning angles in regularly spaced resource configurations (see, for example, Figure 1B). Null sequences generated from such regular traplines would likely follow very similar or identical routes, which would make the null model very difficult to reject in cases where traplining behavior is most pronounced (i.e. high Type II error). This is particularly likely to happen in experimental arrays containing few resources, where most studies that carefully measure traplining occur.

In this paper we address the main shortcomings in the statistical analysis of traplines by proposing and assessing a new null model for comparison with empirical foraging sequence data: a spatially explicit individual-based (IB) null model. In our IB model, we deliberately exclude spatial memory of the modeled "agents" to statistically test whether repeats in observed foraging sequences are driven by the spatial layout of

resources and realistic forager movements. We selected this as our null hypothesis since it is pertinent to the majority of traplining studies, which examine traplining as a model system for complex cognitive processes. We compare the proposed null model with existing methods for trapline foraging: a completely random null model and a sample randomization test (Ohashi et al. 2007) using both real empirical and hypothetical sequence data. Though there is no standard sequence predictability threshold required to definitively classify behavior as "traplining", we were able to compare the likelihood of Type I or II statistical errors between the three null models. We hypothesize that the null model presented here will be less prone type I error compared to a purely random null and less susceptible to type II error compared to a sample randomization null model. We test the IB null model's sensitivity to a range of different resource abundances, sensory inputs, and movement patterns. Finally, we assess the strengths and weaknesses of using IB models for testing the statistical significance of empirical data.

The proposed approach of using an individual-based null model could be expanded to other ecological and behavioral questions, especially for spatially explicit and individual-based processes. Few null models exist for spatial problems, though space plays an important role in maintenance of biodiversity, species invasions, host parasite interactions and disease dynamics (Tilman and Kareiva 1997), and many other ecological processes. There are also very few null models for questions in individual-based ecology, where aggregating at the population level can obscure the mechanism of interest. For example, variation in individual behavior may drive patterns of foraging specialization or niche complementarity (Bolnick et al. 2002, 2003, 2010, Heinrich 1976). We predict that

individual-based, spatially explicit null models such as the one proposed here will become more essential with the growth of these two emerging fields in ecology.

METHODS

Overview

We describe the spatially explicit IB null model for trapline foraging we developed in NetLogo (Wilensky 1999; see online Supplementary Material). This null model is designed to test whether realistic limitations on forager movement and an explicit spatial structure of resources can drive predictability in foraging sequences. We use this null model to test statistical significance of traplining in: 1) novel foraging data; 2) data from the literature; and 3) simulated data. We further compare the performance of our new null model for these three datasets with two alternative null models: a completely random null model and a sample randomization null model (Ohashi et al. 2007). Finally, we test the sensitivity of the null model to several parameters including resource abundance, sight distance, and movement type.

Netlogo Null Model Description

The model is comprised of two agent types: foragers, which may travel throughout the field, and resource points, which are fixed in space. Resources are distributed according to the known locations of resources in empirical experiments of bee foraging. For our sensitivity analysis of resource density, resources are spatially distributed according to a random uniform distribution. We examine one forager per simulation. In

the model, space is unitless but may be assigned units for applications to specific study systems.

Foragers (individual agents) make decisions using several rules, which operate in combination to create foraging sequences (Figure 1). First, foragers scan for a resource within their detection distance (Fig. 1, step 1), and evaluate whether any resources are within sight (step 2). If so, foragers evaluate whether the resource was one of the two most recently visited flowers (step 4). If there are no resources (step 2) or all resources were recently visited (step 4), foragers move one step in the field according to a random or correlated random walk (step 3) and begin the process again by scanning for floral resources (step 1).

If at step 2 there is only one resource available within sight that was not recently visited, that resource is chosen automatically. If multiple resources are available within sight, the forager must choose which to visit (step 5). In our base model, foragers select among multiple flowers in sight at random, but we evaluated other decisions rules in a sensitivity analysis (see Appendix 2 Figure 3). After the forager has chosen a resource, the forager travels directly to the resource in a straight line approach (step 6). The forager handles the resource in one time step (step 7) and begins the process again by searching for another resource (step 1).

Sample Randomization Null Model Description

Sample randomization tests generate null sequences using the distribution of travel distances and turning angles between resources in observed foraging sequences from empirical data (as in Ohashi et al. 2007). To create the null sequences, we randomly

selected distances and turning angles from observed distributions. We chose the next resource in the sequence based on the selected distance and turning angle. We assigned separate distributions to the center and edges of the field as in Ohashi et al. (2007), using the current position of the forager to determine which distribution to draw from. The model was created using the R statistical programming language (R Core Team 2014) and is available online (Appendix 2).

Comparison with Empirical Data

We tested the statistical significance of traplines in individual foraging sequences relative to all three null models using a permutation test (Figure 4). We used two different empirical datasets: (1) the publically available "Lihoreau" dataset, consisting of bumble bee (Hymenoptera: Apidae: *Bombus terrestris*) foraging sequences collected by Lihoreau et al. (2012), and (2) the "Emory" dataset, which consists of observations of *Bombus impatiens* from a laboratory foraging enclosure at Emory University (C. Ayers, B. Brosi, and E. Dobbs, *unpublished*; see the online Supplementary Material for foraging sequences).

The "Lihoreau" dataset (Lihoreau et al. 2012), was collected in an artificial foraging enclosure designed to study the optimality of traplines. The data consist of observations from eight individual bees in a foraging enclosure with six artificial flowers and a nest box. Rewards were calibrated so that bees would return to the next box after approximately six visits. Each bee was observed for 80 foraging bouts, and artificial flowers were refilled at the end of each bout. For our analysis, we split the dataset into

the first and last 100 visits of each individual bee to examine inexperienced versus experienced foraging separately.

For the "Emory" dataset, we measured the traplining behavior of *B. impatiens* in a laboratory foraging enclosure with 32 artificial flowers with four artificial flower "species" differing in sucrose molarity, color, and scent. Artificial flower species were distributed uniformly throughout the enclosure. Sucrose replenishment was computer-controlled, and bee behavior was tracked automatically using RFID-tag technology. The dataset includes foraging sequences from 955 individual *B. impatiens* from 68 trials and ten different *B. impatiens* colonies. Trials were 75 minutes long and each consisted of 16 *B. impatiens* from the same colony. Some individuals were used in multiple trials, so there is variation in foraging experience between individuals. For this analysis, we focused on the eight most active bees from the "Emory" dataset to match the number of observations in the "Lihoreau" dataset (see the online Supplementary Material for foraging sequences). We did not expect to find significant traplining from the "Emory" bees since the foraging setup was not conducive to traplining, particularly due to the close proximity, uniform distribution, and fast replenishment of resources.

To compare the NetLogo model with empirical data, we incorporated the experimental resource layout for each dataset into the model. We ran the model until the number of visits equaled the mean number of visits for each set of observed bees. For all 24 observed sequences, we ran each null model 999 times. We quantified traplining using Determinism (DET) with a minimum trapline length of four resource points (Ayers et al. 2015), and we analyzed the sensitivity of the results to minimum trapline length (see Appendix 2 Table 2).

Comparison of models using simulated data

We also estimated the probability of rejecting the null model using simulated foraging data with differing levels of predictability. We randomly generated trapline sequences by altering the probability of repeating a past transition based on a fixed trapline sequence. We created a short base sequence at the beginning of each generated sequence to set the initial transition pairs. For each additional visit, there was a set probability of repeating the last transition that occurred the previous time the forager visited the current resource. If a revisit did not occur, we a randomly selected one of the remaining resources. We created 100 simulated sequences with a length of 60 resource visits for all 17 levels of predictability between 20% and 100% probabilities of repeating a past transition. For each of the 1700 hypothetical sequences, we ran each of the three null model 99 times. Using a permutation test for significance, we calculated the probability of rejecting each null model at each level of predictability (Figure 3). Since we do not have an objective quantitative definition of what constitutes a trapline, we cannot precisely measure Type I and Type II statistical error. However, we are able to examine the relative propensity of each model to result in Type I or Type II error when compared with empirical data.

Sensitivity Analysis

We performed a sensitivity analysis for several parameters, including resource abundance, detection distance and movement type (random vs. correlated random walks). We also examined model output in the case where foragers are more likely to choose closer resources when multiple resources are in view (see Appendix 2 Figure 3).

We specifically examined the interaction of sight distance with movement type (Figure 5) using a minimum trapline length of six resource points for our DET metric (Ayers et al. 2015). We examine low, medium, and high sight distances (corresponding to model parameters of 10, 20, and 30 respectively). We utilized three different movement types: a random walk, a walk with low levels of correlation, and a high level of correlation (with model parameters of 0, 0.3, and 0.9 respectively). Foragers utilizing a correlated random walk will on average move farther from their initial starting point than foragers using a random walk (Kareiva and Shigesada 1983). In our model, foragers with a highly correlated random walk have a Mean Squared Displacement of 68.7 after 10 movements, while foragers with no correlation will have a Mean Squared Displacement of 9.7 after 10 movements.

For all sensitivity analysis, we used generalized linear models (GLMs) with binomial errors to statistically test the response of DET (e.g. the level of traplining) to changes in null model parameters (as in Ayers et al. 2015). Analyses were performed using R (R core team 2014).

RESULTS

Null model comparison using simulated sequence data

We first compared the proposed null model with completely random and sample randomization null models using hypothetical foraging sequence data with varying levels of predictability. For each level of percent probability of repeating a past transition, we tested for significant traplining (where H_0: absence of traplining behavior and H_1: presence of traplining). We found that a completely random null model has a 95% probability of rejecting the null model with only a 62% chance of repeating a past

transition, corresponding to a determinism score of 0.25 (Figure 3), which by most criteria would not be considered a strong trapline. Our Netlogo model finds significant levels of traplining sequences at 69% probability of repeating a past transition (DET = 0.39; Figure 3), while the sample randomization model would reject the null hypothesis for levels of sequence predictability greater than 72% (DET = 0.45; Figure 3). For intermediate levels of traplining, the sample randomization model is less likely to detect significant traplining than the proposed Netlogo model. Thus, relative to the proposed model, the random model may be susceptible to high Type I statistical error when identifying traplines, while the sample randomization null model may be susceptible to high Type II statistical error (Figure 3). Sample randomization tests may be particularly prone to error when there are few resource points per quadrant (e.g. center, corner, or edge). Since most lab and field studies of traplining use only a small number of resource points, this problem is very likely to occur when applied to empirical sequence data.

Null model comparison using empirical sequence data

We compared null model output with two sources of empirical data (i.e. the "Lihoreau" and "Emory" datasets; see Methods). We divided foraging sequences from "Lihoreau" into experienced and inexperienced foraging using the first and last 100 visits of each bee.

As with the hypothetical sequence data, we found that the proposed null model had an intermediate significance cut-off level compared to the random and sample randomization models (Figure 4A). For the dataset without suspected traplining (i.e. "Emory" in Figure 4A and B), we found that on average all three models correctly failed

to reject the null model. However, the completely random model rejected the null hypothesis for specific individuals (see Appendix 2 Table 3), which contradicted the findings of the Netlogo and sample randomization models. For the Lihoreau (2012) dataset with experienced bees, all three null models easily rejected the null hypothesis ($P < .001$; Figure 4). For the less experienced bees, with unknown levels of traplining, the sample randomization model found that 2 of the 8 bees did exhibit significant traplining, while the proposed spatially explicit model found that 5 out of 8 bees were traplining more than expected by realistic movements and resource layout alone (Figure 4).

Sensitivity analysis of Netlogo null model

We performed a sensitivity analysis of the Netlogo model by varying key parameters including sight distance and the degree of correlation in the random walk. We calculated significance cut-off levels and compared determinism levels with experienced or inexperienced foragers from empirical *Bombus* data (Lihoreau et al. 2012). We expected to find significant traplines for experienced bees, and less significant traplining for the inexperienced bees.

We found that the effect of sight distance on estimated determinism followed a sinusoid pattern that interacted heavily with the degree of correlation in the random walk (Figure 5A). For 5 out of 8 experienced bees, the model found significant traplines for all combinations of parameter values (compare Figure 5 A and B). Many combinations of parameter values resulted in finding significant traplining in the inexperienced bees, where we would expect lower levels of traplining. Using the intermediate parameter values for sight distance (sight = 20 in Figure 5) and the degree of correlated movement

(CRW = 0.3 in Figure 5), however, we found all but one experienced bee had significant traplining, while none of the inexperienced bees used significant traplining.

DISCUSSION

While null models are increasingly used in the field of ecology, a knowledge gap still exists in statistical hypothesis testing at two of the field's frontiers: spatially explicit and individual-based processes. Few null models exist for spatial problems, though space plays an important role in the maintenance of biodiversity and species distributions (O'Dwyer and Green 2010, Rahbek et al. 2007), species invasions (Cadenasso and Pickett 2001), food or oviposition site choices (Lancaster et al. 2003), as well as host-parasite interactions and disease dynamics (Dion et al. 2011, Ramsey and Efford 2010). Existing null models for spatial problems are typically not spatially explicit, including models for migration patterns, species co-occurrences (Gotelli 2000), species ranges over an environmental gradient (Veech 2000, Sanderson 2004, Hofer et al. 1999), and maintenance of beta diversity (Rô Me Chave and Leigh 2002).

There are also very few null models for individual-based processes, which are also at the forefront of ecology. Individual variation, whether through genotypic variation or differences in learning and experience, is an important mechanism in many ecological processes, including foraging specialization or niche complementarity (Bolnick et al. 2002, 2003, 2010, Heinrich 1976). For example, individual niche widths in sticklebacks have been shown to respond differently to decreases in interspecific competition compared to population-level niche widths (Bolnick et al. 2010). In these cases,

aggregating at the population level can obscure the mechanism of interest, yet to our knowledge no IB null model has been used to test the significance of empirical data.

In this paper, we address this knowledge gap in the statistical analysis of spatially explicit and individual-based problems by proposing a new individual-based null model for comparison with empirical data. Traplining is a good candidate for this type of null model, since it is both spatially explicit and individual-based, and it is a non-binary process with no objective quantitative definition of what constitutes a trapline. Using trapline foraging as a case study, we demonstrate how to use individual-based null models to test the significance of suspected traplines in empirical bumble bee foraging data, and we compare results with existing non-spatially explicit null models for traplining.

We compared our proposed model with existing non-spatially explicit models by calculating the relative tendency of the models to result in type I or II statistical error. We found that the proposed null model shows lower tendency to Type I error compared with a purely random model, and lower tendency toward Type II error compared with a sample randomization model. In our analysis of empirical data, the completely random null model incorrectly rejected the null hypothesis (e.g. detected significant traplines; Figure 4) in sequences without suspected traplines, and the sample randomization null model failed to reject the null model for sequences from the less experienced bees (Figure 4). Although we would not expect to see traplining in completely naïve bees, our results show that "Lihoreau" bees achieved a moderate level of traplining within their first 100 flower visits, which was more difficult to detect using the sample randomization null

model. Our use of an individual-based model for null model generation to compare against empirical data is, to our knowledge, a novel approach.

We also demonstrate how to determine which parameters are most important for the formation of the null model by performing a sensitivity analysis of the model to several key parameters. We found the most important factor influencing the degree of sequence repeats in the proposed null model was resource abundance (Appendix 2 Figure 1), which may be straightforward to quantify in laboratory set-ups but potentially difficult in field settings. Generally, sequence repeats were more prevalent in lower resource density settings compared to high-resource settings, though when sight distance was very small this relationship reversed (Appendix 2 Figure 1). The type of movement (e.g. random vs. correlated random walk) interacted with sight distance, such that a forager using a random walk had a greater sequence predictability at very high or very low sight distance ranges, while sequence predictability for foragers using a correlated random walk was highest with intermediate sight distance levels (Appendix 2 Figure 2). For parameters which are difficult to quantify and potentially influential, such as sight distance, it is possible to analyze the null model output over a range of possible parameters (as demonstrated in Figure 5). The relative ratio of parameters may also be important, for instance, in field settings with a large number of resources, foragers with low detection distances would only be able to view a small proportion of total available resources. We would therefore expect very few sequence repeats to occur by chance. Parameters that are particularly influential for a study system may also be targeted for further empirical investigation to narrow the range of possible values.

The null hypothesis tested here, that sequence repeats occur due to the spatial geometry of resources and realistic forager movement, is relevant to the majority of traplining studies, which are typically interested in complex learning and decision-making processes. Such studies occur most frequently in low resource-abundance settings, particularly in artificial foraging enclosures, where we found resource layouts are most likely to drive the level of sequence repeats. A spatially explicit null model is therefore important to test whether observed traplines are due to the cognitive process of interest or the specific geometry of resources in the experimental design.

The use of an individual-based, spatially explicit null model for evaluating empirical data is a novel approach to this problem, and may be applied to test the statistical significance of a wide range spatially explicit individual-based processes. For instance, the use of IB null model as presented here may be useful to test how innate or learned individual variation and space interact to influence processes including maintenance of biodiversity, species ranges, species invasion or migration, and disease spread. IB null models therefore may play an important role in advancing the emerging fields of spatial and individual ecology.

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FIGURES

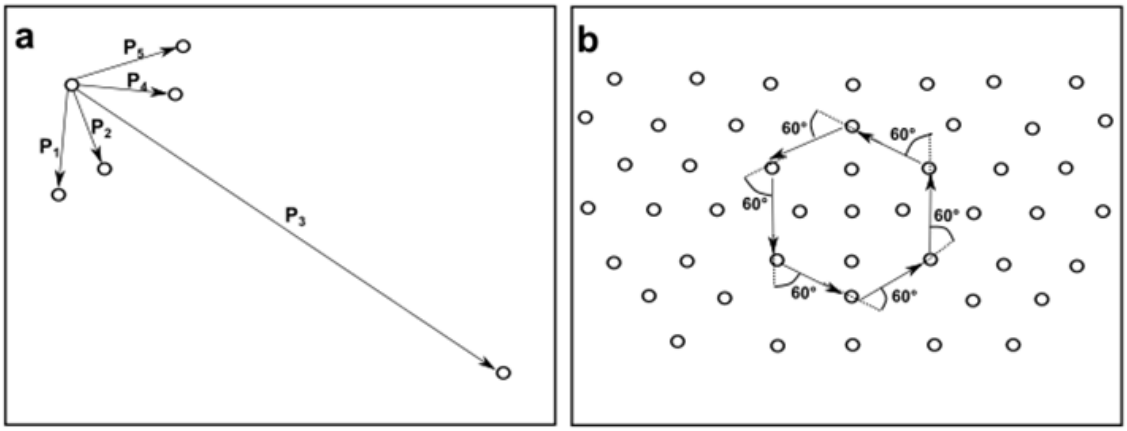

Figure 1. Role of resource layout for testing the significance of spatial memory use in trapline foraging. (Points represent resources and arrows represent foraging movements.) (a) In a completely random null model, there is an equal probability of visiting any resource point ($P_1=P_2=P_3=P_4=P_5$). However, more distant resources are less likely to be encountered by chance due to realistic forager movements ($P_3 < P_1, P_2, P_4, P_5$). Null models which do not incorporate differential probabilities of resource visitation due to spatial layouts may lead to high type I error. (b) Null models which randomize travel distances and turn angles may lead to high type II error when foragers consistently utilize uniform traplines. Strong traplining may result in a decreased range of turning angles and travel distances, which may lead to a large number of sequence repeats in null model sequences

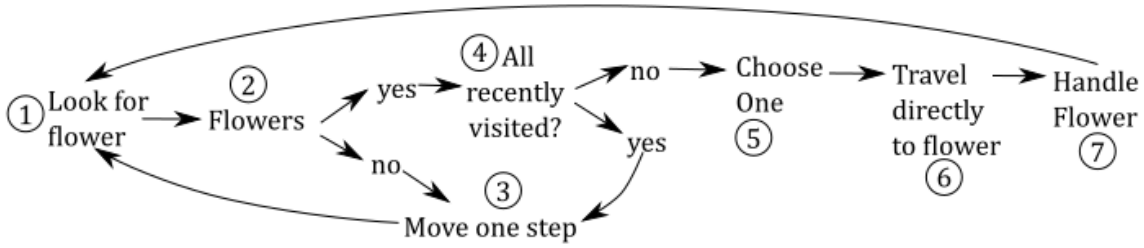

Figure 2. Model flowchart: (1) Look for a resource; (2) determine whether there are one or more resources in the detection distance; (3) move one unit of space according to a random or correlated random walk; (4) determine whether all of the resources in the detection distance are either the last or second to last flower visited; (5) randomly choose one resource which was not visited recently; (6) travel to flower using a straight line approach; (7) handle resource

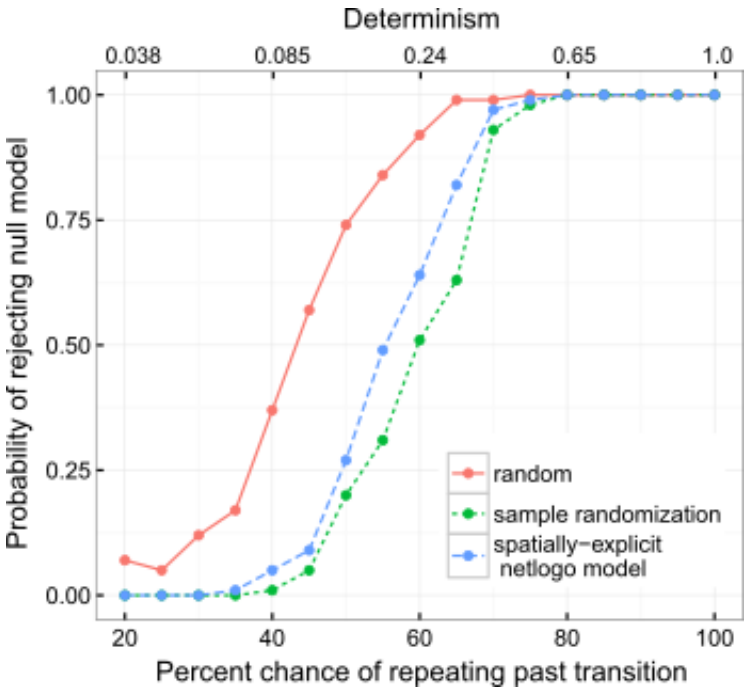

Figure 3. Effect of sequence predictability on the probability of rejecting the null hypothesis (where H0: absence of traplining behavior and H1: presence of traplining) for the proposed Netlogo model and two existing null models. The determinism values (i.e. level of sequence predictability) corresponding to the percent chance of repeating a past transition are shown on the secondary x-axis. We found that the random model is more likely to reject the null hypothesis for low levels of traplining (Type I error), while the sample randomization model is more likely to fail to reject the null hypothesis for intermediate levels of traplining (potential Type II error)

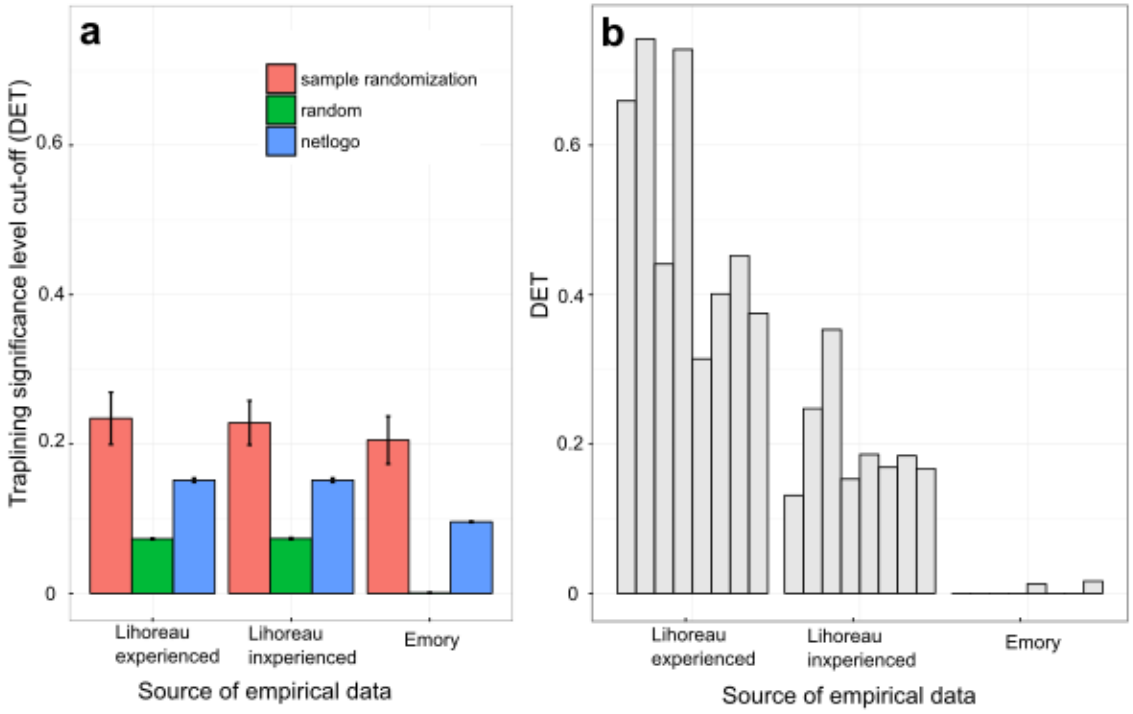

Figure 4. (a) Minimum determinism (DET) levels required to reject the null model with 95% confidence using a completely random null model, our spatially explicit null model, and a sample randomization null model. We used the models to analyze two sources of empirical *Bombus* foraging data, including experienced and inexperienced foraging sequences from Lihoreau et al. 2012. (Error bars are 95% binomial CI) (b) The corresponding determinism level of empirical *Bombus* sequences (calculated with a minimum trapline length of 4 resources)

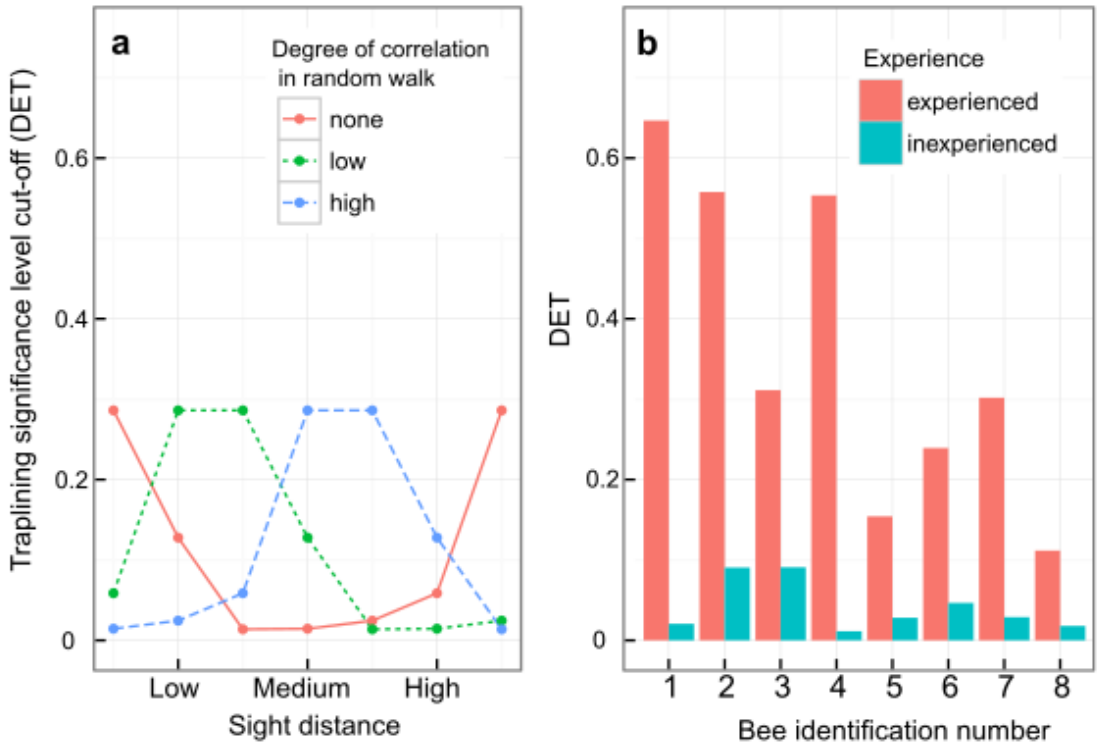

Figure 5. (a) Sensitivity analysis of the effect of forager sight distance and type of correlated random walk on the determinism (DET) level required to reject our spatially explicit null model with 95% confidence. (b) The corresponding DET levels of inexperienced or experienced *Bombus terrestris* foraging sequences from Lihoreau et al. 2012 (calculated with a minimum trapline length of 6 resources)

Chapter 3.

THE EFFECT OF BEE SPECIES RICHNESS ON COMPLEMENTARITY AND RESOURCE SPECIALIZATION ON THE INDIVIDUAL AND SPECIES LEVEL

Carolyn A. Ayers, Emily K. Dobbs, and Berry J. Brosi

ABSTRACT

Given ongoing pollinator declines, it is increasingly important to understand how bee species losses affect plant pollination services. This is in essence a question about the relationship between biodiversity and ecosystem functioning. Traditionally, studies of biodiversity ecosystem functioning (BEF) have focused on fixed differences, particularly between plant species. However, many animals exhibit dynamic behaviors which can respond to the presence of other species. Though these behaviors can be important drivers of ecosystem function, they are rarely ever discussed in a BEF framework. This is particularly true for pollinator species, whose behaviors can have direct functional implications for plant reproduction. In this paper, we used tightly controlled laboratory behavioral experiments with standardized artificial flowers to better understand how species richness (i.e. one to four bee species) affects bee specialization at different organismal hierarchies. We found that greater bee species richness (1) had differential effects on specialization at the individual level across species; (2) increased specialization at the species level for all four species tested; and (3) increased complementarity (i.e. species niche partitioning) at the community level. These results show a direct link

between species richness and bee specialization and complementarity, which are important behaviors for plant pollination and plant-pollinator ecosystem stability.

INTRODUCTION

With global declines in bee species diversity and abundance (Biesmeijer 2006, Potts et al. 2010), there is increasing interest in how bee species losses will affect plant pollination services and ecosystem stability. This problem is essentially a question about the relationship between biodiversity (e.g. the number of bee species) and ecosystem functioning (e.g. plant pollination services). Classic biodiversity-ecosystem functioning (BEF) studies have focused on plants (Tilman et al. 1997), where resource differentiation is often assumed to occur through natural selection for fixed traits (e.g. the ability to fix nitrogen or utilize other resources). However, this assumption is not necessarily valid in plant pollinator systems, since pollinators can rapidly change their foraging patterns in response to competition over ecological timescales (Pimm 1985, Rosenzweig et al. 1991, Bolnick et al. 2010). These dynamic (i.e. plastic) behavioral traits, while little studied in a BEF framework, can have direct consequences for plant pollination and reproduction. Therefore, exploring BEF relationships driven via phenotypic plasticity is a key area of research for BEF generally, and is particularly important for plant pollinators.

Traditional studies of BEF focus on three potential mechanisms. First, niche complementarity, where different species focus on different resources (Loreau et al. 2001), can increase ecosystem productivity (Hooper et al. 2005). Specifically, ecological communities with high complementarity (i.e. low niche overlap) are predicted to have greater total resource acquisition across the community compared to communities with

low complementarity, or high niche overlap. Second, diversity can also promote greater overall resource use through facilitation between species, where one species benefits from the presence of another (Cardinale et al. 2002). Third, BEF can be driven by a sampling effect, where greater numbers of species improves the probability of having a small number of important species which are responsible for driving ecosystem function (Loreau et al. 2001, Hooper et al. 2005). In this paper, we specifically focus on the first mechanism, complementarity, since it stresses overall species numbers rather than the functional importance of specific species.

While traditional complementarity studies have focused on fixed differences in resource use, resource differentiation can also be driven by plastic phenotypic shifts in resource use (including those driven behaviorally). For example, plants with overlapping resource use have been shown to increase their uptake of particular nitrogen chemical forms in response to interspecific competition (Ashton et al. 2010). In a predator-prey system, one study found that a greater diversity of natural predators improves suppression of an aphid prey species, with important implications for pest control in agriculture (Cardinale et al. 2003). In Cardinale et al. (2003), suppression of the target pest species occurred after mixed groups of predators sufficiently suppressed a parasitoid's typical host population, causing the parasitoid to switch to the target pest species.

Research on this topic is limited but growing, and may be relevant to a range of animal-mediated ecosystem functions, particularly for plant pollinators, whose foraging patterns can have direct functional consequences for plant pollination and reproduction. Plastic behavioral shifts in response to interspecific competition can impact ecosystem functioning at three hierarchical levels: individual insects; species; and entire pollinator

communities. At the individual level, pollinator transitions between flowers of the same species (floral fidelity) are important for plant reproduction given plant requirements for conspecific pollen transfer (Morales & Traveset 2008, Flanagan et al. 2009, Waser 1986, Chittka et al. 1999, Brosi & Briggs 2013). Specialization at the species level, where individuals of a particular bee species tend to specialize on the same plant (Brosi 2016), has also been shown to aid in conspecific pollen transfer and seed set (Fründ et al. 2013). Finally, pollinator complementarity (i.e. niche differentiation at the community level), where different bee species specialize on different plant species (Brosi 2016), is associated with greater recruitment of diverse plant communities (Fontaine et al. 2005). Both species-level specialization and community-level complementarity could enhance functioning if particular pollinator species specialize on the plant species for which they have a relatively high pollination efficiency.

Several studies have examined the role of species richness in shaping functionally important pollinator behaviors in an ecosystem functioning context. For instance, one study found that the removal of the most abundant pollinator species led to lower plant seed count via reductions in pollinator floral fidelity (Brosi & Briggs 2013). In addition, interspecific competition between bee and syrphid fly pollinators has been shown to lead to more efficient pollination in mixed-plant communities, via increased pollinator functional group specialization on their target plant group (Fontaine et al. 2005). Honey bees have also been shown to have increased pollination efficiency on sunflower (Greenleaf & Kremen 2006) and almonds (Brittain et al. 2013) when wild bee species are present. In Greenleaf & Kremen (2006) direct interference from wild pollinators forced honey bees to switch more frequently between male and female flowers, which led to

greater sunflower pollination. In almond orchards, where movement between trees of different genotypes (typically planted in different rows) is necessary for nut production, few direct interactions between *Apis* and non-*Apis* pollinators were observed. The presence of non-*Apis* pollinators, however, caused *Apis* to switch between almond rows more frequently (most likely due to exploitation competition), which led to greater pollination and nut crop production (Brittain et al. 2013).

There is, however, an incomplete understanding of how species richness enforces bee behaviors important for plant pollination. This is in part because all of the aforementioned studies were highly dependent on the identity of plant species present in the experiment. Since pollinator species vary in their ability to interact with different pollination syndromes (Fenster et al. 2004), plant-pollinator species identities can drive experimental outcomes. In several studies, pollinator functional diversity (and how well it matched the plant community), rather than species richness per se, was the primary driver of plant seed set (Fontaine 2005, Hoehn et al. 2008). These findings underscore the difficulty of determining the role of species richness in driving bee foraging patterns, independent from the confounding effects of bee and plant functional groups.

Previous studies have shown that interspecific and intraspecific competition have opposing effects on specialization (e.g. Rosenzweig 1991). Interspecific competition decreases niche breadth (i.e. increasing specialization) while intraspecific competition increases niche breadth (i.e. decreasing specialization). When foragers of the same species compete, individuals distribute themselves amongst patches in proportion with patch quality (Fretwell & Lucas 1969, Fontaine et al. 2008). However, in the case of two competing species where one is competitively dominant, studies have shown that the less

dominant species may choose to exclusively utilize a less rewarding resource to reduce competition with the dominant species (e.g. Pimm et al. 1985). For example, in the presence of interspecific competition, bumble bees have been shown to increase specialization on flower species with corolla lengths more closely matching their proboscis length (Inouye 1978). When competing species were removed, the bees utilized both flower types. Resource partitioning can also occur temporally or spatially rather than by plant species or functional group (Morse 1977, Hubbell and Johnson 1978, Walther-Hellwig & Frankl 2000).

In this study, we sought to understand general drivers of pollinator species richness on functionally important behavior through the use of tightly controlled laboratory behavioral experiments with standardized artificial flowers, thus removing plant species identity as a potential confounder of the patterns we observed. While this level of experimental control offers a number of advantages, it comes with the trade-off of not allowing measurement of direct functional outcomes (especially plant reproduction). We thus focused on functionally relevant pollinator behavior, i.e. behavioral patterns that typically correlate with greater pollination function.

In this paper we merged the experimental approach of BEF experiments, in which species richness is manipulated, with the experimental approach of phenotypic plasticity studies where plastic responses are measured in different environmental conditions. In contrast to the typical abiotic focus in reaction norm experiments, we explicitly created "reaction norms" of functionally-relevant behavior in response to variation in the biotic environment, particularly species richness. We tested bee behavior in a laboratory artificial foraging enclosure across a species richness gradient of one to four bee species.

We tested bee foraging specialization on four artificial flower "species" differing only in color, scent, and sucrose molarity. Again, our use of standardized artificial flowers helps to disentangle the effects of competition from the identity of plant species.

We specifically tested how interspecific competition affects bee specialization at the individual, species, and community level. We hypothesized that with greater species richness, we would observe (1) greater individual-level specialization; (2) greater species-level specialization, driven by reduced variation between individuals; and (3) greater complementarity (i.e. resource partitioning at the community level) driven by increased sorting of pollinator species onto different artificial flower types.

METHODS

Overview

We examined the effect of increasing bee species richness on bee foraging behavior using a laboratory foraging enclosure with artificial flowers. All trials consisted of 16 bee individuals, and we tested all combinations of one to four bee species.

Bee species

We ran experiments with four bee species, including two social (*Bombus impatiens* and *Apis mellifera)* and two solitary species (*Osmia lignaria* and *Megachile rotundata*). We maintained multiple *Bombus* colonies, each in their own training enclosure, to avoid colony-level effects. We had access to only one *Apis* colony, but we maintained multiple *Apis* brood frames from that colony in separate training enclosures. For our two solitary bee species, we randomly sorted individuals into one of several training enclosures

assigned to each species. We alternated the spatial position of enclosures and the bee species assigned to them in order to reduce training enclosure-level effects.

Foraging chamber

We examined bee foraging behavior using a laboratory foraging enclosure with artificial flowers. We used RFID technology to precisely track bee foraging behaviors and automatically control energetic rewards. RFID tag readers were embedded inside artificial flowers, which detected RFID tags glued to each bee's thorax. Each time a bee entered a flower and was detected by the reader, the bee and flower identity was automatically recorded. RFID tag readers also triggered automatic computer-controlled sucrose rewards, which were fed to bees inside flowers through a vertical pipette tip located beneath the RFID tag readers.

The laboratory foraging enclosure contained 32 total artificial flowers in four rows of eight, including four species of artificial flower "species". Flower "species" differed in sucrose concentrations (2.0 M, 1.5 M, 1.0 M, and 0.5 M), and were distinguishable by color (blue, white, yellow, pink) and scent (clove, wintergreen, spearmint, and lemongrass respectively). The enclosure was approximately 0.74 m deep by 2.27 m wide by 0.75 m tall. Flower "species" were distributed uniformly inside the chamber. We used incandescent lamps to hold lighting and temperature (28° C) constant across trials.

We automatically tracked bees using mic3-TAG RFID 16 kbit tags (Microsensys GmbH, Erfurt, Germany) which are 1.9 x 1.6 x 0.5 mm and weigh 5.5 mg. We attached tags to the bees' thorax using nontoxic glue (Elmer's Glue-All multi-purpose, Elmer's

Products, Inc, Westerville, Ohio). These tags are commonly used in bee foraging experiments (e.g. Decourtye et al. 2011, Schneider et al. 2012) and do not interfere with bee movement or flight. We used RFID tag readers embedded in each artificial flower to record the timing and identity of each bee visitor.

Whenever a bee was detected in a flower by the RFID tag reader, software (Processing language) determined whether a reward should be released, and if so, the size of the reward to be dispensed. Reward dispensation was effected through interface with Arduino MEGA 2560 R3 hardware (Arduino LLC), which controlled solenoid valves that in turn released sucrose solution from reservoirs located outside the foraging chamber. Once a valve was opened (in microbursts), the sucrose solution was gravity-fed through plastic tubing into the corresponding artificial flower, and the sucrose was dispensed as a droplet at the end of a vertical pipette tip at the bottom of each artificial flower. Solenoid valves released 10 µl of sucrose solution automatically after a bee was detected in a flower unless a bee had been recorded in that flower in the last 30 seconds.

Experimental procedure

Bees trained on flowers with an identical appearance, scent, and sucrose molarity to those in the experimental enclosure inside separate training enclosures. Artificial flowers in the training enclosure, however, were not computer controlled, and instead delivered sucrose solution through wicks embedded in a sucrose reservoir.

At the beginning of each trial, we randomly selected bees from the desired colony from their training enclosures and chilled the bees for one hour to temporarily immobilize them. We then attached RFID tags to their thorax, recorded their tag numbers, and fasted

bees for one hour. Before transferring bees to the primary foraging enclosure, we wiped the chamber with ethyl alcohol to remove bee scent markers remaining from the previous trial. Trials lasted for 75 minutes. We returned bees to their original training enclosures following each trial.

While we could not avoid reusing bees from previous trials (approximately 50.17% of bees tested were used in a previous experiment), we held the proportion of experienced bees essentially constant across the species richness gradient (varying only from 47.7% to 51.6% of experienced bees). We found that experience did increase the number of total flower visits (P = 8.401e-05), but there was no significant interaction with bee species richness (P = 0.763) or species identity (P = 0.512; GLM with quasi-Poisson errors).

Statistical analysis

We used the R statistical programming language (R Core Team 2014) for all statistical analyses. We used the "bipartite" package (Dormann et al. 2008) to calculate measures of specialization, specifically the "H2fun" function (Dormann et al. 2009) for complementarity (H2') and the "dfun" function for d' (Dormann 2011).

First, we tested the level of specialization at the individual level by applying the d' metric (i.e. the standardized Kullback-Leibler distance) to the foraging specialization of individuals within each trial. For d', a value of zero represents an absolute generalist and one represents an absolute specialist (Blüthgen et al. 2006). We used linear models to test the effect of bee species richness on species-level specialization (d'). Before running the model, we log-transformed d' to normalize its distribution.

We also measured floral fidelity by calculating the proportion of transitions between flowers of the same "species". Floral fidelity is a special case of species-level specialization, where bees visit flowers of the same type in a sequential order. We used a generalized linear model (GLM) with quasi-binomial errors to test the effect of species richness and species identity on floral fidelity. In our GLM, we counted a transition between flowers of the same type as a "success" and a transition between flowers of different types as a "failure".

Next, we tested how bee species richness affects species-level specialization (using d') by pooling individuals within each species. As with individual-level richness, we used linear models with log-transformed d' to estimate the effects of species richness and species identity.

We tested the effect of species richness on complementarity, or the degree of niche partitioning at the community level. We measured complementarity using H2', an index which is commonly applied to plant-pollinator networks (Blüthgen et al. 2006). H2' measures the degree of niche partitioning or sorting of pollinator species onto plant species. H2' ranges from zero to one, where a value of one represents a community where all species are entirely specialized on different species, and a value of zero represents a community where all species interact at the rate predicted by their relative number of total visits (Blüthgen et al. 2006). We used linear models to test the effect of bee species richness on complementarity (H2'). Before running the model, we log-transformed H2' to normalize its distribution.

Finally, we used a GLM with quasi-Poisson errors to test the per bee number of flower visits by bee species identity and by bee species richness. We have not yet

compared our multi-species trials with low density *Bombus* trials to analyze the effects of intraspecific versus interspecific competition, but this analysis is planned for the future.

RESULTS

We ran a total of 176 trials with 2,816 bees. We ran 63 single-species trials, 53 two-species trials, 35 three-species trials, and 25 four-species trials.

First, we found no significant overall trend for individual-level specialization with respect to richness (P= 0.117; Table 1). However, we found a significant interaction between species richness and species identity (P= 1.580E-04; Table 1), where individual specialization decreased with species richness for *Megachile* and *Apis,* remained relatively constant for *Osmia,* and increased for *Bombus* (Figure 1). There were highly significant species differences in individual foraging specialization (P= < 2.2E-16) where *Bombus* individuals were the least specialized on average and *Megachile* individuals were the most specialized (Figure 1). We also tested the level of floral fidelity, but did not find any significant differences in fidelity with species identity or species richness (Table 2). We found that the number of flower visits increased with species richness for all four bee species (P = 0.0003055; Table 2).

Second, we found that species-level specialization (d') increases with species richness for all four bee species tested (Table 1; P= 4.07E-05). We also found significant differences between species (P= < 2.2e-16), following the same order as above. The most specialized species was *Megachile,* followed by *Apis, Osmia,* and *Bombus* (Figure 2). The number of total flower visits followed the reverse trend, where *Bombus* was the most

active species, followed by *Osmia*, *Apis*, and *Megachile* across all four species richness levels (Figure 3).

Finally, we found that complementarity increased with greater species richness (Table 3; Figure 4; $P = 0.009$), indicating that bee species diversity drives resource partitioning at the community level. There were several outlying trials with high complementarity, but we could not identify any common feature shared by the majority of outlying trials.

DISCUSSION

With worldwide declines in bee species diversity (Biesmeijer et al. 2006, Potts et al. 2010), it is important to understand how bee species losses will affect plant pollination services. This question can be informed by existing studies on the relationship between biodiversity and ecosystem functioning. However, BEF studies have traditionally focused on fixed differences between species, while pollinators are able to rapidly change their foraging patterns in response to competition from other species. The role of interspecific competition in driving ecosystem functioning via changes in plastic behaviors is an understudied area in the field of BEF. In this paper, we address this knowledge gap by examining the effect of bee species richness on bee foraging behaviors important for plant pollination.

Using a laboratory foraging enclosure with standardized artificial flowers, we were able to test how changes in bee diversity affect bee specialization at individual, species, and community levels. We found that (1) bee species richness differentially affected individual specialization according to species identity; (2) floral fidelity did not change

with species richness; (3) species-level specialization increased with richness for all four species tested; and (4) complementarity (i.e. resource partitioning at the community level) increased with greater species richness.

First, we found that the effect of species richness on individual specialization was highly species-dependent. We found both a significant main effect of species identity and a significant interaction between species identity and richness. Individual specialization increased with species richness for *Bombus* individuals, remained relatively constant for *Osmia* individuals, and decreased with species richness for *Megachile* and *Apis*. The decrease in specialization for *Megachile* and *Apis* may have occurred since the number of flower visits also increased with species richness. The measure of specialization that we used (d') is typically not sensitive to the number of interactions in a network, however d' does increase when the number of interactions is very low (Blüthgen et al. 2006). This can occur since bees with very few visits could by chance only visit a particular flower type, while this is unlikely to occur by chance for bees with very many visits. Since *Megachile* and *Apis* were our least active species, their estimated specialization levels may be vulnerable to the sensitivity of d' to low flower visit numbers. Given the high foraging activity of *Bombus*, the increase in d' we observed in trials with greater species richness most likely represents a meaningful increase in specialization.

We were not able to detect any changes in floral fidelity across the species richness gradient ($P = 0.383$), in contrast to a previous field study that documented reductions in fidelity following single-species removal manipulations (Brosi & Briggs 2013). The lack of significant differences in floral fidelity may have been driven by the fact that our artificial flowers did not differ in shape or handling procedure, so there was no learning

cost associated with switching between flower "species" in our experimental design, which is a commonly proposed mechanism for floral fidelity (Waser 1986, Chittka et al. 1999).

Second, we found that all four species had increased species-level specialization with greater species richness. Since the number of visits increased with species richness for all four bee species, this pattern cannot be explained by the sensitivity of d' to low numbers of flower visits. Therefore, we find that species-level specialization increases with greater bee diversity. This result is consistent with the findings of Fründ et al. (2013), where d' increased in a multiple-species context. However, in Fründ et al. (2013), specialization increased from one to two species, but did not continue to increase from two to four species, whereas we found a consistent increase in specialization. The effect of species richness on specialization differed between the individual and species level for two of the four bee species. For *Megachile* and *Apis*, the level of specialization increased with richness at the species level but decreased at the individual level. This type of decoupling between individual and population-level niche width can occur due to differences in within versus between-individual specialization (Bolnick et al. 2010, Brosi 2016). For *Megachile* and *Apis*, the niche breadth of individuals increased with species richness (i.e. specialization decreased), however differences between individuals decreased. Since between-individual variation was lower in a multiple-species context, we saw a reduction in species-level niche breadth, where all individuals were pooled together.

We also found that, when pooled across the species richness gradient, individuals of each species converged more on the same resource within a trial, leading to greater species-level specialization. The relative rank of each species in terms of specialization at

both the individual and the species levels was inversely related with the mean number of visits for each species. Since two of the bee species had relatively few flower visits, we cannot exclude the possibility that these species rankings for specialization may have been driven by the total number of visits.

Finally, we found that complementarity increased with greater species richness. This indicates that as species-level specialization increased, each species specialized on different flower types within a trial. We detected very low levels of complementarity overall (Figures 3-4). However, our use of standardized artificial flowers with no differences in shape or required handling procedures was a very conservative test for bee specialization, since the cost of learning to handle new flowers is often cited as a mechanism driving floral fidelity in bees (Waser 1986, Chittka et al. 1999). We also found that the flower type chosen by each species varied between trials, indicating that social information (i.e. intraspecific copying), rather than inherent preference, may be driving specialization in this context. Our results demonstrate that differences in flower color, scent, and sucrose molarity are sufficient to drive changes in niche partitioning across a species-richness gradient. These findings stress the importance of diversity as a driver of complementarity and specialization across plant-pollinator networks (Blüthgen & Klein 2011).

One limitation in our experimental setup was the high variation among species in their foraging activity in the foraging chamber, which resulted in variation in the number of total visits. While d' and H2' were designed to account for differences in species abundance and number of visits, d' in particular increases rapidly at very low numbers of visits (Blüthgen et al. 2006). An additional limitation was that intraspecific competition

increased as species decreased, since we held the total number of bees constant. Since decreasing intraspecific competition usually decreases niche width (increasing specialization), release from intraspecific competition could partially drive some of the results found here. This may be an important mechanism for *Bombus,* our most active species, which likely experienced a greater degree of intraspecific competition relative to the other species. Further analyses are planned to disentangle the effects of intraspecific versus interspecific competition in driving *Bombus* specialization.

In future studies, altering competition via decreasing the level of rewards or increasing the number of bees could potentially allow us to better understand the roles of interference versus exploitative competition in driving bee specialization and niche partitioning in response to interspecific competition. Additionally, altering handling procedures between flower types (e.g. by changing flower structure using three-dimensional printing) could be useful for establishing the mechanisms underlying floral fidelity, and it could better enable us to examine the role of bee species richness in driving floral fidelity.

These results show a direct link between species richness and bee specialization and complementarity, which are important for pollination function, plant reproduction, and ecosystem stability. Since we tested specialization using standardized artificial flowers, our results indicate that the previously observed relationship between species richness and specialization and complementarity does not solely depend on the identities of plant species present in the experiment. With global declines in bee species diversity of growing concern (Biesmeijer et al. 2006, Potts et al. 2010), our findings indicate that species losses will lead to decreasing specialization and complementarity, with

potentially negative consequences for ecosystem function and stability. Our findings also demonstrate that plastic behaviors driven by interspecific interactions, which are not traditionally studied in BEF contexts, are excellent candidates for future research on drivers of biodiversity-ecosystem functioning relationships.

ACKNOWLEDGEMENTS

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TABLES

		Individual-level specialization ln(d')			Species-level specialization ln(d')		
	Df	Sum Sq	F value	*P* value	Sum Sq	F value	*P* value
Species identity	3	529.700	157.658	< **2.2E-16**	114.390	32.107	< **2.2e-16**
Species richness	3	2.700	2.447	0.118	20.420	17.198	**4.07E-05**
Species identity * Species richness	9	22.6	6.739	**1.580E-04**	6.57	1.843	0.139

Table 1. ANOVA results for linear models (specialization at species and individual levels). For all models, we used species identity and species richness as explanatory variables. We ran the models using log-transformed data for d’.

		Floral Fidelity		Total visits	
	Df	Chi-sq.	*P* value	Chi-sq.	*P* value
Species identity	3	7.401	0.060	405.25	**< 2.2E-16**
Species richness	3	0.154	0.695	13.04	**3.055E-04**
Species identity * Species richness	9	3.056	0.383	0.75	0.861

Table 2. ANOVA results for a generalized linear model with quasi-binomial errors (floral fidelity), plus ANOVA results for a generalized linear model with quasi-Poisson errors (total visits).

	Complementarity ln(H2')		
	Coef	SE	*P* value
Intercept	-3.315	0.307	**< 2E-16**
Species richness	0.282	0.106	**0.009**

Table 3. Summary of linear model results testing the effect of species richness on complementarity, or niche partitioning at the community level. We used H2' as a metric for complementarity and ran the model using log-transformed data.

FIGURES

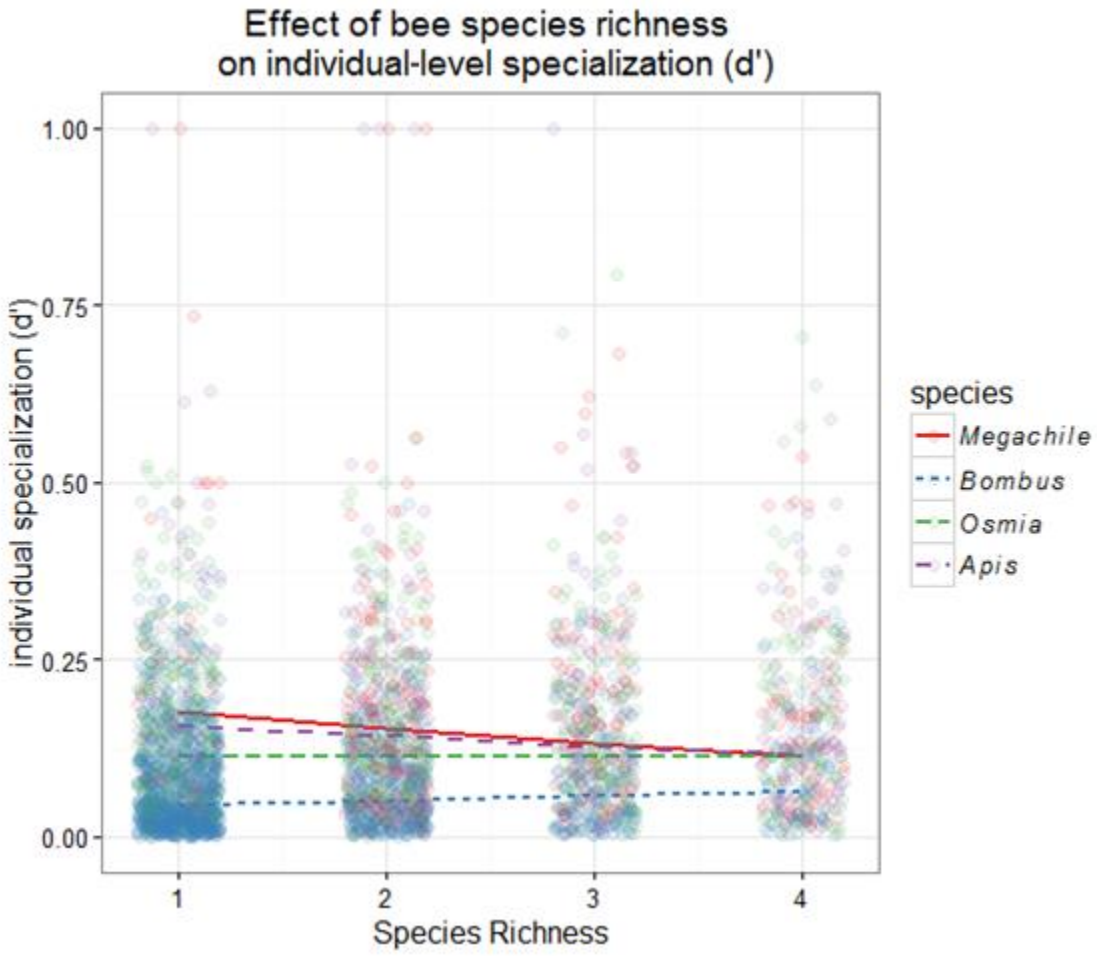

Figure 1. The effect of species richness on individual level specialization (measured using d'). The lines of best fit were determined using a linear model with log transformed d' data. Points represent the specialization of individual bees. We found a significant interaction between species richness and species identity (P= 1.580E-04), where individual specialization decreased with species richness for *Megachile* and *Apis*, remained relatively constant for *Osmia*, and increased for *Bombus*. We found significant species differences (P= < 2.2E-16), where *Bombus* had the lowest level of individual of specialization and *Megachile* had the greatest level of individual specialization.

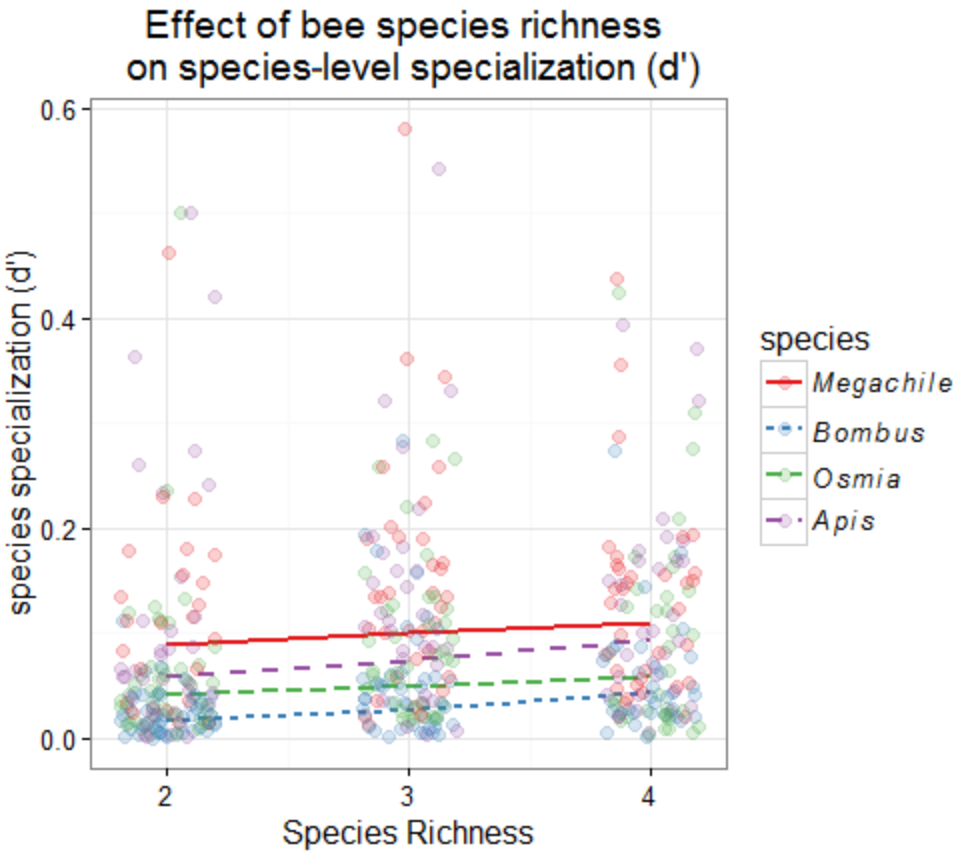

Figure 2. The role of species richness in species-level specialization (d'). The lines of best fit were determined using a linear model, with log-transformed data. Individual points represent the specialization of a particular species within a foraging trial. We found that d' increased with increasing species richness for all four bee species. *Bombus* had the lowest level of specialization, while *Megachile* had the greatest level of specialization.

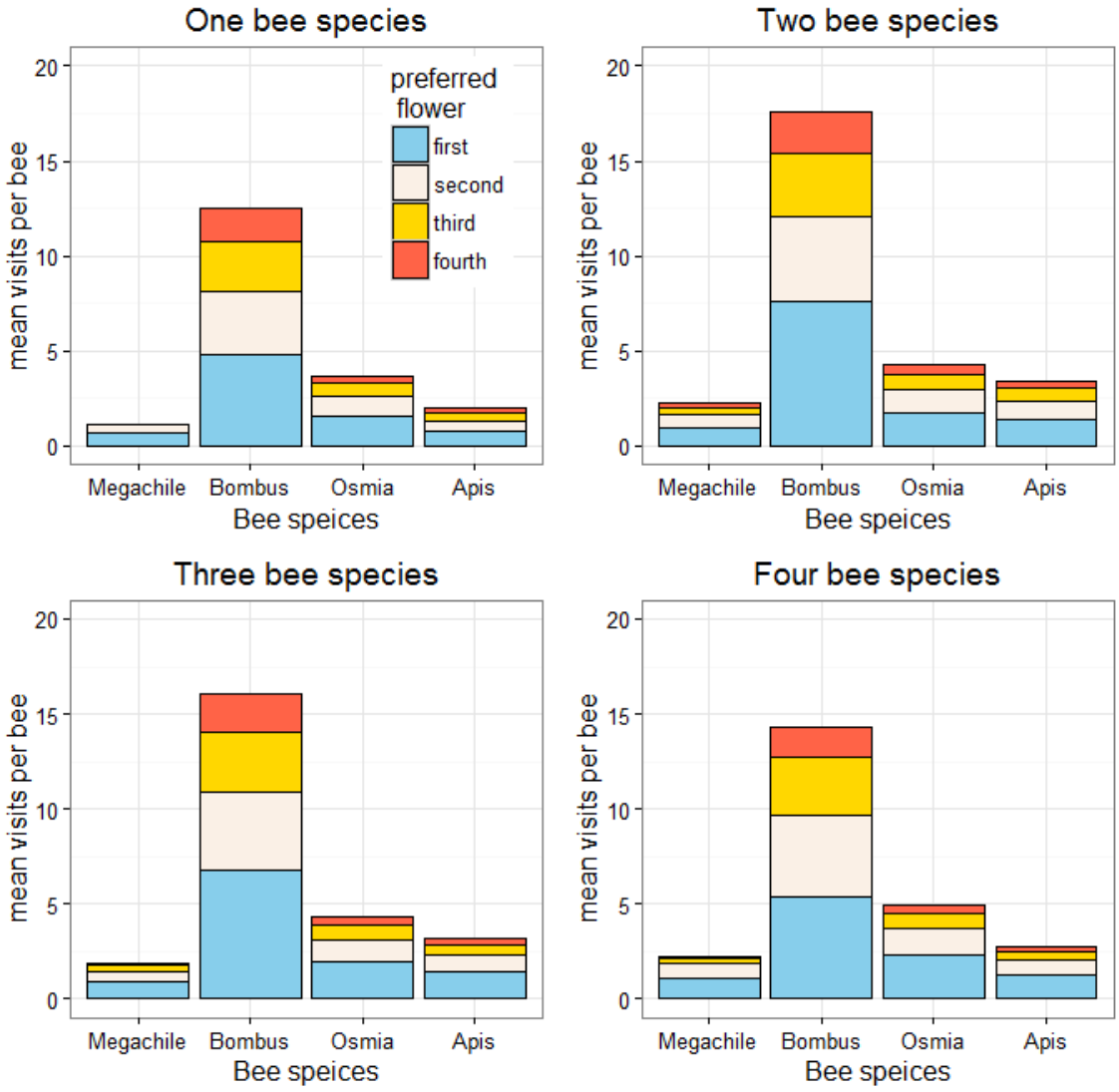

Figure 3. Number of per-bee visits to four artificial flower "species" pooled across experiments containing either one, two, three, or four bee species. Since the most commonly used flower type differed between trials, we ranked each flower species as most visited, second-most, third-most, or fourth-most visited to examine changes in specialization.

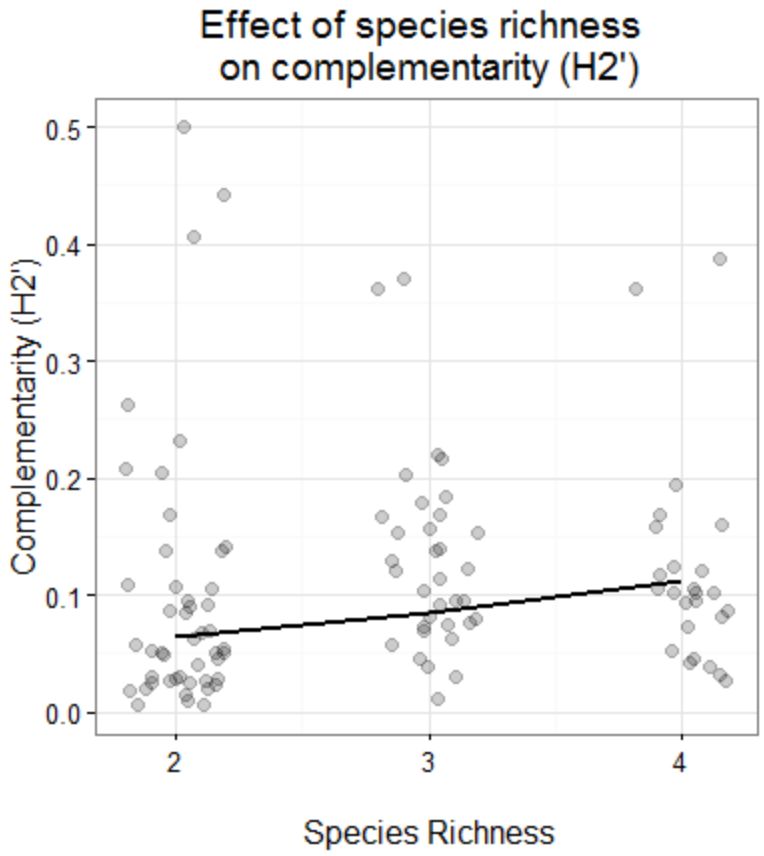

Figure 4. Effect of bee species richness on complementarity (H2'). The line of best fit was determined using a linear model with log-transformed H2' data. We back-transformed the data and the line of best fit before plotting here. Each point represents a single foraging trial. We found that complementarity increases with greater species richness ($P = 0.009$).

Chapter 4.

THE EFFECT OF SUB-LETHAL NEONICOTINOID PESTICIDE EXPOSURE ON FUNCTIONALLY-RELEVANT BEE FORAGING BEHAVIORS IN A SINGLE VERSUS MULTIPLE SPECIES CONTEXT

Carolyn A. Ayers, Emily K. Dobbs, Anna Mayrand, and Berry J. Brosi

ABSTRACT

Exposure to neonicotinoid pesticides has been identified as a contributing factor to global pollinator declines. Many studies have shown that sub-lethal exposure to field realistic levels of pesticides negatively affects bee behaviors important for survival. Yet to our knowledge no one has specifically tested how a multiple-species context can alter the effects of exposure on bee behaviors important for bee fitness. Here we show that sub-lethal neonicotinoid exposure interacts with species richness to negatively affect bee foraging behaviors relevant for bee fitness and plant pollination. We found that a field-realistic dose of neonicotinoid pesticide decreases total visits, decreases bee energy gains, and increases energy expenditures in a multiple species context. We found the opposite in a single-species context, where neonicotinoid exposure increases total flower visits and energy gains. Such a strong interaction between neonicotinoid exposure and species richness indicates that pesticide exposure may negatively affect bee and plant fitness more greatly than previously anticipated by single-species experiments.

INTRODUCTION

Neonicotinoid pesticides have been implicated as one potential factor driving global pollinator declines (Godfray et al. 2014). Neonicotinoids, which are the most commonly used class of insecticides worldwide (Goulson 2013; Van der Sluijs et al. 2013), are systemic pesticides that may be incorporated into the nectar and pollen of plants where they are consumed by pollinators. They target acetylcholine receptors in the insect nervous system, and can have detrimental effects on bees even at very low exposure levels (Mommaerts et al. 2010). For instance, at levels of neonicotinoid exposure commonly found in agricultural fields, several bee species (e.g. *Apis* and *Bombus*) have increased worker mortality (Gill et al. 2012, Henry et al. 2012), reduced colony weight (Whitehorn et al. 2012), and lower worker and queen production (Laycock et al. 2012, Whitehorn et al. 2012). Low levels of pesticides may also impair foraging behaviors including navigation (Henry et al. 2012), ability to learn how to handle flowers efficiently (Stanley and Raine 2016), and ability to collect pollen (Feltham 2014). Furthermore, changes in pollen foraging due to sub-lethal neonicotinoid exposure has been shown to have negative consequences for plant reproduction (Stanley et al. 2015).

There is, however, very little understanding of how field-realistic neonicotinoid pesticide exposure affects bee behaviors important for bee and plant fitness in a single versus multiple species context. Many laboratory studies have examined the effects of disturbances on foraging behavior amongst individuals of the same species (Morandin and Winston 2003, Schneider et al. 2012), however no study to our knowledge has examined changes in behavior in a multiple-species context. While field studies

presumably have multiple species present, no study known to us has specifically examined the interaction between cognitive disturbances and species richness.

We expect that exposure to neonicotinoid pesticides will differentially affect foraging in single versus multiple species contexts, since environmental complexity may increase the difficulty of learning and processing environmental cues (Dukas and Real 1993, Laverty 1994, Naug and Arathi 2007). For instance, a forager in a multiple species context may need to sample a greater number of resources to determine the most rewarding resource, and would need to frequently resample resources to detect changes in the reward landscape (Keasar et al. 2002, Naug and Arathi 2007). There is also some evidence of potential trade-offs in cognitive processes used to store versus process information. For instance, the rate of learning in bumble bees has been shown to decrease when presented with greater numbers of rewarding flower types (Dukas and Real 1993).

There is evidence that bees and other insects modify their behavior in the presence of other bee species, which can have important fitness consequences as well as broader ecological implications (Fründ et al. 2013, Greenleaf & Kremen 2006, Brittain et al. 2013, Brosi & Briggs 2013, Cardinale et al. 2003). For example, pollinator species diversity has been shown to drive short-term foraging specialization, a behavior that promotes conspecific pollen transfer which is required for plant reproduction (Brosi and Briggs 2013, Flanagan et al. 2009, Morales and Traveset 2008, Arceo-Gomez and Ashman 2011). Similarly, a greater diversity of natural enemies can interact to suppress a prey species, with implications for pest control in agriculture (Cardinale et al. 2003). However, there is little understanding of how exposure to pesticides will disrupt these behavioral responses to competition.

In this paper we tested the effect of field-realistic neonicotinoid pesticide exposure and species richness on bee foraging behaviors important for both bee and plant fitness. We tested bee behavior in a laboratory artificial foraging enclosure using RFID technology to precisely track bee movements and energetic rewards. We examined the effect of exposure on the foraging behavior of *Bombus impatiens* across a species richness gradient of one to four bee species. We also examined *Bombus* foraging behavior at low densities in the absence of other bee species to evaluate the confounding effects of interspecific and intraspecific competition. The design of this study is unique in that it allows us to tightly control species abundance, species richness, and neonicotinoid exposure to examine the relative importance of each of these factors for functionally important bee behaviors.

We specifically test how sub-lethal neonicotinoid exposure and interspecific competition interact to affect (1) total bee activity, (2) foraging efficiency (energy gains per flower visit) and total energy gains, and (3) travel distances and flower handlings times. We hypothesize that (1) bees exposed to a field-realistic dose of neonicotinoid pesticides in a multiple species context will have reduced flower visits, (2) reduced energy gains, and (3) increased handling times and distances travelled. These questions will improve our understanding of the mechanisms underlying behavioral responses to competition and how neurological disruptions act on these mechanisms to influence functionally important behaviors.

METHODS

Overview

We examined bee foraging behavior in response to sub-lethal neonicotinoid exposure and bee species richness using a laboratory foraging enclosure at Emory University. We examined four total bee species, including two social bee species (*Bombus impatiens and Apis mellifera)* and two solitary taxa (*Osmia lignaria* and *Megachile rotundata)*. We examined all combinations of one to four bee species containing *Bombus* while holding total bee density constant at 16 bees. We maintained multiple colonies of *B. impatiens* and multiple enclosures of *O. lignaria, M. rotundata,* and *A. mellifera* to account for colony-level and enclosure-level effects. We ran trials with either all exposed bees or all control bees, mimicking exposure in landscapes with seed treatment or spray pesticide treatments.

We held bee density constant at 16 bees for multispecies trials, using eight *Bombus* individuals for two-species trials, either five or six *Bombus* for three-species trials, and four *Bombus* for four-species trials. We also performed single-species *Bombus* trials with densities of sixteen, eight, or four *Bombus* individuals in the absence of interspecific competitors to quantify the effects of intraspecific competition.

Pesticide exposure

Bees in the treatment group were exposed to thiamethoxam ($C_8H_{10}ClN_5O_3S$, Sigma Aldrich), a neonicotinoid pesticide applied to a wide range of row crops in the US (Maienfisch et al. 2001). Bees from the treatment group fed *ad libitum* on sucrose solutions with a field-realistic pesticide concentration of 10 ug/L (Blacquiere et al. 2012)

inside their training enclosures. Bees from the control group fed *ad libitum* on sucrose solutions not containing pesticides. No pesticides were introduced to the primary foraging enclosure or to control bee training enclosures.

We used serial dilutions to reach a thiamethoxam concentration of 10 ug/L (10 ppb). We measured 10 mg of dry thiamethoxam with a microbalance and dissolved the dry pesticide into 1 L of water at 60° C using a magnetic stirrer and hotplate for approximately 15 minutes. Once dissolved the solution was placed in an ice bath. We then added 0.5 mL to 499.5 mL of sucrose solution to reach a desired concentration of 10 ug/L. We made new pesticide and control sucrose solutions every two weeks and stored solutions under refrigeration.

Foraging chamber

The laboratory foraging enclosure contained a foraging array with four artificial flower species differing by color (blue, white, yellow, pink), scent, and sucrose concentrations (2.0 M, 1.5 M, 1.0 M, and 0.5 M). There were 32 total flowers in four rows of 8. The different flower types were distributed uniformly throughout the chamber. The enclosure was approximately 0.74 m deep by 2.27 m wide by 0.75 m tall. Incandescent lamps were used for lighting and to maintain a temperature of 28° C.

The foraging enclosure enabled automatic tracking of bees using mic3-TAG RFID 16 kbit tags (Microsensys GmbH, Erfurt, Germany). Tags were 1.9 x 1.6 x 0.5 mm, and weighed 5.5 mg. We used nontoxic glue (Elmer's Glue-All multi-purpose, Elmer's Products, Inc) to attach tags to each bee’s thorax, and we ensured that tags did not interfere with bee movement or flight. These tags are commonly used in bee foraging

experiments (Morandin and Winston 2003, Schneider et al. 2012). RFID tag readers embedded in each artificial flower record the presence of a bee and activate automatic computer-controlled rewards.

Whenever the RFID tag reader detected a bee, computer-controlled solenoid valves released the sucrose solution from reservoirs located outside the foraging chamber. When a valve was opened, the sucrose solution was gravity-fed into the chamber into artificial flowers. Sucrose was dispensed from a pipette tip embedded in the artificial flower, which was taken up by the bee's proboscis through capillary action. This system allowed us to accurately estimate the amount of energy received by a bee.

We used Arduino MEGA 2560 R3 hardware (Arduino LLC) and Processing software to automatically control sucrose rewards and record flower visits. Whenever a bee entered a flower, a 10 µl reward would be automatically released unless a bee had been recorded in that flower in the last 30 seconds. Otherwise, there was no depletion of resources throughout the trial.

Experimental procedure

We maintained bees in training enclosures, where they trained on flowers with an identical appearance, scent, and sucrose molarity to those in the experimental enclosure. Artificial flowers in the training enclosure, however, differed in the mechanism of sucrose delivery. Training flowers were not computer controlled, and instead used wicks embedded in a sucrose reservoir to deliver the sucrose solution.

Before each trial, we captured bees at random from their training enclosures and chilled them for one hour before attaching RFID tags and recording their tag numbers.

We wiped clean the foraging chamber using ethyl alcohol to remove any bee scent markers remaining from previous trials. We fasted bees for one hour before transferring them to the primary foraging enclosure. We recorded their foraging behavior for 75 minutes before ending each trial. We then recaptured bees and returned them to their original training enclosures. Wherever possible we did not use previously tagged bees, however approximately 58% of bees tested had been used in a previous trial.

Statistical analysis

We used the R statistical programming language (R Core Team 2014) for all analyses. We used linear models to test the effect of bee species richness and pesticide exposure on total bee energy gains and energy gains per flower. We used generalized linear models with Poisson errors to examine the effect of exposure and bee species richness on the number of flower visits. When comparing single versus multiple species contexts, we pooled trials with two, three, and four bee species.

We used mixed-effect models to test the effect of exposure and bee species richness on handling times and distances travelled. We used exposure and species richness as fixed effects, and bee identity as a random intercept. We tested for both the main effects and interactions between species richness and pesticide exposure. We were able to calculate handling times, or the amount of time a bee spent in a flower, by subtracting the time the bee exited a flower from the time it entered, which were both recorded by our RFID tag reader system.

We also compared our multi-species trials with low density *Bombus* trials to analyze the effects of intraspecific versus interspecific competition. Since we held total

bee density constant, the density of intraspecific competitors decreased with greater species richness. Since decreasing intraspecific abundance might affect bee foraging behavior in qualitatively similar ways as increasing species richness, we compared our multi-species trials with low density single-species trials in order to disentangle these two mechanisms. We specifically chose to examine *Bombus* foraging behavior at low densities, since *Bombus* was the most active species and was therefore the most likely to be disproportionately affected by intraspecific competition. For our two-species trials, which contain eight *Bombus* individuals out of 16 total bees, we compared bee foraging behavior with trials containing a total of eight *Bombus* individuals, with no interspecific competitors present. Similarly, for three-species trials, we compared foraging behavior with low density single-species trials containing the same number of *Bombus* (either five or six bees). Finally, for our four-species trials, which contain four *Bombus* individuals, we compared the observed foraging behavior with low-density *Bombus* trials containing only 4 individuals.

RESULTS

Sub-lethal neonicotinoid exposure decreases total bee activity in a multi-species context

We ran 137 trials with 2192 bees from April 2015 to September 2015. We ran 83 control trials with 1328 bees, and 54 treatment trials with 864 bees. We tested 13 different *Bombus* colonies. To test for the role of intraspecific competition, we ran 67 additional trials with four colonies and 612 *B. impatiens* individuals over three different bee densities (23 trials with four bees, 23 trials with eight bees, and 21 trials with 16 bees), which took place from February to March 2016.

First, we measured the mean number of visits per bee per trial across a species richness gradient of one to four bee species. We found a significant interaction between neonicotinoid exposure and bee species richness ($P = 0.00169$; Table 1), where total activity increased slightly with greater bee species richness for control bees, while in exposed bees the mean number of flower visits decreased with species richness (Figure 1A). We also found that the effect of exposure on the number of total visits was opposite in a single versus multiple species context. In a single-species context, exposed bees had increased total activity relative to control bees, while in a multi-species context (i.e. trials containing two or more bee species) exposed bees had reduced total activity (Figure 1A).

We compared these results with low-density *Bombus* trials to examine the confounding effects of decreasing intraspecific competition as species richness increased. We found that release from intraspecific competition was responsible for driving the increased number of flower visits with species richness for control bees. For bees exposed to neonicotinoid pesticides, species richness had no effect on the number of flower visits in the absence of interspecific competitors. (Figure 1B). Therefore, release from intraspecific competition alone was not sufficient to explain the decrease in total bee visits for treated bees as species richness increased.

Exposure decreases total bee energy gains and energy gains per flower visit

Second, we measured the effect of species richness and neonicotinoid treatment on the mean energy gained per bee per trial (Figure 2A). We found that neonicotinoid exposure decreases the per-bee energy intake ($P = 0.0183$). We found a significant interaction between exposure and species richness, where energy gains increased with

species richness for control bees, but decreased with species richness for exposed bees (*P* =0.000112). In our comparison with *Bombus* only trials, we found that the increase in energy for control bees occurred due to release from intraspecific competition as species richness increased, while reduced intraspecific competition was not sufficient to explain the decrease in energy gains for exposed bees (Figure 3).

To determine whether the difference in energy gains was entirely driven by differences in total visits, we examined the mean energy gained per bee per flower visit (Figure 2B). We found a significant interaction between exposure and species richness, where the energy gained per flower by treated bees decreased with bee species richness relative to control bees (P= 0.0348). This indicates that, in addition to visiting fewer flowers, treated bees visited flowers with lower energetic rewards than control bees.

Exposure interacts with species richness to increase flower handling times

Though our dataset does not include information on the mode of bee movement (flying vs. crawling) or their exact travel paths required to precisely calculate energy expenditures, we can examine relevant factors such as the distance traveled between flower visits and the average handling time (i.e. the length of time a bee spends inside a flower). We found that there was a significant interaction between species richness and exposure affecting handling times (*P*= 0.00091). As species richness increased, handling times decreased for control bees and increased for exposed bees (Figure 4). However, we did not find any significant effect of bee species richness or exposure on distance travelled between pairs of flowers. Since exposed bees had significantly greater handling

times than control bees with high species richness, they would likely have greater total energy expenditures.

DISCUSSION

With neonicotinoid pesticides as a potential driver of bee declines, it is important to understand how neonicotinoid exposure can affect functionally-relevant bee foraging behaviors in multi-species environments. Behavioral responses to interspecific competition can be important for individual fitness, and may have ecological consequences. For example, important ecosystem services, including plant pollination and inhibition of agricultural pests are dependent on behaviors driven by interactions between multiple species (Fründ et al. 2013, Greenleaf & Kremen 2006, Brittain et al. 2013, Brosi & Briggs 2013, Cardinale et al. 2003). However, the differential effects of neonicotinoid pesticides on functionally-relevant bee foraging behaviors in a single versus multiple-species context are poorly understood.

Using a laboratory foraging enclosure, we determined the effect of sub-lethal neonicotinoid pesticide exposure on bee foraging behavior in a single versus multiple species context. We found that (1) neonicotinoid exposure increased total visits in a single species context, but decreased the total number of flower visits in a multiple species context; (2) exposure decreased energy gains for neonicotinoid exposed bees, which was mediated by reduced flower visits and visits to less rewarding flowers; and (3) pesticide exposure interacted with species richness to increase handling times in exposed bees, while there was no effect on travel distances. Decreased energy gains and lowered foraging efficiency in a multiple species context would likely have negative implications

for bee fitness, and the strong interaction with species richness indicates that the consequences of pesticide exposure will be especially severe for diverse plant-pollinator communities.

In our analysis of the total number of flower visits per bee, we found a significant interaction between pesticide exposure and species richness affecting total visits (P= 0.00169). We found that the number of total visits increased with species richness for control bees, and decreased for neonicotinoid-exposed bees (Figure 1A). In our comparison with low density *Bombus* only trials, we found that the increase in number of visits for control bees was primarily driven by release from intraspecific competition. This may have occurred since *Bombus* was the most active of the 4 species tested, so the interspecific competitors may not have been sufficiently active to alter *Bombus* foraging patterns. This pattern may have also occurred if *Bombus* avoided flowers recently visited by conspecific competitors (but not from interspecific competitors) through the use of chemical cues. For instance, previous research has shown that bees avoid recently depleted flowers using scent markers (Goulson and et al. 1998, Stout and Goulson 2001). For exposed bees, we found that the decrease in total visits was not fully explained by intraspecific competition alone (Figure 1B), indicating that neonicotinoid exposed bees may be less able to compete with interspecific competitors relative to control bees.

In our analysis of bee energy gains, we found that neonicotinoid exposure decreased bee energy gains overall relative to control bees. However, in a single species context, exposed bees gained more energy than control bees. We found that energy gains increased along with species richness for control bees, while exposure greatly reduced the level of energy gained by exposed bees as species richness increased (Figure 2). We

found that the trend for control bees was driven by release from intraspecific competition similarly as described above for the total number of visits. However, release from intraspecific competition did not fully explain the decreased energy gains in exposed bees (Figure 3), implying that exposed bees were not able to compete as effectively with interspecific species compared to unexposed controls. We also tested whether the quality of bee visits differed between exposed and control bees, and found a significant decrease in energy gain per flower in exposed bees as species richness increased. Therefore, exposed bees not only visited fewer flowers, they also visited lower quality flowers in terms of their energy rewards.

Our tracking system allows us to know which flowers bees visited, but not the method of movement between flowers, so we cannot precisely calculate energy expenditures. However, we were able to evaluate behaviors which require energy expenditures, including distances travelled between flowers and mean flower handling times. We found a significant interaction between species richness and exposure affecting handling times, where handling time increased with species richness for exposed bees, but decreased for control bees (Figure 4; $P = 0.00091$). We did not find a difference in travel distances in control vs. neonicotinoid treated bees or in a single vs. multiple species context. These findings indicate that energy expenditures are higher for exposed bees relative to controls at high species richness levels. Thus, the difference in net energy gains between exposed and control bees in a multiple species context is likely to be greater than the difference estimated by energy gains alone.

Our results stress the importance of examining the community context rather than drawing inferences from single-species experiments. We found that exposure to

neonicotinoids, which acts as a stimulant, increased total flower visits and energy gains of bees in a single-species context. This finding is consistent with other studies on the effect of neonicotinoid exposure in a single species context (Stanley and Raine 2016). However, this relationship reversed in a multiple-species context, where exposed bees were less able to adapt their behavior to competition from other species. Multiple bee species are present and play an important functional role even in areas of intense agriculture (Greenleaf and Kremen 2006), so it is important to understand how neonicotinoid exposure affects behavior in the presence of competing species.

One limitation in the statistical analysis of our data was the lower number of four-species trials, which were limited in number due to a short overlap in the seasonality of the four bee species. Another limitation in our dataset was that foraging activity differed greatly between species. *Bombus* had a very large number of visits, which led us to focus our analysis on *Bombus* behavior, while *Apis* and *Megachile* did not readily learn how to use the artificial flowers and had very few visits.

Future studies are needed to better understand how community context interacts with a broader range of cognitive disturbances. For instance, different types of neonicotinoid pesticides have been shown to differentially affect the insect nervous system with different physiological outcomes (Moffat et al. 2016). Future studies could also test whether the source of environmental complexity changes the observed outcome. For instance, one could examine whether increasing plant species richness or complexity in rewards also interacts with pesticide exposure to alter pollination-relevant bee foraging behaviors.

Future studies are also need to improve our understanding of how neurological disturbances can alter interspecific interactions more generally. Neurological disturbances are globally widespread and can be naturally occurring or anthropogenic. For instance, pathogens and parasites (Klein 2003, Hurd 2003, Gegear et al. 2006) and plant secondary compounds including caffeine and nicotine (Städler 1992, Brenner 2003, Wright et al. 2013, Köhler et al. 2012) have all been shown to drive behavioral changes in insects. Ethanol produced by a diversity of nectar microbes has also been shown to affect the behavior of pollinating wasps (Ehlers and Olesen 1997). With the high prevalence of community-wide cognitive disturbances, potential effects on interspecific interactions could have important implications for many study systems.

With worldwide declines in pollinator species richness (Biesmeijer et al 2006), it will become increasingly important to understand how multiple-species contexts and neonicotinoid exposure interact to influence ecologically-important behaviors. Our findings indicate that field-realistic exposure to neonicotinoid pesticides decreases the number of flower visits and bee energy gains in a multiple-species context. A decrease in energy gains would likely have important effects on bee fitness, while lowered flower visits could potentially harm plant reproduction in pollen-limited environments. This paper is amongst the first to address how species richness and neonicotinoid exposure interact to disrupt important behavioral responses to competition, and the potential implications for bee fitness and plant pollination.

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TABLES

				energy gains					
	total visits			total energy			energy per visit		
Fixed Effects	Coef	SE	*P* value	Coef	SE	*P* value	Coef	SE	*P* value
Exposure	0.414	0.124	0.816	0.580	0.128	**0.018**	0.040	0.031	0.235
Species Richness	0.095	0.043	0.224	0.121	0.046	0.844	0.022	0.011	0.380
Exposure * Richness	-0.220	0.071	**0.002**	-0.279	0.072	**1.120E-04**	-0.037	0.017	**0.035**

	energy expenditures					
	distance travelled			handling time		
Fixed Effects	Coef	SE	*P* value	Coef	SE	*P* value
Exposure	0.074	0.040	0.166	-0.400	0.148	0.639
Species Richness	-0.002	0.014	0.170	-0.086	0.053	0.549
Exposure * Richness	-0.031	0.023	0.164	0.278	0.084	**9.100E-04**

Table 1. Results of a generalized linear model with binomial errors (total visits), linear models (total energy gain and energy gain per visit), and mixed-effects models (distances and handling times). All *P*-values were calculated using an ANOVA.

FIGURES

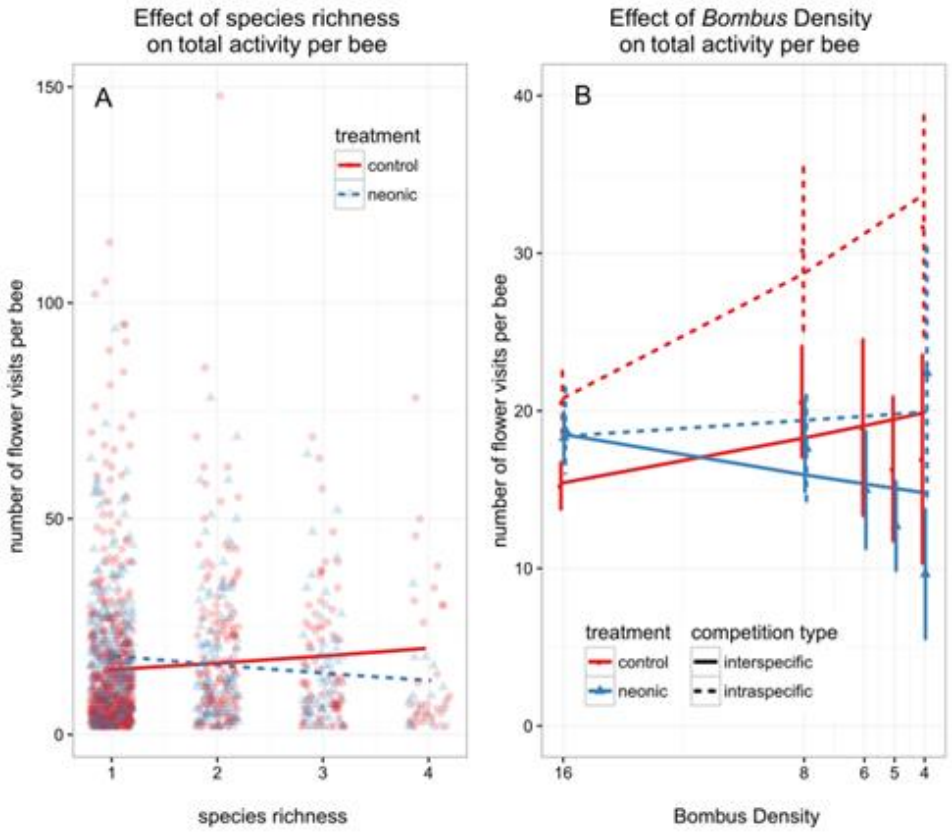

Figure 1. (A) Effect of species richness and sub-lethal pesticide exposure on the number of flower visits by *Bombus impatiens*. The lines of best fit for control (red) and bees exposed to neonicotinoids (blue) were determined using generalized linear models with Poisson errors. Each point represents the number of flowers visited for an individual bee. We found a significant interaction between neonicotinoid exposure and bee species richness affecting total visits (P= 0.00169). (B) Effect of interspecific competition on total visits relative to intraspecific controls. Points and error bars indicate the mean number of visits with Poisson 95% confidence intervals. The lines of best fit were determined using generalized linear models with Poisson errors. We found that interspecific competition (rather than intraspecific competition) drives the decrease in flower visits for treated bees as species richness increases.

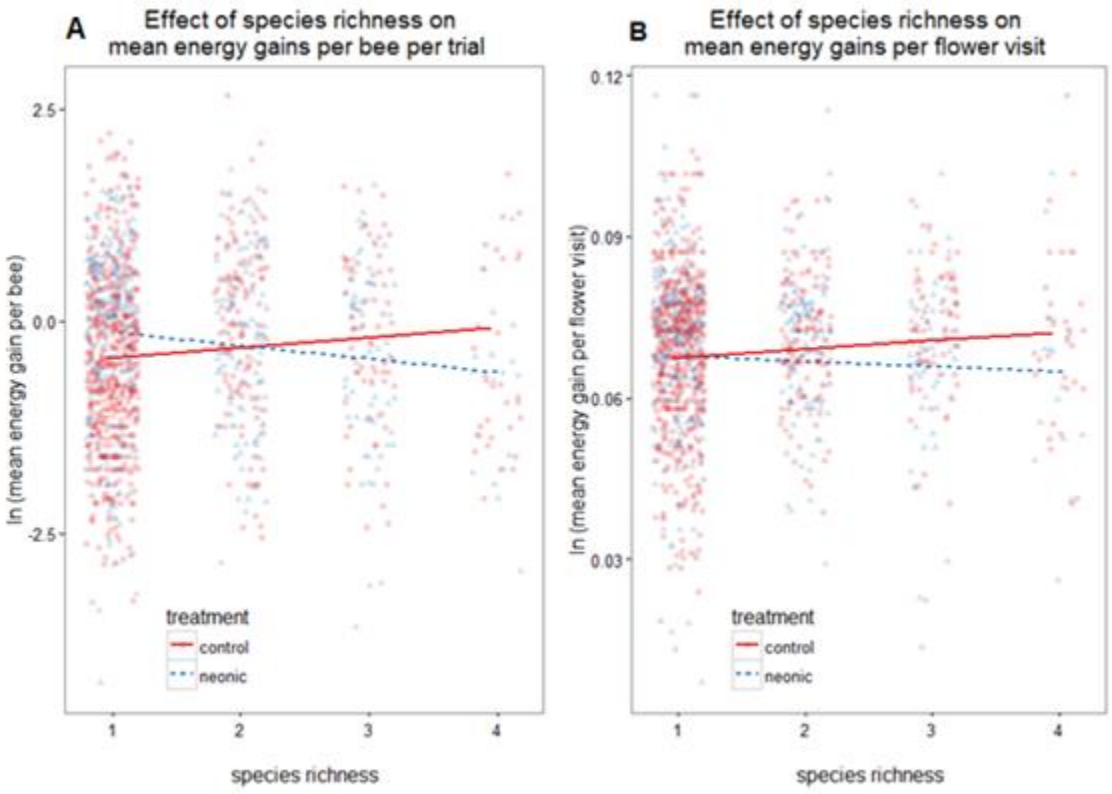

Figure 2. Effect of species richness and sub-lethal pesticide exposure on (A) energy gains (kJ) per bee per trial and (B) energy gains per flower visit. The lines of best fit for control bees (solid line) and bees exposed to neonicotinoids (dashed line) were calculated using linear models. Points represent the energy gains of individual bees. In A, we found a significant main effect of exposure on energy gains per bee (P = 0.0183), and a significant interaction between neonicotinoid exposure and bee species richness (P = 0.000112). In B, we found a significant interaction between exposure and species richness (P= 0.0348).

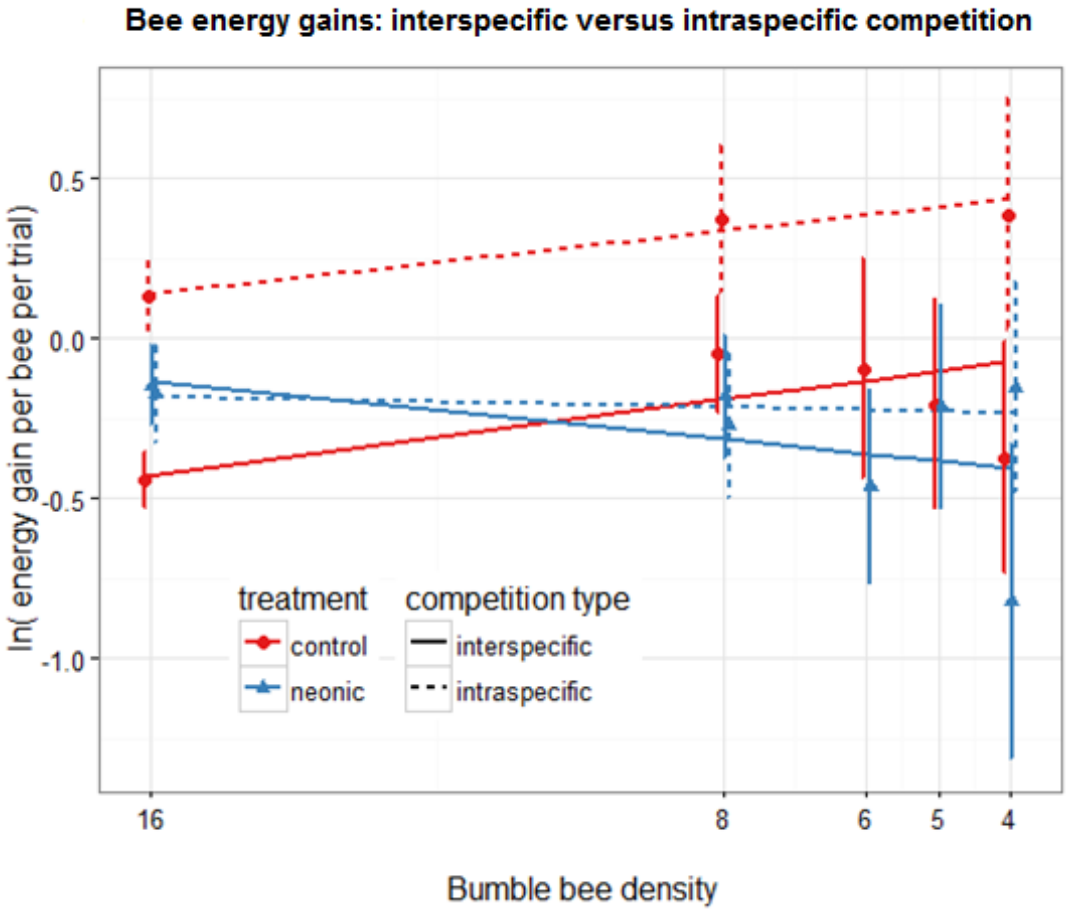

Figure 3. Effect of interspecific competition on energy gains per bee relative to intraspecific controls. Points and error bars indicate the mean number of visits with 95% CI. We determined the lines of best fit using a linear model. We found that intraspecific competition drives the increase in energy gains with species richness for control bees, while interspecific competition primarily drives the decrease in energy gains for treated bees.

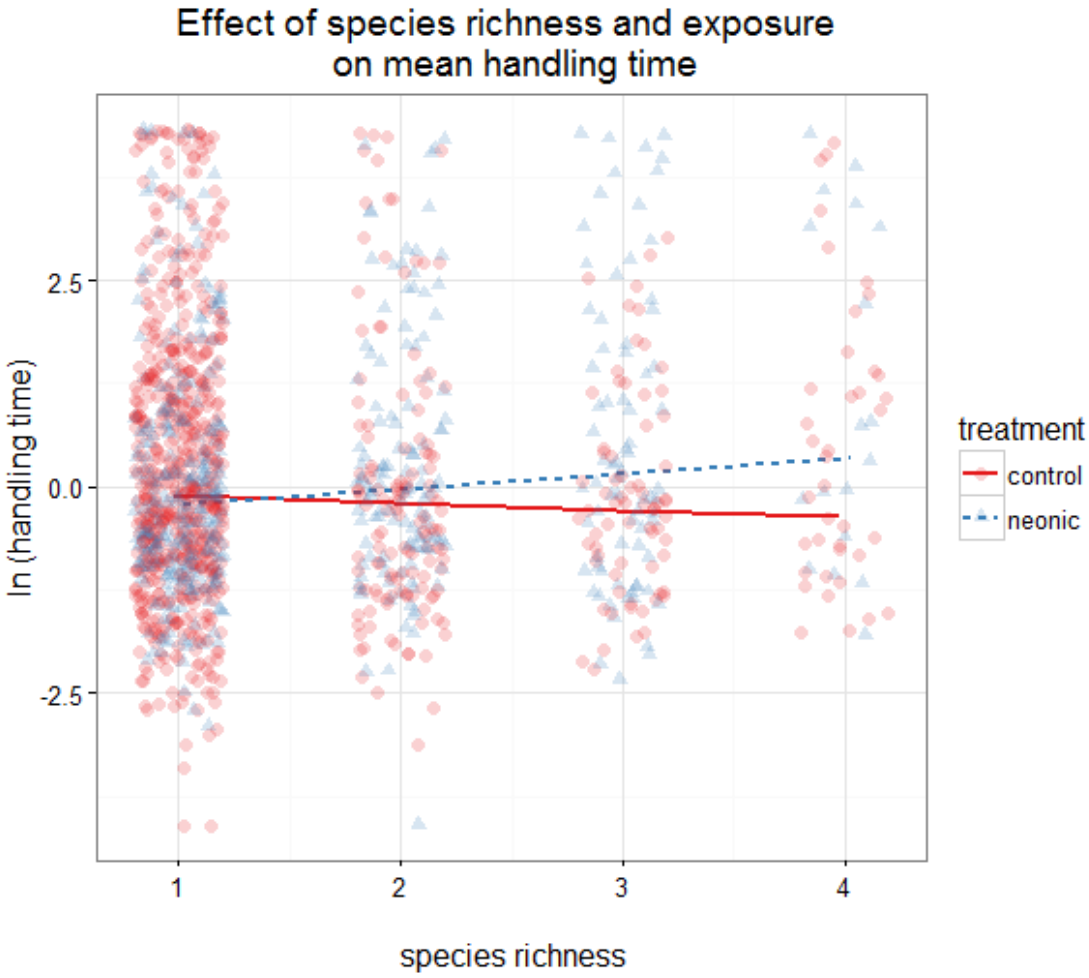

Figure 4. Effect of sub-lethal pesticide exposure and species richness on the mean handling time per flower visit (in minutes). We determined the lines of best fit using a mixed-effects model with random intercepts for each bee. Points represent the mean handling time of individual bees. We found a significant interaction between pesticide exposure and species richness affecting handling times (P= 0.00091).

CONCLUSION

In this dissertation, I use both a statistical and experimental approach to study important bee foraging behaviors. First, I developed new statistical techniques to quantify and test the significance of trapline foraging, a behavior in which foragers repeatedly visit spatially fixed resources in a predictable sequence. Using empirical data to compare metrics, I found that my proposed metric, determinism, offers an improvement over other metrics since it does not depend on the arrangement of resources or experimental design, which allows for comparisons between differing environments. I found that the spatially explicit individual-based null model I developed is less prone to Type I or II statistical error relative to existing models. The type of model proposed here may also be useful for other ecologically important spatially explicit and individual-based processes.

Second, I used a foraging enclosure with artificial flowers to examine the effects of bee species richness and sub-lethal neonicotinoid pesticide exposure on functionally important bee foraging behaviors. I found that species-level specialization and complementarity increase with bee species richness. I also found that neonicotinoid exposure decreases total flower visits and bee energy gains in a multiple species context. These findings are important for better understanding how ongoing pollinator species losses and exposure to neonicotinoid pesticides, the most widely used insecticide worldwide, can affect bee fitness and the stability of plant pollination services to both agricultural and natural ecosystems.

My first two dissertation chapters, in which I propose a new metric and an individual-based spatially explicit null model, are both important for testing the statistical

significance of suspected traplines. A metric for traplining, as presented in Chapter 1, is required for quantifying the degree of traplining, which allows for comparisons across studies and against a null distribution. The spatially explicit individual-based null model presented in Chapter 2 builds on Chapter 1, as it demonstrates a method for building a null distribution for testing the significance of traplines by purposefully excluding the mechanism of interest (e.g. spatial memory). There are currently no widely used metrics or null models which can be used to easily compare between differing studies and environments. The methods presented in these two chapters offer an improvement over existing methods, since they allow for easy comparisons across different resource densities or spatial arrangements.

The third and fourth chapters both address changes in bee behavior as the result of increasing species richness. Chapter 4 adds an additional layer of complexity to Chapter 3 by testing how a cognitive disturbance (i.e. neonicotinoid pesticide exposure) alters bee behavioral responses to species richness. Together, these chapters test how species losses and sub-lethal exposure to neonicotinoid pesticide might interact to affect bee foraging behaviors important for bee and plant fitness. Both of these chapters are vitally important for understanding how bee species losses will affect bee foraging behaviors with potential negative consequences for plant reproduction.

The statistical component of this dissertation can also inform the metrics used for specialization in the experimental component. Specifically, in Chapter 3, d’ (a commonly used metric for species-level specialization) was sensitive to the number of visits at very low visitation numbers. This occurs since a forager visiting few flowers has a greater probability of visiting all flowers from the same species compared to a forager who

visited a very large number of flowers. A null model as presented in Chapter 2 could address this problem by controlling for the probability of visiting the same flower species. A spatially-explicit null model would be especially useful, since it would account for the spatial layout of flowers in determining the probability of transitioning between flowers of the same species.

Future laboratory studies could focus on the mechanisms underlying the results observed in the experiments presented here. For instance, one could alter the level of competition via decreasing the level of rewards or increasing the number of bees to better understand the roles of interference versus exploitative competition in driving bee specialization and niche partitioning in response to interspecific competition. Additionally, altering handling procedures between flower types (e.g. by changing flower structure using three-dimensional printing) could be useful for establishing the mechanisms underlying floral fidelity, and it could better enable us to examine the role of bee species richness in driving floral fidelity. Future studies are also needed to better understand how community context interacts with a broader range of cognitive disturbances, including different types of insecticides or environmental contaminants. Additionally, future studies could also test whether the source of environmental complexity changes the observed outcome. For instance, one could also examine whether increasing plant species richness or complexity in rewards also interacts with pesticide exposure to alter pollination-relevant bee foraging behaviors. The experiments could also be repeated with a different set of bees to test whether the identities of the bees chosen for the experiment alters the outcome.

The effect of species diversity and cognitive disturbances on bee foraging behaviors important for plant reproduction could also be tested in a greenhouse or field setting. In controlled greenhouse experiments, one could directly measure the effects of sub-lethal neonicotinoid pesticide exposure on the reproductive fitness of plants mediated via changes in pollinator behavior. In a field setting, one could temporarily remove bee species in or near agricultural areas treated or not treated with neonicotinoid pesticides to examine whether the remaining species respond differently to species removals.

My dissertation has broader implications for statistical methods in ecology, as well as for conservation and sustainable agriculture. The statistical methods presented in the first component of my dissertation (Chapters 1-2) offer an improvement over existing methods for traplining. These methods may be applied to many other spatially explicit and individual-based processes, which are currently at the forefront of ecology. To our knowledge, we are the first to propose a spatially explicit individual-based null model specifically designed to test the statistical significance of empirical data.

The second component of my dissertation (Chapters 3-4) has important implications for BEF and for pollinator conservation. My results show that phenotypic plasticity, as opposed to fixed traits, can be an important mediator of BEF relationships. This indicates that bee species losses may alter bee behavior more greatly than predicted by studies based on fixed-traits. We also found evidence of complementarity in the absence of pollinator trait matching, which may have been mediated by the use of social information. Finally, we found that sub-lethal exposure to neonicotinoid pesticides may alter bee behavior more greatly in complex multi-species environments than would have been predicted by experiments in single species environments, where the majority of

studies take place. Together, these studies indicate that bee species losses and exposure to pesticides may have greater negative implications for bee fitness and plant pollination in natural and agricultural systems than previously anticipated.

Appendix 1.

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SUMMARY

Appendix 1 contains four components: (1) calculation of determinism (DET) with and without including reverse sequences (i.e. perpendicular diagonals on recurrence plots) or immediate repeats of behavior (i.e. horizontal/vertical lines on recurrence plots) in the numerator of DET, (2) R code for calculating the prevalence of reverse sequences, (3) R code for generating random sequences, (4) a sensitivity analysis of DET with regard to resource abundance and minimum trapline length for high resource densities, and (5) a statistical analysis of the effect of experience on the degree of traplining (with and without inclusion of reverse sequences in the DET calculation).

CALCULATION OF DETERMINISM

Determinism may be calculated using the contour map tool from the 'spatstat' statistical package in R. First, we created a matrix resembling a recurrence plot, where a 0 or 1 was placed to represent the absence or presence of a recurrence, respectively. We set all values on the main diagonal to 0 so these would not be included in calculations of determinism. One half of the sum of the entire matrix gives the denominator in our calculation of DET. The 'spatstat' package may be used to assign unique numbers to each contiguous set of 1s in the matrix. This package, which was created for designing contour maps, assigns a unique number to all contiguous sets of points. We created a table to identify the number of points in each contiguous set, and restricted the table to only

include sets larger than the required minimum length. Half of the sum of the table represents the numerator in our calculation of DET.

The R package ‘fNonlinear’ may also be used to quickly create recurrence plots.

```
#=============================================================#
#-This R code contains a function to calculate Determinism--#
#-and create recurrence plots using the fNonlinear package--#
#=============================================================#

#Load required packages
library(fNonlinear)
library(spatstat)

#=============================================================#
#------Begin function to calculate Determinism--------------#
#=============================================================#

#x is a vector of numbered resource visits/behaviors
#minl is the minimum length of a diagonal to be considered in the
#numerator of the determinism calculation

determinism <- function(x, minl){

#Depending on the dataset it may be desirable to filter out consecutive
visits
#to the same flower. See function below and delete ‘#’ in the line
below to use
#x = filterout(Ldata = x)

#-----set up matrix resembling a recurrence plot, where a 1 indicates a
repeat
#-----visit and 0 indicates the absence of a repeat.

det1 = matrix(cbind(rep(x,length(x))),nrow=length(x))
tdet = t(det1)
det = ((det1 - tdet) == 0) * 1

#set the main diagonal equal to zero so it won't be included in the
calculation

diag(det) = 0

#Use spatstat package to create a 'countour map' of the matrix,
#which assigns all sets of contiguous 1's a unique number
yi <- as.im(det)
ycOut <- connected(yi, background = 0)
 yc <- ycOut$v

#Depending on the dataset it may be desirable to filter out diagonals
perpendicular to #the main diagonal. Code is provided for the
‘removeperpdiag’ function below.
#Delete “#” from the line below to filter out perpendicular diagonals
```

```
#yc = removeperpdiag(yc,minl)

#Note: this code may take several minutes to run for very long
sequences

#---- filter out short repeats: a 'trapline' should include more unique
resources
#---- than the minimum cutoff (minl)

#make an alternative DET matrix that contains the resource IDs
det2 = matrix(rep(x,nrow(det)),nrow=nrow(det),byrow=TRUE)*det
#make a dataframe with the number of times each resource appears in a
diagonal
listofseq = data.frame(group = yc[1:length(yc)],
seq=det2[1:length(det2)])
#how many unique resources are in the diagonal
uniquevisits = rowSums((table(listofseq)>0)*1)
#only count diagonals with at least 'minl' number of unique resources
longenough = (uniquevisits >= minl)*table(yc)
#find the numerator:
#(remember this still includes both the top and bottom halves of the
matrix)

contig = sum(longenough)

denominator= sum(det)

#This also still includes top and bottom halves of the matrix


#------------------- total DET score
#divide the numerator and denominator in half before calculating DET
for just
#the top half of the matrix

print((contig/2)/(denominator/2))
}

#=========================================================#
#---optional function to filter out visits to same flower in a row
#=========================================================#

filterout <- function(Ldata){
for (i in 2:length(Ldata)){
if(Ldata[i] == Ldata[i-1] ){Ldata[i - 1]= NA}
}
Ldata=Ldata[!is.na(Ldata)]
Ldata
}


#=========================================================#
#---optional function to filter out perpendicular diagonals
#=========================================================#

removeperpdiag = function(yc,minl){
```

```
#first, remove observations that are too short to save time
remove = names(table(yc)[table(yc)< minl])
for (i in remove){
yc[ yc == i ] = NA
}

#Only do these steps if there are perpendicular diagonals longer than
minl
if(sum(!is.na(yc))!= 0){

#-------remove sequences perpendicular to the main diagonal
#save list of levels (aka groups of continuous points) that weren't
removed in the previous step
newlevels= levels(droplevels(yc))
#use a loop to go through each level and remove all that are not
parallel
for (i in 1:length(newlevels)){

#only look at matrix positions of current contiguous group
set = which(yc == newlevels[i])

#make a list of all possible parallel points
pardiag=c(seq(set[1], length(yc), (nrow(yc) +1))[-1],seq(set[1], 0, -
(nrow(yc) +1))[-1])

for(i in 1:length(set)){
pardiag = c(pardiag,c(seq(set[i], length(yc), (nrow(yc) +1))[-
1],seq(set[i], 0, -(nrow(yc) +1))[-1]))
}

#remove points that don't fall in these positions
keepers = set[set %in% pardiag]
toberemoved = setdiff(set,keepers)

if (length(toberemoved) > 0){ yc[toberemoved] = NA }
}}
yc
}


#=========================================================#
#------Example DET calculation-----------------------------#
#=========================================================#

x= c(1,2,3,8,9,10,2,3,4,5,7,3,8,9,10,7,5,4,8)
determinism(x, 3)


#=========================================================#
#------Make recurrence plot--------------------------------#
#=========================================================#

recurrencePlot(x, m=1, d=0, eps = 1, nt = 1,end.time = 800, pch = 16,
cex = .1)
```

CALCULATING THE PREVALENCE OF REVERSE SEQUENCES

In this code, we quantify the prevalence of reverse sequences, which appear as diagonals perpendicular to the main diagonal on recurrence plots. We quantify the number of recurrences which belong to a perpendicular diagonal, out of the total number recurrences which belong to a recurrent series of any type (all diagonals, and vertical/horizontal lines when applicable). In other words, we examine what proportion of recurrence points included in the numerator of the DET calculation are removed if we do not include perpendicular diagonals.

```
#load required package
library(spatstat)

propperp <- function(x, minl){

#This function outputs the proportion of recurrence points which are
removed from the #numerator of the DET calculation if perpendicular
diagonals are not included
#Note that this code requires the 'removeperpdiag' function provided
above
#This code may take several minutes to run for very long sequences

#optional: use function to filter out immediate repeats if needed
#x = filterout(Ldata = x)

det1 = matrix(cbind(rep(x,length(x))),nrow=length(x))
det2 = t(det1)
det = ((det1 - det2) == 0) * 1

diag(det) = 0
rr = sum(det)  + nrow(det)   #diagonal + other 1's

det

yi <- as.im(det)
ycOut <- connected(yi, background = 0)
 yc <- ycOut$v
 yc

#------ calculate the numerator of DET first for matrix with
perpendicular diagonals
det2 = matrix(rep(x,nrow(det)),nrow=nrow(det),byrow=TRUE)*det
listofseq = data.frame(group = yc[1:length(yc)],
seq=det2[1:length(det2)])
uniquevisits = rowSums((table(listofseq)>0)*1)

longenough = (uniquevisits >= minl)*table(yc)
contig = sum(longenough)

#------------ now calculate the numerator of DET again without
perpendicular diagonals
#use the function provided above in part 1 to remove perpendicular
diagonals

yc2 = removeperpdiag(yc,minl)
```

```
det2 = matrix(rep(x,nrow(det)),nrow=nrow(det),byrow=TRUE)*det
listofseq2 = data.frame(group = yc2[1:length(yc2)],
seq=det2[1:length(det2)])
uniquevisits2 = rowSums((table(listofseq2)>0)*1)

longenough2 = (uniquevisits2 >= minl)*table(yc2)
contig2 = sum(longenough2)

#------------------- total proportion

#optional:
#uncomment the line below to also print out additional information
related to DET
#print(c(contig,contig2,nrow(det), rr))

#print the proportion only
print((contig - contig2)/contig)

}
```

CALCULATION OF SIMULATED SEQUENCES

```
R code to generate foraging sequences:
#================================================#
#------This R code may be used to generate hypothetical foraging
sequences
#------with varying levels of predictability
#================================================#
#p is used to set the probability of repeating the last transition
#s is used to set the abundance
# a short sequence is created to be used as a reference of past
transitions
generate_seq = function(p,s){
starter = c(1:s,1)
hypseq= starter
a=1:(length(starter) - 1)
i=length(starter)
#where i is the ith visit in the sequence
#set the length of sequences to be generated here. It is currently set
at 100
while(i < 100){
#what is the current position?
current = which(starter == hypseq[i])[1]
#We use the sample function to determine if the forager succeeded in
#repeating the past transition
#according to the probability 'p'
#If successful, set the next entry in the sequence to the past
transition
#made in the reference sequence
#if not successful, choose a different resource
if(sample(1:100,1) <= p){hypseq[i+1]=starter[current + 1]}
else {hypseq[i+1]=sample(a[- c(starter[current],starter[current +
1])],1)}
#update i to move to the next item in the sequence
i = i + 1}
```

```
#find the determinism---
#comment the next line out to output the sequence instead
#remember to set a minl value below
b = determinism(hypseq,minl)
}
```

SENSITIVITY ANALYSIS:

THE EFFECT OF RESOURCE ABUNDANCE ON DETERMINISM

Sensitivity of DET to Resource Abundance for High Resource Densities

We repeated the analysis in Chapter 1 Figure 2 and Table 2 with an increase in resource abundances by one order of magnitude. Specifically, we used a reference group of 50 resources, and compared with 100, 250, or 500 resources. As in Chapter 1 Figure 2, we simulated 1,044 sequences each with a length of 1000 resource visits. To better fit the generalized linear model, we simulated a greater number of sequences with intermediate and high levels of sequence predictability. For the range of 40% to 50% chance of repeating a prior transition, we simulated sequences at intervals of 0.5%. For the range of 50.25% to 90% percent chance of repeating a past transition, we simulated sequences at intervals of 0.25%. For the range of 90.125% to 100%, we generated sequences at intervals of 0.125%.

We found significant effects of abundance and significant interactions between abundance and sequence predictability (Table 1). Thus, corrections for resource abundance would be required to compare DET across studies with large numbers of resources. We also found very little sensitivity to resource abundance at very high levels of traplining, with the greatest sensitivity occurring at intermediate values of traplining.

Comparisons across studies with large differences in resource density are still possible after correcting for resource abundance, which may be facilitated by sensitivity

analyses such as the one presented here (Figure 1). In these analyses, GLMs are used to determine the predicted DET values for sequences with a given level of predictability but different resource densities. Conversions between resource densities may then be performed using the underlying predictability level. For example, sequences with 50 resources and a mean DET of 0.5 occurred when there was a 79% chance of repeating a previous transition. For the same sequence predictability (i.e. a 79% chance of repeating a past transition) and 250 resources, the mean DET was equal to 0.61. Therefore, a DET value of 0.5 for 50 resources may be converted to 0.61 for comparison with sequences observed for 250 resources.

Sensitivity analysis of minimum length 'l'

We calculated the mean determinism (DET) values for simulated sequences with five, 10 and 50 resources, with different minimum required trapline lengths (Table 2). We found that DET decreased as the minimum required trapline length increased.

THE EFFECT OF BEE FORAGING EXPERIENCE ON THE DEGREE OF TRAPLINING

We used a mixed effects model with binomial errors to test the effect of bee foraging experience on the degree of traplining. Specifically, we compared the first and last quarter of flower visits for each bee. Since repeated measures were obtained from individual bees (before and after gaining experience on a foraging array), we included a random effects for both the slope and intercept.

We calculated the results when reverse sequences (perpendicular diagonals on recurrence plots) were included in the calculation of DET (Table 3, Figure 2), and the

results when reverse sequences were excluded from the calculation of DET (Table 4, Figure 3). Overall, we found that 27.1% of all recurrences in a recurrent series belonged to a perpendicular diagonal (i.e. a reverse sequence). Despite the high prevalence of reverse sequences, the results of our above analysis were not very sensitive to the exclusion of these sequences from the DET calculation.

TABLES

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*
Intercept	-10.48	0.14	-72.77	< 2E-16
factor(abundance)100	0.62	0.24	2.6	0.00965
factor(abundance)250	2.25	0.29	7.71	4.46E-14
factor(abundance)500	3.12	0.39	8.09	2.71E-15
percent	0.13	0.002	73.96	< 2E-16
factor(abundance)100:percent	-0.01	0.003	-2	0.04587
factor(abundance)250:percent	-0.02	0.004	-6.2	9.81E-10
factor(abundance)500:percent	-0.03	0.005	-5.61	2.90E-08

Null deviance: 2047821 on 683 degrees of freedom
Residual deviance: 54218 on 676 degrees of freedom
Dispersion parameter for quasi-binomial family taken to be 71.2

Table 1. The effect of sequence predictability and abundance on determinism for 50, 100, 250, and 500 resources.

Sensitivity Analysis of DET by Minimum Trapline length *'l'*

Abundance: 5					
	Percent chance of repeating transition				
	0%	**25%**	**50%**	**75%**	**100%**
$l = 3$	0.35	0.32	0.42	0.73	1
$l = 5$	0.02	0.01	0.05	0.47	1

Abundance: 10					
	Percent chance of repeating transition				
	0%	**25%**	**50%**	**75%**	**100%**
$l = 3$	0.06	0.1	0.3	0.64	1
$l = 5$	0.001	0.01	0.05	0.36	1

Abundance: 50					
	Percent chance of repeating transition				
	0%	**25%**	**50%**	**75%**	**100%**
$l = 3$	0	0.07	0.43	0.71	1
$l = 5$	0	0.02	0.13	0.56	1
$l = 10$	0	0	0	0.21	1

Table 2. Mean determinism (DET) values for simulated sequences with five, 10 and 50 resources, with different minimum trapline lengths.

Statistical Model:
cbind(success, failure) ~ experience + (1 + experience | as.factor(Bee.ID))

Random effects:				
Groups	*Name*	*Variance*	*Std. Dev.*	*Corr.*
Bee ID	Intercept	1.0196	1.0098	
	Experience	0.1799	0.4242	-0.93
Number of observations: 16, groups: Bee ID, 8				

Fixed effects:				
z	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
(Intercept)	-3.1807	0.3621	-8.784	< 2e-16
experience	0.6905	0.1509	4.575	4.77e-06

Table 3. Effect of bee experience on DET: Mixed-effects model with binomial errors (written in R syntax using the lme4 package) and a summary of model results. Reverse sequences were included in the numerator of DET.

Statistical Model:
cbind(success, failure) ~ experience + (1 + experience | as.factor(Bee.ID))

Random effects:				
Groups	*Name*	*Variance*	*Std. Dev.*	*Corr.*
Bee ID	Intercept	0.9093	0.9536	
	Experience	0.1856	0.4308	-0.89
Number of observations: 16, groups: Bee ID, 8				

Fixed effects:				
z	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
(Intercept)	-3.7251	0.3451	-10.795	< 2e-16
experience	0.7635	0.1537	4.969	6.74e-07

Table 4. Mixed-effects model with binomial errors (written in R syntax using the lme4 package) and a summary of model results. Here, reverse sequences were not included in the numerator calculation of DET.

FIGURES

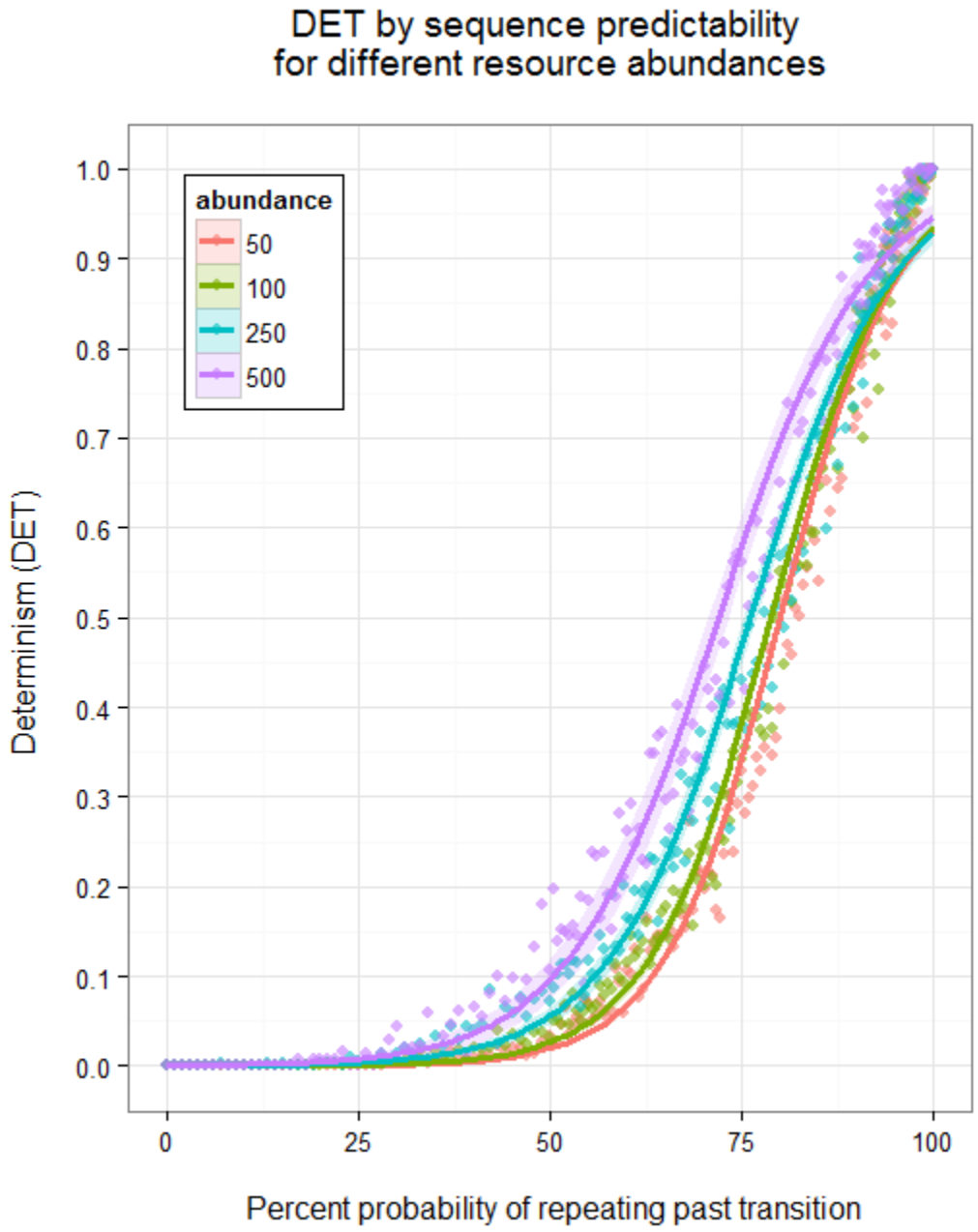

Figure 1. Determinism (DET) values from hypothetical foraging sequences varying in sequence predictability and resource abundance. The line of best fit and 95% CI were calculated using GLM's with quasi-binomial errors.

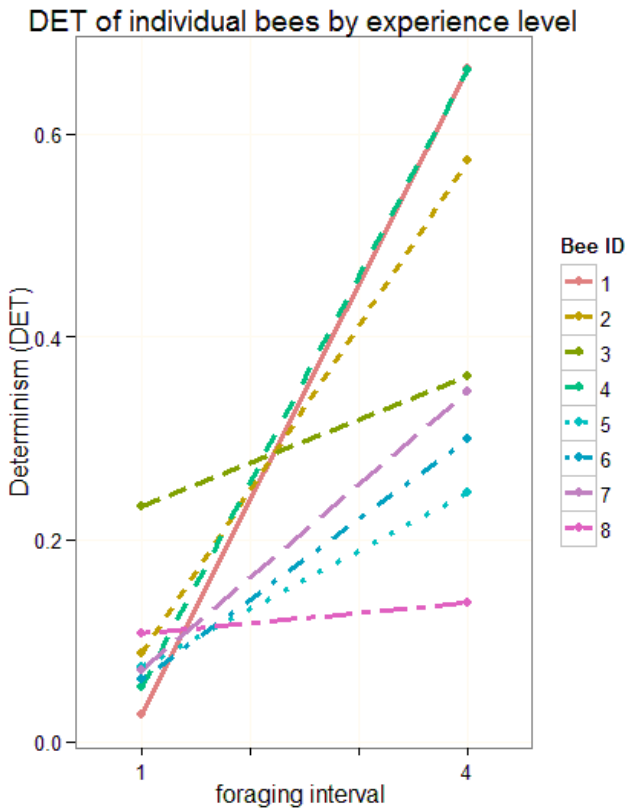

Figure 2. Observed DET values for the first and last quarter of flower visits for eight individual bumble bees. Foraging intervals were each comprised of approximately 110 flower visits. Reverse sequences were included in the numerator of DET.

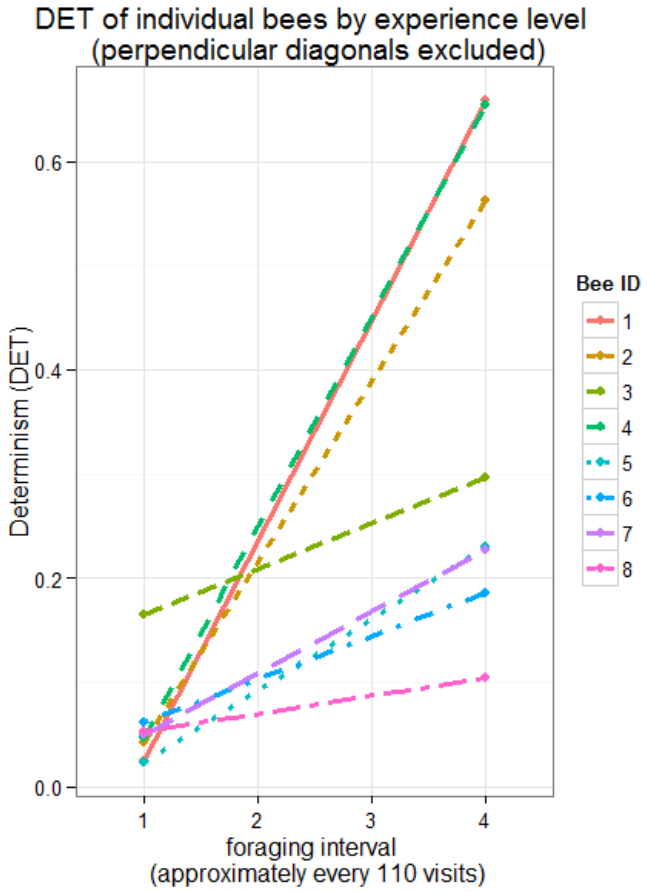

Figure 3. Observed DET values for the first and last quarter of flower visits for eight individual bumble bees. Foraging intervals are each comprised of approximately 110 flower visits. Reverse sequences were not included in the numerator of DET.

Appendix 2.

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SENSITIVITY ANALYSIS OF RESOURCE DENSITY, DETECTION DISTANCE, AND MOVEMENT TYPE

We tested the sensitivity of the null model to a range of resource abundances, forager movement patterns, and sensory perception distances. We hypothesize that, first, traplines emerge more frequently at low resource abundances, since forager movements are more predictable and repeatable where fewer choices are available. Second, for forager movement, we hypothesize that a random walk will result in less traplining than a correlated random walk. Since a random walk resembles a more localized search pattern, there is a greater probability a forager will visit the same resource each time it enters a specific region of the field. For a correlated random walk, the resources visited will depend highly on the initial direction of travel. Third, for sight distance, we hypothesized that medium distances will result in the highest frequency of sequence repeats. Foragers with low sight ranges are more likely to bypass nearby flowers, while foragers with high sight distances have very many resources to choose from. In both cases, it may be difficult to predict which resources will be visited, resulting in fewer sequence repeats relative to foragers with medium sight distances.

Interaction of sight distance and resource abundance

We tested the effect of sight distance on the frequency of traplining in our proposed null model, and tested for interactions with resource abundance. We found that foragers with medium sight distances had the greatest level of traplining for all resource

abundances, while foragers with low or high sight distances had relatively low levels of traplining.

In general, the level of traplining decreased with increased resource abundances (Figure 1). However, when the sight distance was small, the relationship reversed. A forager with low sight range was more likely to repeat sequences in a high resource abundance setting. This interaction between abundance and sight range was statistically significant (<2e-16, See Table 1).

Interaction between sight distance, resource abundance, and movement type

We examined the effect of movement type (a random or correlated random walk) on the frequency of traplines, and tested for interactions with resource abundance and sight distance.We found that sequence repeats occurred at all resource abundances, detection distances, and different movement types. However, very few sequence repeats emerged at low resource abundance with low sight distance with a correlated random walk, or at high resource abundance with high sight distance.

When sight distance was low, sequence repeats occurred more frequently for foragers with a random walk relative to a correlated random walk for all abundance levels (Figure 2). For medium sight distances, a random walk increased the frequency of sequence repeats relative to a correlated random walk, but only at low resource abundances. When the sight distance was high, type of movement had very little effect. This interaction between movement type and sight distance was statistically significant (<2e-16, see Table 1).

This pattern may occur since the resources visited by a forager using a correlated random walk depend highly on the initial direction of travel. As a result, this type of forager is much less likely to revisit the same resource from a given position compared to a forager using a random walk.

DECISION RULES FOR SELECTING AMONGST MULTIPLE RESOURCES

The observed decrease in traplining at high detection distances may be dependent on the decision rules a forager uses to choose the next resource. In cases where foragers are very predictable in their choices (such as always choosing the nearest neighbor resource) traplining may remain high even at high sight distances. However, foragers exclusively following nearest neighbor rules would be more representative of a medium or low sight distance forager since more-distant flowers would never be considered or visited. Since many species preferentially visit closer resources, we ran a sensitivity analysis to compare the effect of foragers using random versus distance-dependent decision rules to select resources.

We quantified sequence predictability in simulations where the probability of a forager choosing a resource was inversely correlated with the resource distance (Figure 3). We found that determinism was elevated only for cases with high sight distance and low-medium resource abundance, and where distance was heavily weighted:

$$\text{Probability of choosing flower}_i = 1 - (\text{distance of flower}_i)^3 / \text{sum}((\text{distance of each flower in sight})^3)$$

There was no difference in determinism when less weight was given to flower distance:

Probability of choosing $flower_i$ = 1 - (distance of $flower_i$) / sum((distance of each flower in sight))

R CODE: SAMPLE RANDOMIZATION NULL MODEL

```
#---------------------- Sample randomization model
# First, we need to know distances and angles between resources

#required packages
      library(stats)
      library(reshape2)
      library(dplyr)


#distance matrix
      #first calculate the distances between resources
      #use this to calculate the distances from the "Lihoreau"
      dataset

      sim.pos = data.frame(x= 0, y= 0)
      sim.pos[1,]=c( -12, 11)
      sim.pos[2,]=c( -11, -8)
      sim.pos[3,]=c( -5, -8)
      sim.pos[4,]=c( 9, -12)
      sim.pos[5,]=c( -5, 4)
      sim.pos[6,]=c( 4, 4)


      d = dist(sim.pos, method = "euclidean", diag = FALSE, upper
      = TRUE, p = 2)

      sim.distmat <- melt(as.matrix(d), varnames = c("row",
      "col"))


#For a given sequence, we need to know the distance travelled

distancefunction = function(sim.seq){


      #let's save the x positions to a vector
      sim.x = sim.seq
      sim.x[sim.seq == "1"] = -12
      sim.x[sim.seq == "2"] = -11
      sim.x[sim.seq == "3"] = -5
      sim.x[sim.seq == "4"] = 9
      sim.x[sim.seq == "5"] = -5
      sim.x[sim.seq == "6"] = 4

      #let's save the y positions to a vector
```

```
      sim.y = sim.seq
      sim.y[sim.seq == "1"] = 1
      sim.y[sim.seq == "2"] = -8
      sim.y[sim.seq == "3"] = -8
      sim.y[sim.seq == "4"] = -12
      sim.y[sim.seq == "5"] = 4
      sim.y[sim.seq == "6"] = 4


      #find x and y differences
      sim.x.diff = sim.x
      sim.x.diff = c(NA,diff(sim.x))

      sim.y.diff = sim.y
      sim.y.diff = c(NA,diff(sim.y))

      sim.dist = sqrt(sim.x.diff^2 + sim.y.diff^2)
      sim.dist
}

#-------------Find the angles of movement

#short function to find the angle between three points
anglefunction = function(a,b,c){

      a.dist= sim.distmat[sim.distmat$row == a & sim.distmat$col
      == b,3]
      b.dist= sim.distmat[sim.distmat$row == a & sim.distmat$col
      == c,3]
      c.dist= sim.distmat[sim.distmat$row == b & sim.distmat$col
      == c,3]

      angle = acos((a.dist^2 + c.dist^2 -
      b.dist^2)/(2*a.dist*c.dist))
      if (!is.na(angle)) {if(angle == 0){angle = pi}}
      if(is.na(angle)){angle = 0}
      angle
}

#now find the angles of movement used in the sequence

sim.angle.function = function(sim.seq){

      sim.angle=sim.seq

            for (i in 2:(length(sim.seq)-1)){
            a= sim.seq[i - 1]
            b= sim.seq[i]
            c=sim.seq[i + 1]
            sim.angle[i] = anglefunction(a,b,c)

            }
```

```
      sim.angle[c(1,length(sim.angle))]= NA
      sim.angle
}

#now make a function to define outside and center regions
      #--- 1's are outsides
      #2's are insides

fieldfunction = function(sim.seq){

      sim.field = sim.seq
      sim.field[sim.field == 1 | sim.field == 2 | sim.field == 4]
      = 1
      sim.field[sim.field == 3 | sim.field == 5 | sim.field == 6]
      = 2

      sim.field
}

#we also need to save a general key for which resource
#is in which region
fieldfunction2 = function(sim.seq){

      sim.field = sim.seq
      sim.field[sim.field == 1 | sim.field == 2 | sim.field == 4]
      = 1
      sim.field[sim.field == 3 | sim.field == 5 | sim.field == 6]
      = 2

      field.mat = data.frame(row = 1:6, field = c(1,1,2,1,2,2))
      field.mat
}


#------------------ Sample randomization function

sampleran = function(sim.seq){

      myseq=t(data.frame(sim.seq))
      mydist = distancefunction(sim.seq)
      myangle = sim.angle.function(sim.seq)
      sim.field = fieldfunction(sim.seq)
      field.mat = fieldfunction2(sim.seq)

      start = sample(1:ncol(myseq) , 1, replace = TRUE, prob =
      NULL)
      sr.seq= as.numeric(myseq[start])

      startquad= sim.field[start]
      newdist = sample(na.omit(mydist[sim.field == startquad]) ,
      1, replace = TRUE, prob = NULL)
```

```
      nextup= as.numeric(which(abs(newdist -
      sim.distmat[sim.distmat$row == as.numeric(myseq[start]),3])
      == min(abs(newdist - sim.distmat[sim.distmat$row ==
      as.numeric(myseq[start]),3]))))
      sr.seq =c(sr.seq, nextup)
      sr.dist = c(NA, sim.distmat[sim.distmat$row ==
      as.numeric(myseq[start]),3][1])

      #for the rest of the sequence elements, it's possible to
      start
      #using turning angles as well
            for (i in 3:length(sim.seq)){
            nextup = tail(sr.seq,1)
            newfield = field.mat[field.mat$row == nextup,2]
            newdist = sample(na.omit(mydist[sim.field ==
            newfield]) , 1, replace = TRUE, prob = NULL)

            pool = abs(newdist - sim.distmat[sim.distmat$row ==
            as.numeric(myseq[start]),3])

            #remove current and second from last flower from the
            pool of choices
            pool[tail(sr.seq,2)[1]] = NA
            pool[tail(sr.seq,1)] = NA

            #arbitary cutoff for which resources are
            #close enough in distance
            #to start considering turning angles
            nextup= na.omit(which(pool < 3))

            if(length(nextup) > 1){
                  newangle = sample(na.omit(myangle[sim.field ==
            newfield]) , 1, replace = TRUE, prob = NULL)

                        anglepool=data.frame(id = nextup, angle=0)
                        for (a in 1:length(anglepool)){
                        anglepool$angle[a]=
            abs(anglefunction(tail(sr.seq,2)[1],tail(sr.seq,1),nex
            tup[a]) - newangle)
                        }
            nextup = anglepool[anglepool$angle ==
            min(anglepool$angle),1]

            } else {nextup=which(pool == min(na.omit(pool)))}


            sr.seq =c(sr.seq, nextup)

            }
      sr.seq #print out new randomized sequence

} #-----end of function
```

NETLOGO CODE: NULL MODEL FOR TESTING THE STATISTICAL SIGNIFICANCE OF TRAPLINES

See the online resource "nullmodel_Lihoreau.nlogo" for the NetLogo code using the Lihoreau dataset resource layout.

BEE FORAGING SEQUENCES FROM "EMORY" DATASET

See "the online resource Emorysequences.csv" for the foraging sequences of the eight most active bees from the Emory dataset.

SENSITIVITY OF DETERMINISM TO MINIMUM TRAPLINE LENGTH

In the determinism metric (DET), the parameter '*minl*' sets the minimum number of consecutive sequence repeats required to be considered part of a trapline. We found that as the minimum length required increased, determinism decreased (Table 2).

TABLES

	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-0.5477741	0.08281	-6.615	3.82E-11
abundance	-0.0102044	0.0044303	-2.303	0.021271
sight	0.0780247	0.0131812	5.919	3.29E-09
movement type (CRW)	-0.4763253	0.1232837	-3.864	0.000112
abundance:sight	-0.0059355	0.0007609	-7.8	6.51E-15
abundance:movement type (CRW)	0.0173798	0.0066384	2.618	0.00885
sight:movement type (CRW)	0.0407735	0.0192464	2.119	0.034146
abundance:sight:movement type (CRW)	-0.0015469	0.0011167	-1.385	0.166005

Table 1. An analysis of the effect of null model parameters on the predictability of sequences (DET) using a generalized linear model with quasi-binomial errors. Movement type was treated as a factor. All variables and two-way interactions were statistically significant. The three-way interaction, however, was not statistically significant

	Determinism			
Source	***minl* = 3**	***minl* = 4**	***minl* = 5**	***minl* = 6**
Lihoreau inexperienced	0.314028	0.131274	0.033462	0.020592
Lihoreau inexperienced	0.446429	0.247449	0.118622	0.090561
Lihoreau inexperienced	0.577922	0.353247	0.224675	0.090909
Lihoreau inexperienced	0.355696	0.153165	0.046835	0.011392
Lihoreau inexperienced	0.4083	0.186078	0.064257	0.028112
Lihoreau inexperienced	0.37766	0.168883	0.05984	0.046543
Lihoreau inexperienced	0.353508	0.184319	0.081155	0.028886
Lihoreau inexperienced	0.392265	0.167127	0.085635	0.017956
Lihoreau experienced	0.91199	0.659439	0.659439	0.646684
Lihoreau experienced	0.895086	0.742364	0.60425	0.557769
Lihoreau experienced	0.7023	0.441137	0.359946	0.311231
Lihoreau experienced	0.839286	0.728316	0.598214	0.553571
Lihoreau experienced	0.493911	0.313938	0.216509	0.154263
Lihoreau experienced	0.673582	0.401107	0.280775	0.239281
Lihoreau experienced	0.732713	0.452128	0.324468	0.301862
Lihoreau experienced	0.550532	0.375	0.111702	0.111702
Emory	0	0	0	0
Emory	0.014035	0	0	0
Emory	0.065574	0	0	0
Emory	0.035714	0	0	0
Emory	0.031949	0.01278	0	0
Emory	0.051852	0	0	0
Emory	0.026087	0	0	0
Emory	0.065574	0.016393	0	0

Table 2 Determinism of individual foraging sequences across different minimum trapline lengths.

Table of determinism for individual foraging sequences and null model output

Model	Source	Individual Number	Determinism	Total visits
sample randomization	Lihoreau inexperienced	1	0.322277221	100
sample randomization	Lihoreau inexperienced	2	0.216507082	100
sample randomization	Lihoreau inexperienced	3	0.258898825	100
sample randomization	Lihoreau inexperienced	4	0.218344696	100
sample randomization	Lihoreau inexperienced	5	0.203913063	100
sample randomization	Lihoreau inexperienced	6	0.212007328	100
sample randomization	Lihoreau inexperienced	7	0.187288213	100
sample randomization	Lihoreau inexperienced	8	0.208517162	100
sample randomization	Lihoreau experienced	9	0.322348399	100
sample randomization	Lihoreau experienced	10	0.301463737	100
sample randomization	Lihoreau experienced	11	0.231262352	100
sample randomization	Lihoreau experienced	12	0.230043487	100
sample randomization	Lihoreau experienced	13	0.188573482	100
sample randomization	Lihoreau experienced	14	0.194406322	100
sample randomization	Lihoreau experienced	15	0.19711342	100
sample randomization	Lihoreau experienced	16	0.210526316	100
observed	Lihoreau inexperienced	1	0.131274131	100
observed	Lihoreau inexperienced	2	0.24744898	100
observed	Lihoreau inexperienced	3	0.353246753	100
observed	Lihoreau inexperienced	4	0.153164557	100
observed	Lihoreau inexperienced	5	0.186077644	100
observed	Lihoreau inexperienced	6	0.168882979	100
observed	Lihoreau inexperienced	7	0.18431912	100
observed	Lihoreau inexperienced	8	0.167127072	100
observed	Lihoreau experienced	9	0.659438776	100
observed	Lihoreau experienced	10	0.742363878	100
observed	Lihoreau experienced	11	0.441136671	100
observed	Lihoreau experienced	12	0.728316327	100
observed	Lihoreau experienced	13	0.313937754	100
observed	Lihoreau experienced	14	0.401106501	100
observed	Lihoreau experienced	15	0.45212766	100
observed	Lihoreau experienced	16	0.375	100
random	Lihoreau inexperienced	1	0.071343734	100
random	Lihoreau inexperienced	2	0.075981549	100
random	Lihoreau inexperienced	3	0.07064459	100
random	Lihoreau inexperienced	4	0.072557749	100

random	Lihoreau inexperienced	5	0.075473029	100
random	Lihoreau inexperienced	6	0.073487233	100
random	Lihoreau inexperienced	7	0.072929396	100
random	Lihoreau inexperienced	8	0.074040127	100
random	Lihoreau experienced	9	0.074967158	100
random	Lihoreau experienced	10	0.072117947	100
random	Lihoreau experienced	11	0.071914998	100
random	Lihoreau experienced	12	0.074486781	100
random	Lihoreau experienced	13	0.072110401	100
random	Lihoreau experienced	14	0.070984922	100
random	Lihoreau experienced	15	0.072490706	100
random	Lihoreau experienced	16	0.07493502	100
netlogo	Lihoreau inexperienced	1	0.156968877	100
netlogo	Lihoreau inexperienced	2	0.149282976	100
netlogo	Lihoreau inexperienced	3	0.15093688	100
netlogo	Lihoreau inexperienced	4	0.151821527	100
netlogo	Lihoreau inexperienced	5	0.151634916	100
netlogo	Lihoreau inexperienced	6	0.144343303	100
netlogo	Lihoreau inexperienced	7	0.15156038	100
netlogo	Lihoreau inexperienced	8	0.156291391	100
netlogo	Lihoreau experienced	9	0.156968877	100
netlogo	Lihoreau experienced	10	0.149282976	100
netlogo	Lihoreau experienced	11	0.15093688	100
netlogo	Lihoreau experienced	12	0.151821527	100
netlogo	Lihoreau experienced	13	0.151634916	100
netlogo	Lihoreau experienced	14	0.144343303	100
netlogo	Lihoreau experienced	15	0.15156038	100
netlogo	Lihoreau experienced	16	0.156291391	100
sample randomization	Emory	17	0.303854521	95
sample randomization	Emory	18	0.199109491	109
sample randomization	Emory	19	0.174757263	100
sample randomization	Emory	20	0.209172686	119
sample randomization	Emory	21	0.181631572	95
sample randomization	Emory	22	0.146362892	111
sample randomization	Emory	23	0.212266686	112
sample randomization	Emory	24	0.21523394	97
observed	Emory	17	0	95
observed	Emory	18	0	109
observed	Emory	19	0	100
observed	Emory	20	0	119
observed	Emory	21	0.012779553	95

observed	Emory	22	0	111
observed	Emory	23	0	112
observed	Emory	24	0.016393443	97
random	Emory	17	0.000157046	95
random	Emory	18	0.000595637	109
random	Emory	19	0.001664018	100
random	Emory	20	0.000313198	119
random	Emory	21	0.0012197	95
random	Emory	22	0.000614494	111
random	Emory	23	0.000255986	112
random	Emory	24	0.000604202	97
netlogo	Emory	17	0.094664168	95
netlogo	Emory	18	0.096223216	109
netlogo	Emory	19	0.096334602	100
netlogo	Emory	20	0.096230438	119
netlogo	Emory	21	0.095470299	95
netlogo	Emory	22	0.097676433	111
netlogo	Emory	23	0.095420956	112
netlogo	Emory	24	0.093865991	97

Table 3. Determinism values of observed foraging sequences and model outcomes for individual bees.

FIGURES

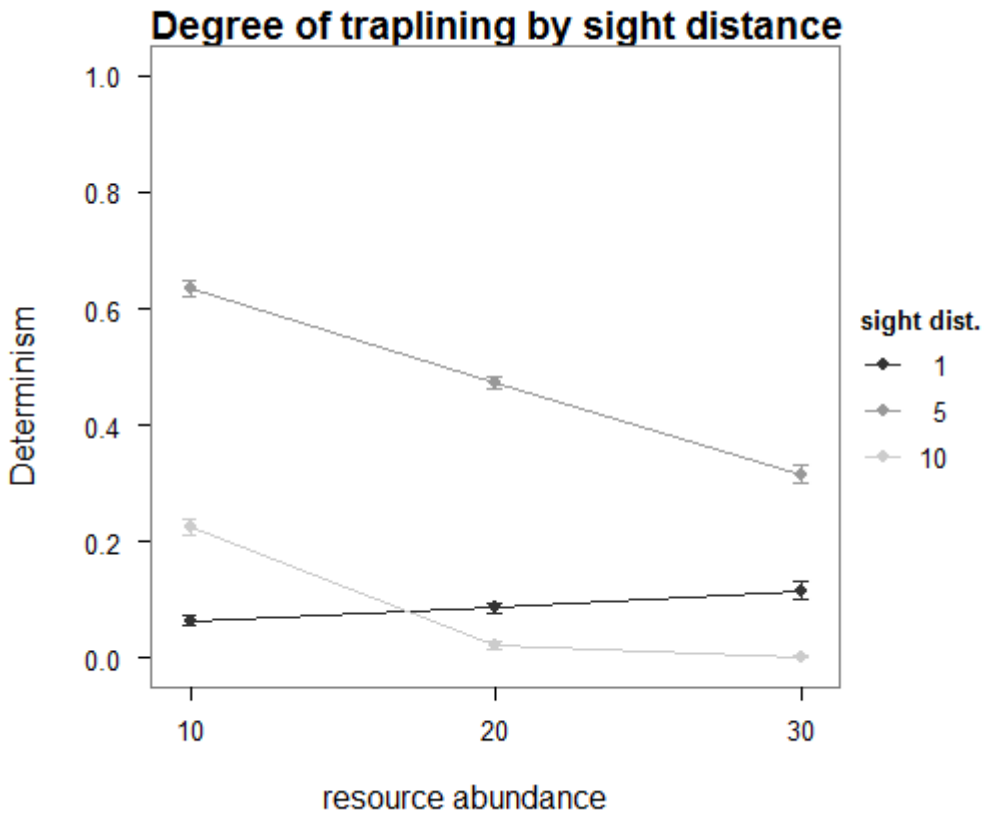

Figure 1. Degree of traplining by resource abundance and sight distance. There is a statistically significant interaction between resource abundance and detection distance. (Error bars are 95% quasi-binomial CI)

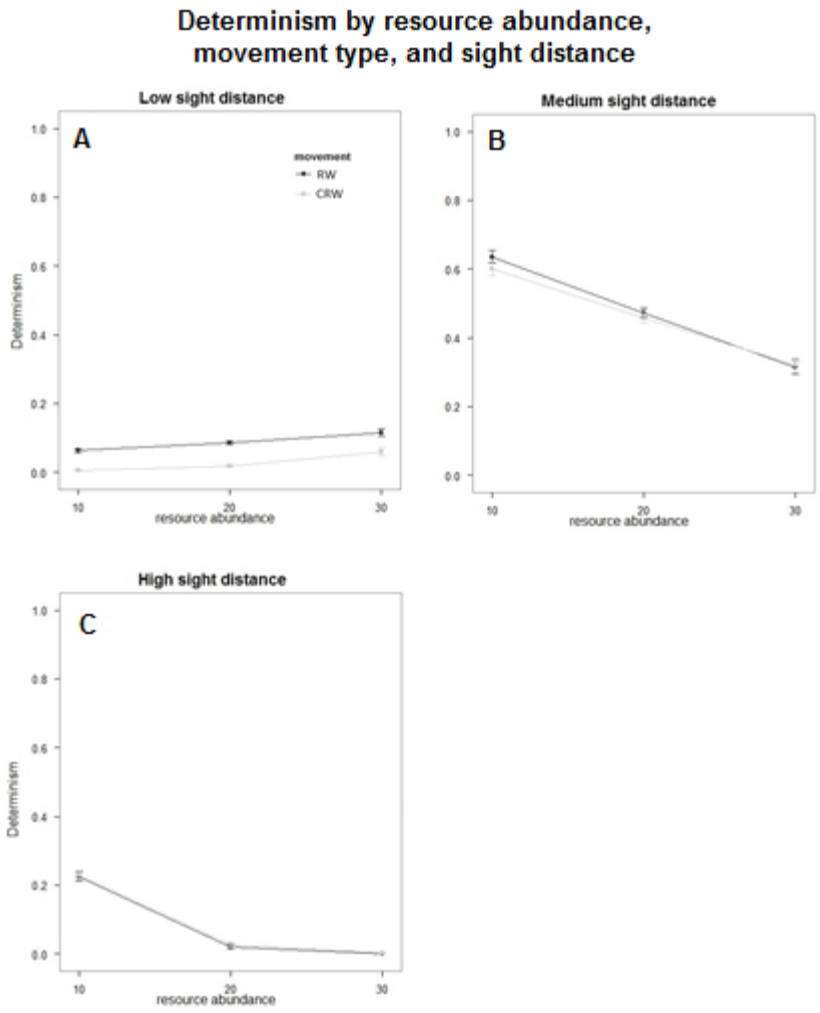

Figure 2. Frequency of sequence repeats (Determinism) by resource abundance and movement type with: (A) high sight distance, (B) medium sight distance, and (C) low sight distance. (Error bars are 95% quasi-binomial CI)

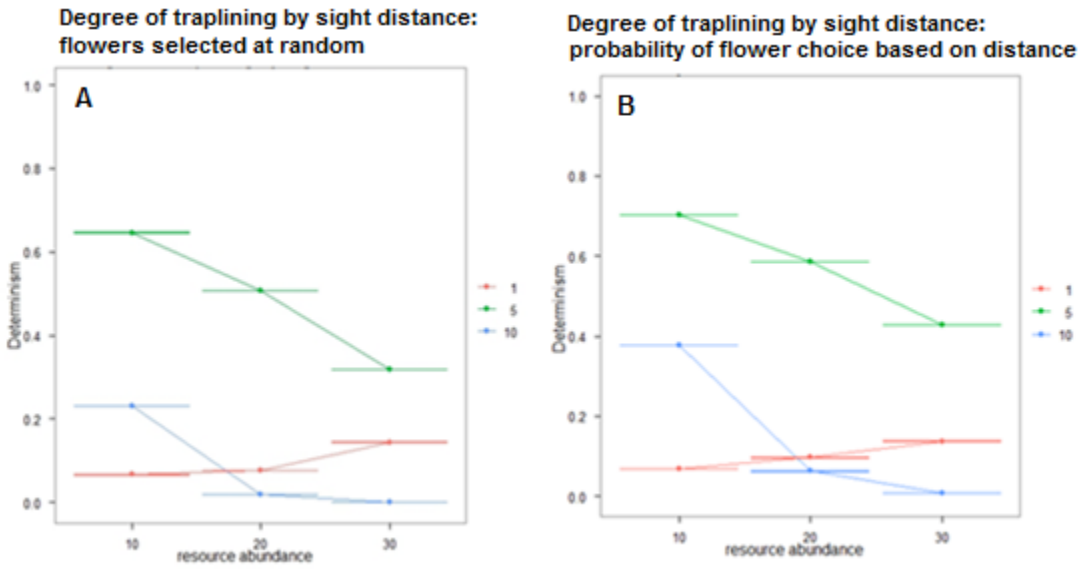

Figure 3. The level of sequence predictability when (A) foragers choose resources at random versus (B) when foragers choose resources according to a probability inversely related to resource distance. We analyzed the interaction of resource density with three sight distance levels: low (red lines), medium (green lines), and high (blue lines).