

The Wisdom of the Acorn: Social Foraging in *Temnothorax* Ants

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2014 by the
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May 2014

ABSTRACT

The coordination of group behavior in the social insects is representative of a broader phenomenon in nature, emergent biological complexity. In such systems, it is believed that large-scale patterns result from the interaction of relatively simple subunits. This dissertation involved the study of one such system: the social foraging of the ant *Temnothorax rugatulus*. Physically tiny with small population sizes, these cavity-dwelling ants provide a good model system to explore the mechanisms and ultimate origins of collective behavior in insect societies. My studies showed that colonies robustly exploit sugar water. Given a choice between feeders unequal in quality, colonies allocate more foragers to the better feeder. If the feeders change in quality, colonies are able to reallocate their foragers to the new location of the better feeder. These qualities of flexibility and allocation could be explained by the nature of positive feedback (tandem run recruitment) that these ants use. By observing foraging colonies with paint-marked ants, I was able to determine the 'rules' that individuals follow: foragers recruit more and give up less when they find a better food source. By altering the nutritional condition of colonies, I found that these rules are flexible – attuned to the colony state. In starved colonies, individual ants are more likely to explore and recruit to food sources than in well-fed colonies. Similar to honeybees, *Temnothorax* foragers appear to modulate their exploitation and recruitment behavior in response to environmental and social cues. Finally, I explored the influence of ecology (resource distribution) on the foraging success of colonies. Larger colonies showed increased consistency and a greater rate of harvest than smaller colonies, but this advantage was mediated by the distribution of resources. While patchy or rare food sources exaggerated the relative success of large colonies, regularly (or easily found) distributions leveled the playing field for smaller colonies. Social foraging in ant societies can best be understood when we view the colony as a single organism and the phenotype - group size, communication, and individual behavior - as integrated components of a homeostatic unit.

ACKNOWLEDGMENTS

I am very grateful to my wonderful committee members – Stephen Pratt, Marco Janssen, Jennifer Fewell, Juergen Liebig, and Bert Hölldobler for their teaching, advice, and helpful criticism throughout my graduate education. In particular, I am grateful to my advisor, Stephen Pratt, who has been my intellectual ‘spirit guide’. Whatever crackpot or half-baked idea I mention to Stephen he always makes me feel valued with his respectful and thoughtful responses, and if there is any actual value in my suggestion he helps to me to ‘mine it’ - to unearth the scientific legitimacy hidden therein. In order to adequately sing Stephen’s praises, I would need another chapter, so in the interest of brevity I will simply say that I am grateful and proud to claim him as my mentor.

I also thank my collaborators past, present, and future on projects that have been completed or are works in progress: Stephen Pratt, Takao Sasaki, Ted Pavlic, Aurelie Buffin, Jessie Ebie, Jennifer Fewell (co-advisor and teacher in my excursions into ant foundress research), Marco Janssen, Brian Haney, James Waters, Jon Harrison, CJ Klok, Andrew Quitmeyer, Tucker Balch, Gage Schaper, Clint Penick, Juergen Leibig, Rebecca Clark, Sara Dreyer, Yung Chang, Manfred Laubichler, Jacob Evans, Kevin Ngo, Tatsuya Kameda, Nick Poetsch, and any others I may have temporarily forgotten. Many other colleagues helped me tremendously along the way including: Adrian Smith, Dani Moore, Tate Holbrook, Josh Gibson, Kevin Haight, Bob Johnson, Adam Dolezal, Kelly Dolezal, Peter Marting, Yohan Cho, Nicola Plowes, Jon Jackson, Ioulia Bespalova, Kirsten Traynor, Athena Aktipis, Marty Anderies, Brigitte Hogan, Manuela Gonzalaz, Anna Dornhaus, Gro Amdam, Juergen Gadau, Rick Overson, Fernando Vonhoff, Arianne Cease, and John VandenBrooks. In particular, I want to thank Takao Sasaki – my friend and also teacher – who always makes me laugh.

I am grateful to the excellent administrative staff of the graduate program (both faculty, managers, and supporting staff) for all of their help and support during my education: Wendi Simonson, Yvonne Delgado, Juergen Gadau, and Kenro Kusumi (when he filled in for Juergen), Elaine Finke, Juergen Liebig, Rob Page and Brian Smith.

I thank my family and friends locally, especially the Parra family, Todd Miller and the Miller clan, and the Lindemberger/ Provost family. I am especially grateful to my parents, Pam and Warren, for their example as educated people and for their continued financial and emotional support. My parent's financial generosity extended not only to me but also to many friends and family in my orbit, and made it possible for me to focus on my studies completely. I appreciate my talented sister Leah (Shaffer model 2.0), who even though she is 8 years younger, I consider to be the wise older sibling. I thank my kids, Ella and Phoenix (both of them smart young people – who I nonetheless managed to often dragoon into the tedious job of feeding ants). Finally, I thank my wife, Jaxinta. Thank you for listening about my scientific successes and failures and in either regard taking a walk with me and later on baking chocolate chip cookies.

This research was supported by the National Science Foundation and ASU's School of Life Sciences University Special Fellowship and doctoral completion RA.

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CHAPTER 1

INTRODUCTION

What are the origins of biological complexity? In the last quarter century, a field has developed that aims to explain the complex phenomena of nature through a reductionist approach. The thesis of the complexity paradigm is that underneath many complex events, simple agents and simple rules are at work. The complex result we observe is an emergent outcome from the interaction of these simple agents. The study of emergence and self-organization is the center of a broad field of inquiry: systems as diverse as the immune system (Detrain & Deneubourg, 2008; Holland, 1992), the nervous system (Koch & Laurent, 1999), and economic markets (May, Levin, & Sugihara, 2008) are often described in such terms. One of the classic model systems in this field has been the collective behavior of social insects (Beckers, Deneubourg, Goss, & Pasteels, 1990; Detrain & Deneubourg, 2008). Sometimes described as a 'distributed intelligence', the social insects demonstrate several of the key features of complex systems: (i). The individual social insects are relatively simple agents with no central leader. Their actions are self-organizing. (ii). Positive and negative feedback influences group behavior in an often non-linear fashion. (iii). Global or group behavior emerges from the interactions of the many agents at lower levels. The leaf nests of *Oecophylla*, nest-site selection of honeybees or *Temnothorax* ants, the construction of vast termite mounds – all emergent feats of the social insects (Beckers et al., 1990; Bonabeau et al., 1997; Seeley, 1986). But collective foraging may have pride of place as the most studied of emergent collective behaviors.

The feats of foraging social insects are familiar to any person who has observed a trail of ants at work or honeybees gathering on flowers. What are the capabilities of these foraging groups? Social insect colonies are able to utilize recruitment to bring nest-mates to a food source (Frisch, 1967; Seeley, 1986; Wilson, 1962b) In this manner, colonies are able to focus their foraging resources on some distant (and perhaps hard to find) food source. Colonies can optimize and find the shortest path to that resource (Beckers et al., 1990; Detrain & Deneubourg, 2008; Reid et al., 2011). In addition, social insects are able to choose from a range of resources (of differing quality) and direct foragers to the most profitable food source (Beckers et al., 1990; Hölldobler &

Wilson, 2009; Seeley, 1986). Depending on the species, colonies are able to respond to changes in the environment (changes in relative food quality) and reallocate foragers flexibly (Seeley, 1986).

Given the high degree of social cohesion and the apparent 'choices' manifested by social insect colonies, it might be tempting to imagine that they are following some blueprint or central leader. Thus far, this has proved to not be the case. Instead, the mechanisms of collective behavior have been shown to involve a few key parameters of individual behavior. For example, individuals are more likely to recruit to better resources over poorer ones. Individual social insects cease exploitation of resources through attrition over time. These attrition rates can also be quality dependent with individuals abandoning poor sources at a higher rate than good sources. While recruitment provides positive feedback for foraging, queuing delays may provide the negative feedback that communicates the nutritional state of the colony. For example, when a honeybee forager returns from a nectar source laden with an abdomen full of liquid, she must seek out a nest-mate to whom she can deliver this food (before she can return to forage more). Longer delays in finding a receiver may increase the attrition rate of returning foragers or decrease the likelihood of that forager initiating recruitment. Queuing delays may be the manner in which a forager learns the colony is 'full'. Taken together, these mechanisms (differential recruitment, attrition, and queuing delays) have been shown to interact and control the social foraging of insect colonies (Detrain & Deneubourg, 2008; Seeley, 1995).

While the study of the proximate mechanisms by which colonies achieve social homeostasis is valuable, it is incomplete without an equal appreciation for the 'ultimate' origins of social behavior. Eusocial ant colonies are increasingly viewed as 'superorganisms' or simply 'organisms' (Hölldobler & Wilson, 2009; Queller & Strassmann, 2009; Wheeler, 1910). In these societies, individual ants gain fitness through the success of their colony as a whole, and it is the colony ultimately that is the 'unit of selection'. As such, the behavioral rules followed by individual social insects can be viewed as adaptations serving the group. Two such aspects of colony phenotype are group size and communication. The ecological constraints model has been useful in providing a context from which to understand why social groups form (Emlen, 1982), and a subject of

continuing research is the question of how this ecological context can influence the utility of group size and communication (Donaldson-Matasci et al., 2013; Dornhaus et al., 2012; Dornhaus & Chittka, 2004).

My thesis describes my investigation of a complex system, the collective foraging of the ant, *Temnothorax rugatulus*. From colony level behavior down to individual ant, I have attempted to describe and understand their social foraging: the proximate mechanisms by which collective behavior emerges and the ultimate context driving the evolution of communication and group size.

Temnothorax is a genus of myrmicine ant that has a worldwide distribution. Physically tiny, *Temnothorax* is one of the most-used model organisms in the study of collective decision-making in the social insects. It is popular in part for its durability and its small colony size: an entire insect society (usually less than 200 individuals) is housed in an acorn or small rock cavity. Highly eusocial, it also demonstrates other interesting behaviors (dominance hierarchies) and ecological relationships (it is parasitized by closely related inquilines). In terms of collective decision-making, the primary context of investigation has been house-hunting (Franks, Pratt, Mallon, Britton, & Sumpter, 2002; Mallon, Pratt, & Franks, 2001; Sasaki & Pratt, 2011). Little research has focused on the social foraging or food choices of these ants (Bengston & Dornhaus, 2012; Gottlieb, Phillips, Sendova-Franks, & Franks, 2013; Wheeler, 1910).

What could this ant add to the tradition of studying emergence in social insects? The study of social insect foraging has previously focused on two systems: mass-recruiting trail-laying ants and honeybees. These systems feature large colonies, but the much smaller size of a *Temnothorax* colony allows a detailed study of individual behavior that would be difficult to match in the larger insect societies. In addition, *Temnothorax* utilizes a form of recruitment, the tandem run, unexplored in the tradition of collective foraging. Where large insect societies have been studied in this context, the self-organization of small insect societies remains unexplored. Do small societies follow the same rules as large societies in their coordination of social foraging? Is there the same division of labor between foragers and receivers? Could 'informed' individuals play a larger role in shaping colony-level behavior? The small colony size and slow 'pace of life'

of *Temnothorax* ants also provides an opportunity to pursue questions of an ecological dimension in the laboratory. Colonies in the wild can have an effective foraging range of less than two meters (Bengston & Dornhaus, 2012), thus arena experiments can provide ecologically meaningful challenges in terms of resource discovery and exploitation. Experimental manipulations that would be very difficult to achieve with wide-ranging species such as honeybees become tractable with *Temnothorax* colonies. These and other questions make *Temnothorax* appealing as a study organism in social foraging.

My thesis addresses the following questions:

1. ***What can they do?*** What are the colony level patterns and dynamics in *Temnothorax* collective foraging?
2. ***How do they do it?*** How do individual behaviors lead to the group-level dynamics? Do informed individuals play a role in guiding the colony level response?
3. ***Can we model the process?*** Do our simulations and analytical models produce a similar result as that empirically documented?
4. ***What is the influence of nutritional state?*** How does nutritional state affect the behavior of *Temnothorax* colonies? What is the control mechanism exerting negative feedback on foraging and recruitment behavior?
5. ***How does resource distribution influence the utility of group size and communication?*** How do ecological circumstances shape the phenotype of groups? *Temnothorax* colonies provide a model to test the applicability of the ecological constraints hypothesis in dictating the utility of group size and communication in ant societies.

In this series of studies, I investigated proximate and ultimate questions surrounding the social foraging of *Temnothorax* ants. In order to test the ramifications of linear recruitment, I assessed colony-level behavior, investigated individual behavior, and validated an emergent model of collective choice (Appendix A). Then, I further characterized the behavioral repertoire of individual ants and assessed the potential role of direct comparisons (Chapter 2). Next, I investigated the role of nutritional state in social foraging (Chapter 3). Finally, I explored the role

of resource distribution in mediating the advantages of group size and communication in social foraging (Chapter 4).

CHAPTER 2

HOW ANTS ASSESS AND RESPOND TO FEEDER QUALITY

Abstract

Colonies of social insects, similar to individual animals, attempt to maximize the profitability of the resources they exploit. But how best to choose among multiple options? One potential strategy would be for individuals to sample several options and make a direct comparison. Previous research has shown that even while individual social insects may have the capacity to make such direct comparisons, the colony level choice results instead through an emergent process that relies on a kind of parallel processing. In societies such as honeybees and mass-recruiting ants, collective choice results from a competition between resources, where individuals seem to act based on local knowledge only. In earlier work, we showed that this kind of emergent process is sufficient to explain collective food source choice by colonies of the ant *Temnothorax rugatulus*. However, our model of decision-making neglected many details of foraging behavior, in particular the possibility that individual ants can directly compare options. In this study, I analyzed the behavior of individual ants to answer two questions: First, does direct comparison on the part of individuals influence colony foraging choice? Second, what is the full repertoire of effects of food quality on individual behavior? I found that while many ants (both foragers and receivers) had the opportunity to make direct comparisons, they did not act on this information to alter the colony foraging response. I furthermore found no influence of food quality on forager behavior, beyond the previously reported effects on recruitment and attrition. These results support the view that small societies of social insects rely on the same emergent framework for collective choice as large societies.

Introduction

One aim of the study of biological complexity is to understand the means by which collective animal behavior results from the interactions of individuals (Camazine, Deneubourg, Franks, Sneyd, & Bonabeau, 2003; Sumpter, 2010). In the eusocial insect societies, complex colony-level behavior is increasingly viewed as an emergent phenomenon (Sumpter, 2006). A central tenet of this growing consensus is that colony-level behavior is not reliant on any central authority or

globally-informed individual, but instead emerges from interactions among locally informed individuals (Detrain & Deneubourg, 2008; Mallon et al., 2001; Sasaki & Pratt, 2011; Seeley, 1995; 2010). For example, when a colony of the ant *Lasius niger* is presented with several food choices, each forager typically visits only one feeder, recruiting nest-mates to it with a strength that depends on the quality of the resource (Beckers et al., 1990). How do colonies compare resource options? It has been suggested that for collectives, the locus of comparison is shifted from the individual to the group with a competition occurring for the attention of available foragers. In this view, better resources 'win' out by being the subject of more vigorous positive feedback (such as recruitment) than poorer resources (Beckers et al., 1990; Beckers, Deneubourg, & Goss, 1992; Camazine, Visscher, Finley, & Vetter, 1999; Franks, Dornhaus, Fitzsimmons, & Stevens, 2003; Lihoreau, Deneubourg, & Rivault, 2010; Mallon et al., 2001; Marshall, Dornhaus, Franks, & Kovacs, 2006; Passino & Seeley, 2005; Seeley, 1986).

But how purely emergent is the collective behavior of insect societies? It has been shown that individual social insects have sophisticated cognitive capacities (Chittka, Dyer, Bock, & Dornhaus, 2003; Collett, 2009; Dukas & Real, 1993; Gould, 1990; Menzel & Giurfa, 2001). Given these abilities, it would be surprising if individual social insects couldn't directly compare resource quality. And if individual social insects can compare resources, what role might this capacity have for shaping the behavior of the group? This question has begun to be examined in the context of nest-choice experiments for ants in the genus *Temnothorax*. Detailed observations of individual ant decision-making demonstrated that recent experience influences the behavior of house-hunting scouts (Franks et al., 2007; Mallon et al., 2001; Robinson, Feinerman, & Franks, 2012). While colonies of *Temnothorax* ants are able to migrate to the best nest among several without individual comparisons, Mallon et al. (2001) found that the minority of ants that visited more than one candidate nest were more likely to choose the better nest than predicted if they were not making comparisons. Another study demonstrated that isolated scouts are influenced by recent experience when making nest choices (Sasaki & Pratt, 2011). Recently, some of the same authors have suggested that these results do not necessarily provide evidence for individual

direct comparison (Robinson et al., 2011; Robinson, Smith, Sullivan, & Franks, 2009), but the degree to which individuals are truly able to make direct comparisons and the possible influence of this potential for colony-level choice remains an open question. It is the purpose of this paper to address this question in the context of social foraging in *Temnothorax* ants.

Earlier work described the abilities of *Temnothorax rugatulus* colonies to distinguish between feeders that differ in sucrose concentration (Shaffer, Sasaki, & Pratt, 2013). Colonies were able to distribute their foragers according to feeder strength, and to flexibly re-allocate foragers to track changes in strength. Individual foragers were found to adjust their behavior according to the strength of the feeder they are exploiting, with higher concentration making them more likely to recruit nestmates, and less likely to abandon the feeder. These ants recruit via tandem runs, in which a successful forager leads a single recruit all the way to the feeder. The effectiveness of tandem running is linear in recruitment effort, meaning that the number of ants recruited is directly proportional to the number of tandem runs led. An agent-based model showed that this linearity, combined with quality-dependent recruitment and attrition, can predict the observed colony-level ability to flexibly allocate labor according to feeder quality.

This model is consistent with a purely emergent mechanism of collective decision-making, in which each forager has direct knowledge of only one feeder, which she assesses by comparison to an internal standard (Figure 2.1c). This assessment then determines her probabilities of recruiting nestmates or abandoning the feeder. However, it is also possible that feeder assessment is influenced by better-informed ants with knowledge of multiple feeders. For example, a forager could visit multiple resources and make direct comparisons among them (Figure 2.1a). Alternatively, comparisons could instead be made within the nest by the receiver ants that offload food brought back by successful foragers. Food exchange, or trophallaxis, distributes nutrition through the colony, but can also spread information (such as the scent of a food source) (Farina, 1996). By accepting sugar water from multiple returning foragers, receivers could be in a position to compare the quality of different feeders. Such 'globally informed'

receivers could influence the collective foraging process in a number of ways. If the receiver spurned offerings from a forager bearing relatively dilute nectar, this might make the forager less likely to recruit to the food source in question or more likely to abandon it (Figure 2.1b). Another possibility is that a globally informed receiver could refuse sugar water from poorer sources, forcing rejected foragers to spend more time searching for a recipient. Even without an alteration in the forager's own feeder assessment, this increased transaction time would shift the colony-level exploitation towards the food sources favored by informed receivers.

Beyond the question of the information a forager uses in assessing a feeder, how does this assessment influence her subsequent behavior? The colony-level choice can be visualized as a rate of flow of food being directed back to the nest, with the rates determined by the behavior of individual ants in response to food source quality (Figure 2.2). We have already documented quality-based differences in recruitment and attrition, but we have not examined the question of transaction and transition times individual ants display towards feeders unequal in quality. We define transaction times as the time of interaction with nest-mates and transition times as travel times between nest and feeder. For example, it has been shown in honeybees that workers have different transaction times based upon feeder quality, spending less time in the nest and offloading nectar more quickly when the nectar is more concentrated.

This paper is intended to answer two questions: a) Do individual foragers or receivers use direct comparisons to influence the colony-level foraging effort? b) What is the full repertoire of behavior that ants display toward feeders unequal in quality, and how might these differences contribute to the emergence of a collective response? To answer these questions, we video-recorded paint-marked colonies engaged in foraging, and made detailed behavioral descriptions (ethograms) of the tasks that foragers were engaged in, as well as describing the overall patterns of foraging and interaction.

Materials and Methods

Experimental subjects

The subjects were 5 colonies of individually paint-marked ants. Colonies of *T. rugatulus* were collected from rock crevice nests in the Pinal mountains near Globe, Arizona, (N 33° 19.000' W 110° 52.561) between April and June 2010. In the laboratory each colony was housed in an artificial nest made from a 2.4 mm thick balsa wood slat with a rectangular central cavity measuring 3.8 x 6.4 cm. The slat was sandwiched between 50 x 75 mm glass microscope slides that served as floor and ceiling. A 3 mm wide entrance was cut through the side of the slat. Each nest was placed in an 11 x 11 cm plastic box and kept in a Sheldon diurnal incubator at a 16:8 day:night schedule, with a daytime temperature of 23°C and a night-time temperature of 15°C. Colonies were provided weekly with water and an agar-based diet (Bhatkar & Whitcomb, 1970). Colonies had queens, ample brood, and an average worker population of 73 ± 7 (SE). In each colony, every worker received a unique combination of paint dots on the head, thorax, and abdomen, using Pactra R/C Car Lacquer paint. To insure an adequate foraging response, colonies were deprived of food for 14 days prior to the foraging experiments. Colonies were placed into the foraging arena 24 hours in advance of the experiments to allow for orientation.

Experimental design

Ant colonies were presented with a choice between two sucrose solution feeders of unequal quality (0.1 M versus 0.8 M), located 30 cm from the nest and 40 cm from one another. Feeders consisted of depression well slides with an initial 0.1 ml droplet of sugar water (replenished at 30-minute intervals throughout the experiment). The arena measured 75 x 60 cm, with 7 cm high walls that were coated with Fluon to prevent ants from escaping. Colonies were allowed to forage for 4 hours while their behavior was recorded with four video cameras (two Panasonic HDC-SD60P/PC and two Canon Vixia HG20) trained on the nest, each feeder, and the entire arena.

Analysis of individual behavior in relation to feeder quality

In order to examine the proximate mechanisms behind the individual forager's assessment of feeder profitability, we reviewed the video records and created ethograms based on the following events: time of food discovery, initiation of feeding, termination of feeding, antennation of food without feeding, entry and exit times at the nest, the time of each trophallaxis bout initiation and termination in the nest, and the maximum number of concurrent trophallaxis partners. In addition, we recorded the time of initiation of each recruitment event (tandem run) and the identity of followers, the start and stop time of each tandem run (even ephemeral tandem runs), and the success or failure of the tandem run. We defined a successful recruitment as one in which the follower exploited the feeder within 5 minutes after the termination of the tandem run. These behaviors were recorded for each ant that exploited either feeder during the initial 60-minute period after food discovery. Unless otherwise noted, we restricted analysis to this period because 60 minutes is long enough for the choice of colonies to become apparent, but not so long that colonies grow satiated.

To detect effects of feeder quality on behavior, we assigned ants to either 'good' or 'poor' feeders based upon the most recent feeder visited. We then compared the two groups of ants for several behavioral measurements derived from the individual ethograms. The 'time at feeder' was defined as the total time between the initiation of drinking (after leaving the nest) and the final termination of drinking before returning to the nest (ant feeder exploitation is often discontinuous). 'Total round trip' duration was defined as the time it took for an ant to travel from the feeder to the nest and back to the feeder. 'Nest to food' time was measured as the time for an ant (that had already begun exploiting a feeder) to travel from the nest back to the food. 'Food to nest' was the opposite. In the nest, recruitment behavior was quantified by observing returning scouts that initiated tandem runs. The identities of all followers and total duration for each tandem run was determined (even short-lived recruitments). 'Search time for 1st receiver' was the duration from the entry of an engorged forager into the nest to the first successful initiation of trophallaxis. 'Maximum trophallaxis receivers' was determined by observing the peak number of receivers that

a returning forager off-loaded to during the course of a continuous trophallaxis bout. 'wait time (after last trophallaxis)' was the period of time between the conclusion of the last trophallaxis bout and the forager leaving the nest to return to the feeder.

Results

Colony-level behavior

At the colony level, our study showed greater exploitation and recruitment to the better feeder, and this translated into more recruitments/ minute to the good feeder (Table 2.1). In earlier work we have shown that colonies are able to collectively choose between 0.1 M and 0.8 M sucrose feeders. However, this earlier work utilized an exploitation index (sequential periodic sampling) to assess exploitation and recruitment, whereas in this study we captured each event precisely: the total number of foragers, exploitation events, and recruitments. Within a single foraging trial, there was a small sub-set of active foragers with an average of 13 ± 3.6 unique ants (an average of 18% of the colony population) engaged in exploitation of the feeders in the first 60 minutes after discovery. On average, 5.4 ± 2.6 individuals (7% of colony population) exploited the poor feeder and 9.8 ± 2.5 individuals (13% of the population) exploited the good feeder.

A detailed description of individual ant behavior

We used our video footage and detailed behavior logs to assemble individual-level summaries of ant activity over time (Figure 2.3). Here we describe the behavior of a typical forager. Upon discovering one of the feeders, foragers would begin a cyclical pattern of exploitation that began with simply drinking the sugar water. Drinking at the feeders tended to be discontinuous, with successful discoverers drinking then pausing, roaming off, and perhaps returning. Video of feeding ants reveals that their abdomens swell noticeably, becoming translucent with liquid once engorged. Successful foragers invariably return to the nest where they advertised their success with motor displays, offerings of sugar water to nest-mates, and sometimes tandem recruitment. Food was offered in a stereotypical display in which the successful forager stands still in front of a nestmate, opens her mandibles, and extrudes a small bubble of liquid on her glossa. This offering

may or may not be accepted by the would-be receiver. After a period of time in the nest, the foragers would return to the feeder (sometimes leading a tandem run), and the pattern would repeat itself. On average, foragers made 2.60 ± 0.18 visits to feeders over the course of the first hour of foraging. Some ants visited both feeders while most visited only one (Figure 2.4).

The potential role of direct comparisons by scouts

We found (in all but one trial) that a subset of ants visited both feeders, leaving open the possibility that direct comparisons on the part of individuals could play a role in the colony decision. In addition, a relatively small number of ants seem to mediate the foraging process by acting as recruiters. On average, there were 3.4 ± 0.4 recruiters per colony (an average of 5% of the colony population). The small number of foragers (along with the relative importance of a few recruiters) leaves open the possibility that a minority of well-informed ants could direct the colony foraging effort. In the first hour after discovery an average of 2.75 ± 1.18 ants visited both feeders, representing an average of 17% of the total first hour forager population (4% of the total population). However, in only one instance did an informed forager recruit during the first hour. This individual first visited the poor feeder and then made 4 subsequent trips to the good feeder, recruiting to the good feeder on her third trip to that source. All other recruitment in the first hour after discovery was initiated by scouts that were exploiting a single feeder (Figure 2.4). Thus, even while a minority of foragers could potentially gain broad knowledge of resources outside of the nest, almost none acted upon this potential knowledge to direct colony-level foraging.

The potential role of direct comparisons by receivers

Receiver ants in the nest have the opportunity to compare feeders indirectly through their interactions with returning foragers. If so, we predicted that foragers returning from poor feeders would experience weaker responses from receiver ants, measured as the time needed to find their first receiver, the maximum number of concurrent receivers during a visit, and the total time in the nest for each foraging trip. We found no significant difference between any of these metrics for foragers bearing good or poor sugar water (Table 2.2). Thus, while receiver ants may have

access to broad information about feeder quality they do not act upon it in their interactions with foragers.

Individual behaviors in response to feeder quality

Most metrics of forager behavior showed no difference between foragers to good and poor feeders (Table 2.3). In particular, there was no significant difference for transition and transaction times, including drinking time, round trip travel time, nest to food travel time, and food to nest travel time. There was, as reported earlier, a strong effect of feeder quality on recruitment and attrition behavior, with ants at the better feeder showing a higher probability to lead tandem runs and a lower probability of ceasing exploitation. Related to the difference in attrition, ants showed differences in feeder switching behavior: after a first visit to a poor feeder a significantly greater number of ants switched to the good feeder on the subsequent trip than the opposite (Figure 2.5). In a given trip home, ants bearing the poorer quality nectar showed a non-significant trend towards greater mean wait time before returning to the feeder than ants exploiting the good feeder (the time from the end of their final trophallaxis to their leaving the nest to return to the feeder).

Discussion

Our study illuminates a number of important features in the emergence of social foraging in *Temnothorax rugatulus*. First of all, our more detailed analysis confirms and expands upon our previous report of the ability of colonies to make collective food choices. Secondly, we were able to examine the possible role of direct comparisons on the part of individual ants in shaping the colony foraging effort. We found that many foragers and receivers had the opportunity to make such comparisons, but there was no evidence that they used this potential information to alter the colony's foraging response. Finally, we were able to make a fuller accounting of potential differences in the behavior individual ants directed toward feeders of unequal quality (particularly in regard to transition and transaction times).

While individual ants may have the capability to compare nectar quality (either directly at the source or upon reception in the nest), it seems that colony-level choice is independent of this potential individual comparison. Tracking the resources available in a colony's environment is a challenging task, as even a currently exploited source could become depleted or change in quality over time (Donaldson-Matasci et al., 2013; Latty & Beekman, 2013). It would place a large burden on individual social insect foragers to constantly maintain 'complete' knowledge of their environs. Just as in the colonies of honeybees and mass-recruiting ants, small societies must make decisions and harvest resources from the environment. The competing communication of rival foragers provides a democratic forum where all foragers are able to advertise the resource they are exploiting (Seeley, 2010). The assessments of individuals become important in this context – as the foragers choose whether to return to the feeder, recruit, or abandon the food source – but it may not be necessary (or even optimal) for individuals to make direct comparisons. Small societies, such as those of *Temnothorax* ants, may benefit from a decision algorithm that defers to the 'wisdom of crowds' over the unlikely fortunes of 'omniscient individuals'.

Along with supporting the emergent framework described in our model, we found that differences in quality-based recruitment and attrition are enough to explain the collective behavior of colonies: we did not find other differences in individual behavior toward feeders of unequal quality.

However, this study highlighted one specific consequence of foraging attrition: switching between feeders. Attrition in general could be either the result of an ant's own internal calculations of feeder profitability or it could result from interaction with nest-mates (including recruitment) or some combination of both. Examining all of the cases of feeder switching (after a first trip), we found not one instance of this switching being due to recruitment. How then to explain the one-way directionality of switching? One possibility is that ants exploiting poor quality feeders are less internally dedicated to that feeder – and more likely to explore (and thus potentially finding richer food sources) or delay return. In our study we found a trend toward greater wait times in the nest (after final trophallactic exchange) that might support this line of thinking. Other authors have emphasized the potential importance of such quality-based wait times for collective choice in ant societies (Robinson et al., 2009; 2011). But there is again the possibility of another kind of direct comparison. As returning foragers bring nutriment home to the colony, trophallactic exchanges are set in motion between all colony members. Thus, a nest worker can act as a donor, even to a returning forager. Such a returning forager bearing dilute nectar might be exposed to more concentrated nectar offered by a nest worker, and this information might induce the forager to search for the other food source. In either scenario, attrition becomes an emergent property (but in one case it is an emergent property coupled with direct comparison on the part of the individual). To test this hypothesis, it would first be necessary to compare attrition in the presence of two feeders (our current study) to attrition where only a single feeder is present.

While *Temnothorax* colonies can make a collective decision based upon emergent decision rules (and not relying on individual comparison), it could be that the sophistication of individuals has other long-term advantages for the colony. For example, the knowledge of multiple food sources (or loyalty to a single poor feeder) could become important if resources change in quality later. Individual ant memory may be an important part of long-term optimization of colony feeding. Honeybee scouts act as monitors of poor quality feeders and it is believed this facilitates the reallocation of foragers during quality switching experiments (Granovskiy, Latty, Duncan, & Sumpter, 2012). While initial colony choice (regardless of the scale of the society) may be largely independent of individual comparisons, we should not discount the role that memory,

heterogeneity, and independence of individual social insect foragers could play in optimizing the long-term collective foraging of societies.

Table 2.1. Metrics of colony-level behavior for colonies presented with two feeders unequal in quality. A strong (but non-significant) trend is shown that colonies allocate more foragers and recruitment toward the better feeder (0.8M sucrose solution) in preference to the worse feeder (0.1 M sucrose solution). P-values indicate the outcome of a 2-tailed paired t-test. For all measures, n = 5 (the number of colonies observed in this study)

Colony-level behavior	<u>0.1 M Sucrose solution</u>	<u>0.8 M Sucrose solution</u>	Significance
Visits to feeder	12.2 ± 6.35	24.80 ± 6.48	= 0.137
Attempted recruitments	1.2 ± 0.8	6.0 ± 1.76	= 0.116
Successful recruitments	0.8 ± 0.8	4.6 ± 1.77	= 0.159
Attempted recruitments/minute	0.02	0.17	
Successful recruitments/minute	0.007	0.08	

Table 2.2. Metrics of individual behavior in relation to the reception of foragers by receiver ants. Upon returning to the nest, foragers bearing high or low quality sugar water demonstrated similar search times for the first receiver, they spent a similar amount of time in the nest, and they attracted a similar number of maximum concurrent receivers. P-values indicate the outcome of Mann-Whitney U tests with replicate numbers (individual ant trips) indicated in the table beside each measurement.

Individual-level behavior	0.1 M Sucrose solution		0.8 M Sucrose solution		Significance
	Measure	Sample size (ant trips)	Measure	Sample size (ant trips)	
Search time for 1st receiver (minutes)	1.41 ± 0.22	59	1.36 ± 0.28	102	= 0.752
Total time in nest (minutes)	8.87 ± 1.04	57	7.52 ± 0.48	108	= 0.678
Maximum trophallaxis receivers	2.15 ± 0.16	59	2.35 ± 0.14	108	= 0.497

Table 2.3. Metrics of individual behavior in relation to the quality of the feeder being exploited.

Times given are minutes (for the top five rows). P-values are for Mann-Whitney U test. Replicate numbers (individual ant trips) are given next to each measurement.

Individual-level behavior	0.1 M Sucrose solution		0.8 M Sucrose solution		Significance
	Measure	Sample size (ant trips)	Measure	Sample size (ant trips)	
Time at feeder/ trip	1.98 ± 0.44	61	1.78 ± 0.09	124	= 0.224
Round trip time (food to nest to food)	16.32 ± 1.95	37	14.82 ± 0.79	97	= 0.964
Nest to food time	3.08 ± 0.39	37	2.92 ± 0.22	95	= 0.933
Food to nest time	2.97 ± 0.27	61	3.32 ± 0.36	124	= 0.130
Wait time (after last trophallaxis)	3.56 ± 1.07	57	1.42 ± 0.28	97	= 0.967

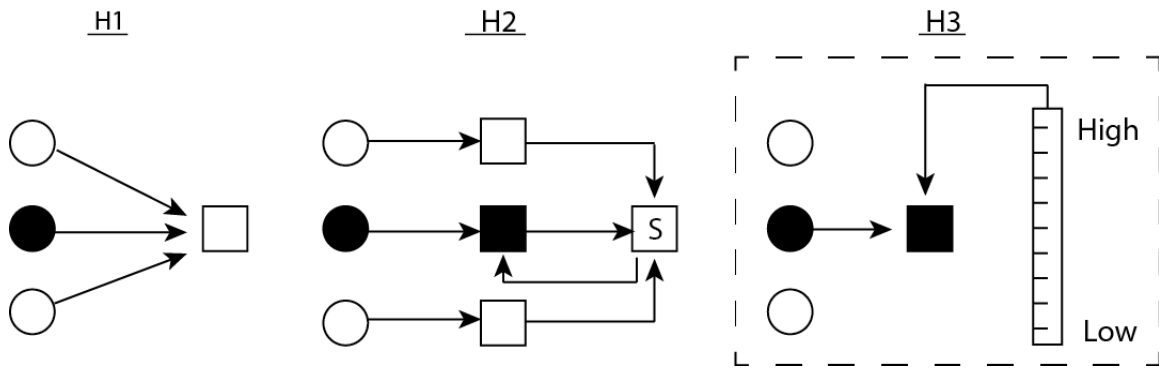


Figure 2.1. Three hypotheses for how a forager can assess the value of her currently exploited food resource. In the schematic, a square represents an ant forager and a circle denotes a food resource (sugar water). Arrows show the flow of information. The shaded square represents the focal individual, and the shaded circle the focal food source. The three hypotheses are distinguished by locus of assessment (forager or receiver ant) and by the amount of information possessed by the focal forager (global or local knowledge). In H1, the forager possesses global knowledge based upon visits to multiple food sources and can assess the most profitable food source through a direct comparison. In H2, a receiver ant, S (for supervisor), acquires broad knowledge about patch quality from her experiences with multiple foragers, makes comparisons, and is able to impart this knowledge to the focal forager. In H3, the forager uses only knowledge about her currently exploited resource, and is able to assess its quality based upon an internal scale imposed by her nervous system. Adapted from Seeley (Seeley, 1995).

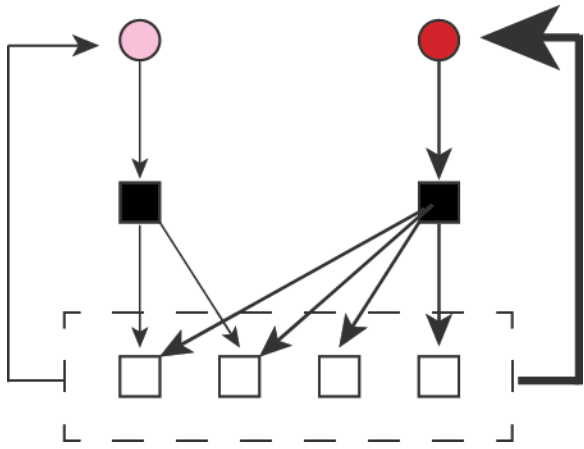


Figure 2.2. The schematic depicts the colony level choice of one food source over another – which results from differential ‘flow’ of exploitation toward the more profitable food source. One goal of this study was to assess how individual ants behave in relation to feeders of unequal quality – and determine how these behaviors can lead to differences in colony-level exploitation.

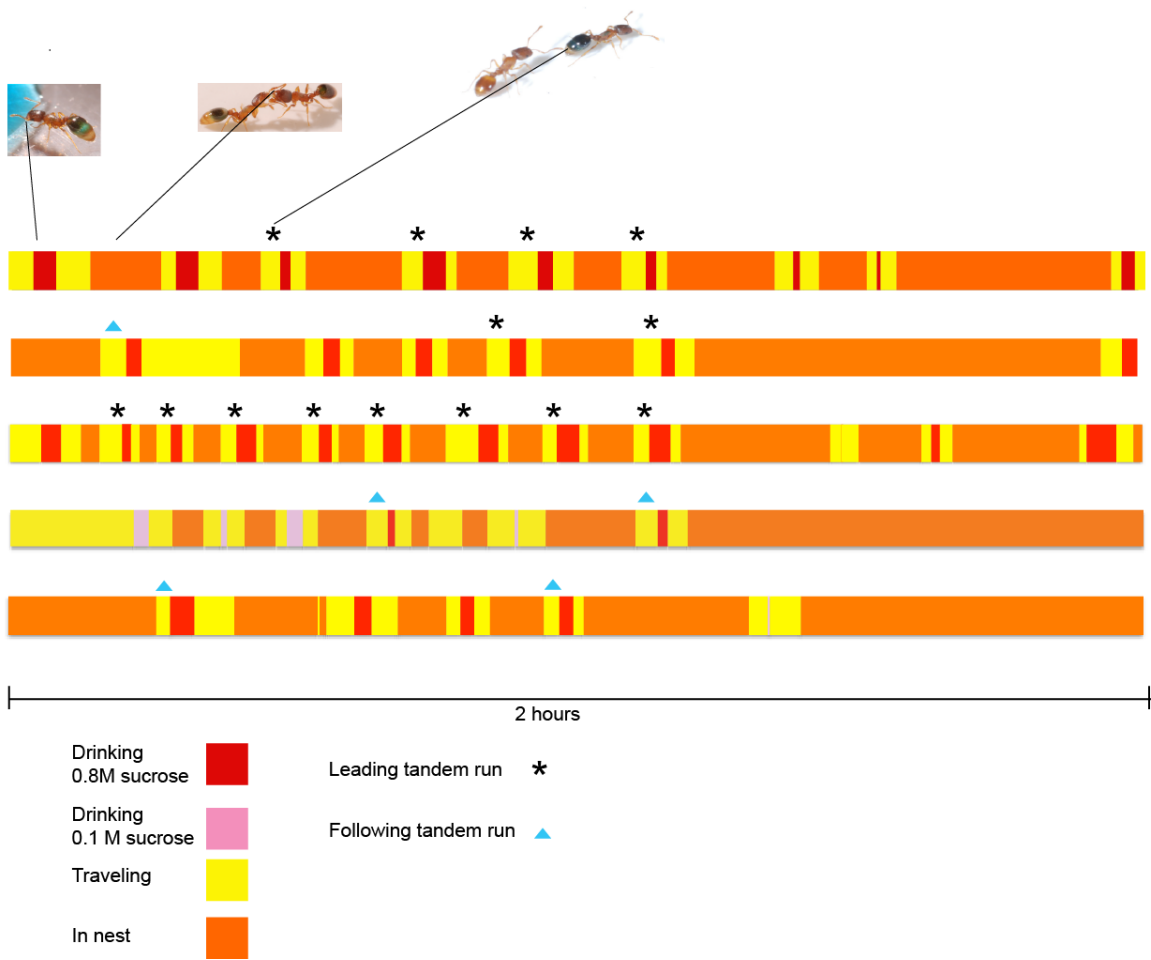


Figure 2.3. Ethograms of ant behavior for a focal ant colony. Each bar represents the behavior of a foraging ant over the course of 2 hours of colony foraging time. Asterisks and triangles indicate that the ant either led or followed a tandem run, respectively. Colors indicate the activities the ants were engaged in over time.

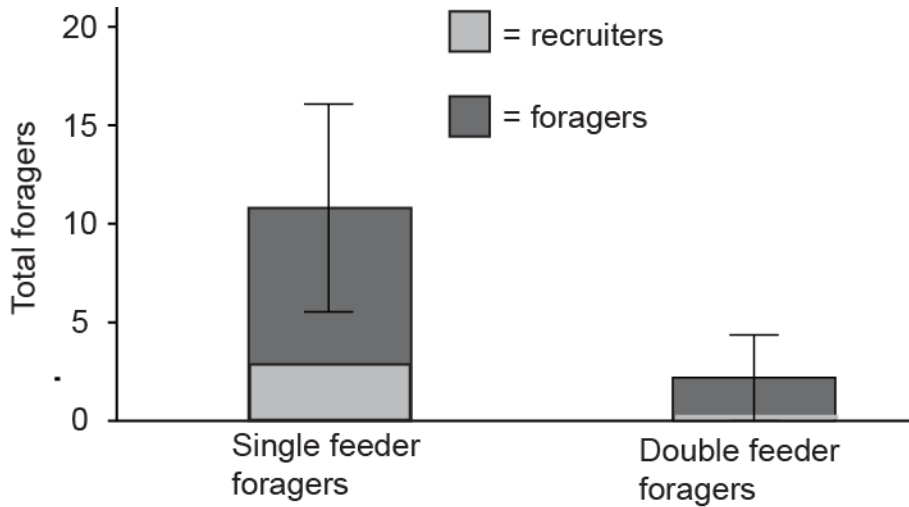


Figure 2.4. Bars show the average number of foraging ants that over the course of the first hour of exploitation visited a single feeder or both feeders (with standard error bars). The lightened portion in the lower part of each bar shows the average number of ants that recruited (recruiter error bars left out for visual clarity). Though tiny, a lightened band is present in the lower portion of the double forager bar: for foragers that visited both feeders 0.2 ± 0.45 individuals became recruiters (a single ant in all five trials).

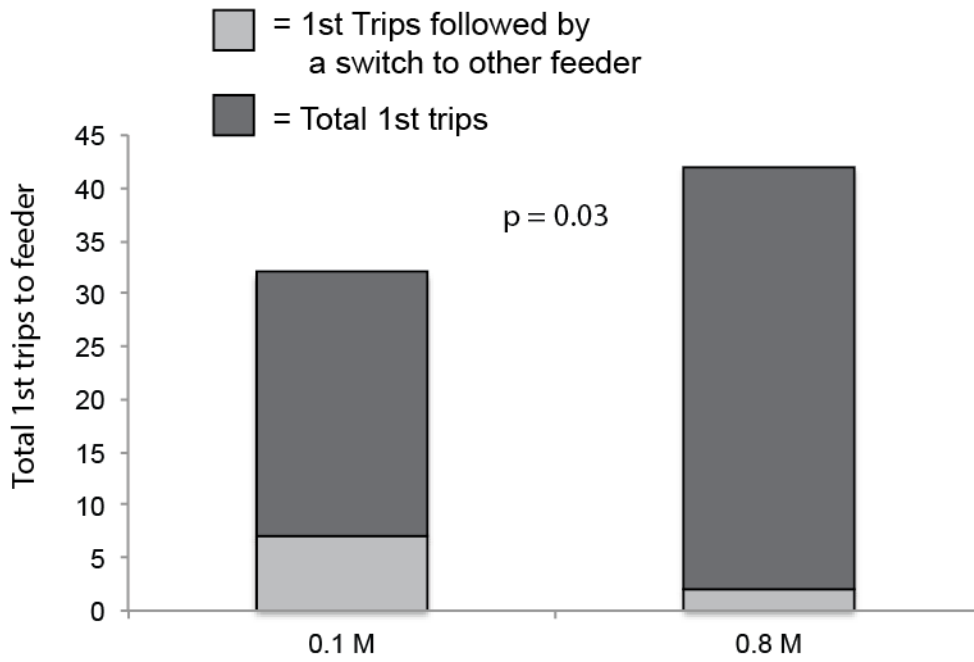


Figure 2.5. After a first visit to a feeder, a greater proportion of ants switched from exploiting the poor feeder to exploiting the good feeder than the reverse. Statistical significance was determined with Fisher's exact test for count data.

CHAPTER 3

THE INFLUENCE OF NUTRITIONAL STATE ON SOCIAL FORAGING

Abstract

Social insect colonies regulate foraging to match the needs of the group. Ants in the genus *Temnothorax* provide a tractable model organism for exploring how this regulation emerges from decentralized interactions among workers with limited information. Here, we report on the effect of colony-level nutritional state on individual exploration, exploitation, and recruitment. We performed laboratory foraging experiments in which colonies were either starved or fed ad libitum for 2 weeks prior to the introduction of sucrose feeders. Colonies were presented with two feeders of unequal quality (0.1 M and 1.0 M) and observed during two hours of foraging. Starved ant colonies allocated more foragers for overall exploration, exploitation, and recruitment, compared to well-fed colonies. However, colonies allocated a consistent and greater proportion of foragers to the better feeder, regardless of starvation. In addition, by filming colonies with marked individuals, we were able to observe how forager behavior changed as colony satiation increased. Starved colonies initially showed robust recruitment and exploitation, but over time this leveled off and then fell. We found that the cessation of recruitment by foragers correlated with longer search times and reduced numbers of trophallaxis receivers, likely cues of colony satiation. Similar to honeybees, *Temnothorax* foragers seem to rely on local behavioral cues to adaptively modulate their foraging behavior.

Introduction

Eusocial insect societies achieve a level of coordination more typical of unitary organisms (Hölldobler & Wilson, 2009) - with individual behavior being tuned to the colony's homeostatic needs and the 'state' of the group. This decentralized and yet coordinated behavior has historically been one of the central mysteries of the insect societies and continues to be the subject of extensive scientific inquiry. One example of this coordinated behavior is the ability of colonies to adaptively respond to nutritional demands. Many studies have demonstrated the ability of social insect colonies to choose efficient foraging strategies in accordance with

competition, food quality, food quantity, distance to food source, or predation. Of all potential variables, starvation has been shown to be one of the most important influences on the collective foraging of social insects. For example, it has been shown that the starvation level of colonies influences the exploration behavior of ants (Gottlieb et al., 2013; Mailleux, Devigne, Deneubourg, & Detrain, 2010b) and bumblebees (Cartar & Dill, 1990). Prior nutritional state also dramatically influences the total exploitation of resources in ants and honeybees (Fewell & Winston, 1992; Seeley, 1995) as well as both the initiation and response to recruitment signals (Hölldobler, 1971; Mailleux, Buffin, Detrain, & Deneubourg, 2010a; Mailleux, Detrain, & Deneubourg, 2006; Roces & Hölldobler, 1996). Nutritional regulation in the eusocial insect societies has been shown to be robust to conditions such as colony size or specific metabolic demands (Cassill & Tschinkel, 1999; Cassill, Stuy, & Buck, 1998; Dussutour & Simpson, 2009; Tschinkel, 2006). While the flexibility and effectiveness of eusocial insect nutritional regulation has been demonstrated in these many systems, questions remain concerning the mechanisms of behavior and interaction that guide individual social insect response.

By manipulating the nutritional state of colonies between extremes (replete or starved) pronounced differences can be shown in the aforementioned individual behaviors (exploration, exploitation, and recruitment). However, foraging and nutrient exchange is a dynamic process, and colonies need to be able to respond to changes in nutritional state in a time-scale varying from minutes to months. For example, honeybees were shown to adjust to deficiencies in pollen in a time-scale of several weeks (Fewell & Winston, 1992). In contrast, outward bound harvester ant foragers respond to interactions with returning successful foragers in a time-scale that varies from seconds to minutes (Pinter-Wollman, Bala, Merrell, & Queirolo, 2013). Individual social insects need to be able to respond flexibly to the 'moving target' that is the colony nutritional state. Even as food is arriving and recruitment is initiated, trophallactic exchanges are occurring, and 'state' of the colony is shifting (Brian & Abbott, 1977; Buffin, Goldman, & Deneubourg, 2012; Sendova-Franks et al., 2010). Aside from the obvious physical benefits of spreading nutriment

through the society, trophallaxis is also a potential avenue for information transfer (Farina, 1996; Hart & Ratnieks, 2001).

Martin Lindauer first proposed that social insect foragers (in honeybee colonies) might be informed of the nutritional status of their colony through their ease of offloading to nectar receivers in the nest (Lindauer, 1949). This was experimentally confirmed by Seeley (Seeley, 1989) who showed that foragers modify the intensity of dance recruitment in response to this information. Periods of high nectar flow result in many occupied receivers (themselves attempting to offload and process nectar), and thus a simple cue (wait time) guides the decision rule of honeybee foragers. While the role of queuing delays has received some attention as one mechanism by which colonies partition work (Anderson & Ratnieks, 1999), other than in honeybees there has been little investigation of the signals or cues that inform social insect foragers of the nutritional status of the colony.

We investigated the role that nutritional state plays in modulating the colony and individual-level response of ants in the context of choice experiments using the species *Temnothorax rugatulus*. *Temnothorax* ants are good biological models to study the individual behavioral strategies and colony-level regulation of social foraging in social insects. Colonies are small in size and experimentally tractable. While the collective behavior of *Temnothorax* ants has been well-studied in the related context of house-hunting only recently has an effort begun to understand the coordination of social foraging including such questions as the emergence of collective choice and trophallactic exchange networks during starvation (Sendova-Franks et al., 2010; Shaffer et al., 2013). Gottlieb et al. (2013) found that the nutritional state of colonies influenced both individual-initiated and socially-mediated information gathering of *Temnothorax* colonies. Similar to that study, our experiments aimed to address the question of how colony nutritional state influences the exploration, exploitation, and recruitment behavior of colonies of *Temnothorax* ants. In addition, by providing nutritionally managed colonies with a choice of feeder (one, a concentrated sucrose solution, and the other dilute), we could test whether colony state

influences the proportional allocation (choosiness) of colonies in terms of exploitation or recruitment. We predicted that starved colonies might be either more or less choosy in selecting a food source than well-fed colonies. For example, starved colonies might recruit indiscriminately to feeders of any strength and thus allocate a higher proportion of foragers to the worse feeder than when the same colonies were well fed. Or the reverse might be true. Starved colonies, precisely because of their deprived state, might allocate even more strongly towards the better feeder than when they were well fed. Honeybees have been shown to adjust their selectivity towards feeders in relation to the availability of natural forage (and the nutritional status of the hive) (Seeley, 1995) and so we might expect a similar response from ants.

The second goal of our study was to observe the behaviors and interactions of returning foragers in the nest (in individually paint-marked colonies) with the goal of elucidating the cues that guide the recruitment and exploitation decisions of foragers as the nutritional state of the colony fluctuates. Ultimately, foragers must balance their assessment of the intrinsic quality of resources with socially acquired information of the state of the colony (Figure 3.1), and we predicted that we could observe this process occurring in real time as colonies foraged to satiation.

Materials and Methods

Our experiments had two components: In the first, we observed multiple nutritionally controlled colonies for several hours in foraging choice experiments. In the second we documented the interactions of individually paint-marked foragers returning to the nest over a period of two hours as the colonies were engaged in social foraging. The first component aimed to test for gross, colony-level effects of nutritional state. The second component aimed to explore the changes in individual behavior that occur in real-time as colonies grow satiated.

Experimental subjects

The subjects were 21 colonies of *T. rugatulus*, which were collected from rock crevice nests in the Pinal mountains near Globe, Arizona, (N 33° 19.000' W 110° 52.561) between April and June

2010. In the laboratory each colony was housed in an artificial nest made from a 2.4 mm thick balsa wood slat with a rectangular central cavity measuring 3.8 x 6.4 cm. The slat was sandwiched between 50 x 75 mm glass microscope slides that served as floor and ceiling. A 3 mm wide entrance was cut through the side of the slat. Each nest was placed in an 11 x 11 cm plastic box and kept in a Sheldon diurnal incubator at a 16:8 day:night schedule, with a daytime temperature of 23°C and a night-time temperature of 15°C. Colonies were provided weekly with water and an agar-based diet (Bhatkar & Whitcomb, 1970). Sixteen colonies were used for arena-based foraging experiments. These colonies were large in size, averaging 300 ± 27 (SE) ants. For individual paint-marking, five queen-right colonies with brood were chosen with an average population size of 73 ± 7 (SE) ants. In each of these colonies, every worker received a unique combination of paint dots on the head, thorax, and abdomen, using Pactra R/C Car Lacquer paint. The large un-marked colonies were either given ad libitum Bhatkar diet (the fed treatment) or deprived of food (the starved treatment) for 14 days prior the arena foraging experiments. Marked colonies were deprived of food for 14 days prior to the foraging experiments. Colonies were placed into the foraging arena 24 hours in advance of the experiments to allow for orientation.

Effect of nutritional state on colony decision-making

In the first component of the project, ant colonies that had been starved or fed ad libitum were presented with a choice between two sucrose solution feeders of unequal quality (0.1 M versus 1.0 M). These experiments took place in 16 (75 x 60 cm) arenas located on four banks of shelves. The feeders were located 30 cm from the nest and 40 cm from one another. The feeders were depression well slides with an initial 0.1 ml droplet of sugar water (replenished at 30-minute intervals). Colonies were observed for two hours after the introduction of the feeders. Data was recorded as an observer rotated between arenas (at approximate 7 minute intervals). The following details were recorded: the number of ants in the arena prior to the introduction of feeders, the number of ants drinking at each feeder per observation (exploitation), and recruitment (tandem runs). The experiment took place on two days: April 27, 2011 and May 20,

2011. Half of the colonies received the fed treatment and the other half the starved treatment on each of the two days with all colonies receiving both treatments.

In the second component of the experiment, paint-marked ant colonies were presented with a choice between two sucrose solution feeders of unequal quality (0.1 M versus 0.8 M). With separate cameras trained on the nest, each feeder, and the entire arena, the colonies were recorded for 4 hours as they foraged. The feeders were located 30 cm from the nest and 40 cm from one another. The feeders were depression well slides with an initial 0.1 ml droplet of sugar water (replenished at 30-minute intervals). The arena measured 75 x 60 cm with fluon treated walls. Cameras were two Panasonic high definition video cameras (model HDC-SD60P/PC) and two Canon video cameras (Vixia HG20). These filmed experiments took place between May and June, 2011.

Analysis

Colony level experiments (unmarked colonies)

The response of colonies was analyzed using the software SPSS. Comparisons were related samples Wilcoxon Signed ranks tests or paired t tests (unless otherwise indicated in the results and figure legends).

Individual-level analysis: behavior as colonies forage and grow satiated

In order to examine how the behavior of individual ants changes in response to colony satiation, we viewed the recordings of ant behavior (for the five paint-marked colonies) at the two feeders, the arena, and the nest. Our analyses were performed using the first 120 minutes after initial food discovery (of either feeder), unless otherwise indicated. 120 minutes is long enough for the choice of colonies to become apparent and to witness the beginnings of satiation. We recorded the following events: time of food discovery, initiation of feeding, termination of feeding, antennation of food without feeding, entry and exit times at the nest, the time of each trophallaxis bout initiation and termination in the nest, and the maximum number of concurrent trophallaxis

partners. In addition, we recorded the time of initiation of each recruitment event (tandem run) and the identity of followers, the start and stop time of each tandem run (even ephemeral tandem runs), and the success or failure of the tandem run. For these analyses, we defined recruitment based upon a leader's attempt to lead recruitment, a 'discrete' tandem run. Very often a leader initiating a tandem run will have several ephemeral followers – with only a subset of tandem runs lasting long or resulting in a follower being taken to the food source. A discrete tandem run was defined for our analyses as a tandem run attempt that occurred on a distinct trip home from the feeder (regardless of the number of prospective followers or success of any pairing). These behaviors were recorded for each ant that exploited either feeder during the initial 120-minute period after food discovery.

From the record of these events, individual behaviors were quantified for each foraging ant. In the nest, recruitment behavior was quantified by observing returning scouts that initiated tandem runs. 'Discrete' tandem runs were recorded for each trip home by a successful forager. 'Search time for 1st receiver' was the duration from the entry of an engorged forager into the nest to the first successful initiation of trophallaxis. 'Maximum trophallaxis receivers' was determined by observing the peak number of receivers that a returning forager off-loaded to during the course of a continuous trophallaxis bout. 'Total time in nest' was the time an ant remained in the nest until she left – to return to the feeder. Individual behaviors were analyzed for 120 minutes after the initial discovery of either of the feeders. These behaviors were analyzed based upon a one-hour cut-off: comparing the behavior of foragers during the first hour of foraging after food discovery to their behavior during the second hour. We used survival analysis in SPSS (Log-rank Mantel-Cox) to compare the search time durations between the first and second hour of foraging. Ants that did not find a receiver or leave the nest by the end of the second hour of foraging were given a duration and treated as censored data. Maximum trophallaxis receivers were compared between the two hours using a parametric t-test in SPSS. Recruitment between the first and second hours was compared by performing Pearson's chi-square test in R with a 2 x 2 matrix of the number

of recruitments observed with the expected number of recruitments being the total number of recruitments in the 120 minute period divided by 2.

Results

Colony-level experiments (unmarked colonies)

We found that starved colonies had a significantly greater number of ants exploring in the arena (before the feeders were introduced) than fed colonies (Wilcoxon signed ranks, $Z = -3.181$, $df = 15$, $p = 0.001$; Figure 3.2). In addition to the generally elevated number of explorers in the starved condition, the number of exploring ants scaled with colony size (Figure 3.3). The average total exploitation was lower for the fed treatment than for the starved treatment when exploitation was combined for both feeders (paired sample test, $t = 2.699$, $df = 15$, $p = 0.016$). In addition, total average recruitment was lower for the fed treatment relative to the starved treatment (paired sample test, $t = 3.695$, $df = 15$, $p = 0.002$). Analysing the high and low feeder separately showed that most of the shift in exploitation and recruitment could be attributed to a shift in behavior towards the high feeder (Figure 3.4 and Figure 3.5). In general, feeding shifted exploitation and recruitment downward for both feeders (Figure 3.4 and Figure 3.5). Feeding treatment did not alter the proportional allocation (choosiness) of total exploitation towards the feeders (paired samples test, $t = 1.263$, $df = 15$, $p = 0.226$). Nor did feeding treatment alter the proportional allocation of recruitment (paired samples test, $t = -0.888$, $df = 15$, $p = 0.389$).

Individual-level analysis: behavior as colonies forage and grow satiated

Temnothorax colonies showed a predictable pattern in terms of feeder exploitation with exploitation events growing, reaching a peak, and then declining. However, the timing of this peak and the decline varied by colony. Even though each of the five paint-marked colonies was starved for the same period of time (14 days) prior to the experiment, their rate of satiation varied.

For each of the five colonies, we compared behavior during the first hour after food discovery with the same behavior during the second hour after food discovery. In most cases, the reception of foragers by receiver ants correlated to changes in recruitment behavior and exploitation (Figures

3.6 – 3.10). High numbers of maximum trophallaxis receivers, short search times for first receivers, and short total nest trip durations were generally correlated with continued recruitment behavior and robust exploitation on the part of foragers. This was the case for two colonies: A48 (Figure 3.8) and colony A195 (Figure 3.10) both of which showed a peak of exploitation in the second hour. In contrast, colonies that demonstrated a decline in maximum trophallaxis receivers, longer search times for receivers, and longer total nest trip durations usually showed a sharp decline or complete cessation of recruitment and the beginnings of a decline in exploitation. This was the case for three colonies: A15 (Figure 3.6), A25 (figure 3.7) and A146 (Figure 3.9).

Discussion

Our study reaffirms and builds upon several of the general principles established in previous work on the influence of nutritional state in social insect foraging. We found that independent ant exploration increases with starvation, an effect that scales with colony size. By examining the effect of nutrition in the context of feeder choice experiments, we were able to test whether colony state influences the proportional allocation (choosiness) of exploitation or recruitment. We did not find a significant alteration in choosiness. Colonies allocated a statistically similar proportion of total foragers to the good and poor feeders regardless of nutritional state. We found that exploitation and recruitment shift upward or downward (depending on nutritional condition) in what appears to be a classic shift in threshold response. Karl von Frisch noted this phenomenon in honeybees – noting that the likelihood of a dance occurring varied depending upon the availability of natural forage (Frisch, 1967) and his observations were later built upon by Lindauer (1948) and Seeley (1995). Similar results have been shown in leafcutter ants where the likelihood of a recruitment signal (stridulation) changed with nutritional state (Roces & Hölldobler, 1996).

Our detailed analysis of forager interactions in the nest, while not providing manipulative experimental evidence, provides correlative evidence that foragers rely on interaction cues to guide their exploitation and recruitment decisions. While our individually paint-marked colonies

were all starved equally prior to the video-recorded feeder choice experiments, each responded dynamically in a distinct way depending upon discovery time and unknown aspects of colony biology. This idiosyncratic response allowed for comparison between colonies. Reduced recruitment and exploitation over time correlated generally with increased search times in the nest and reduced maximum trophallaxis receivers. In contrast, recruitment activity and robust exploitation correlated with larger numbers of receivers and short nest search times. What is the actual signal or cue that is informing foragers of the state of their colony? In all likelihood, an evolved signal is not required when informative cues abound. Foragers could potentially rely on any number of single nest-mate cues to guide their recruitment and exploitation decisions (Seeley, 1995; 1998), or their decision could be an integration of multiple cues.

Societies of social insects rely on interaction or encounter rates to guide individual decisions including food exploitation and recruitment decisions (Greene & Gordon, 2007; Leadbeater & Chittka, 2009; Pratt, 2005). Recruitment signals (and the response of nest-mates) are perhaps the clearest example of this phenomenon (Cassill, 2003; Couvillon, 2012; Mailleux, Buffin, Detrain, & Deneubourg, 2011). These behavioural responses likely are probabilistic in nature with individual responses being influenced by the physiological and neurological state of the recipient. For any single interaction the individual's response represents an estimate of local conditions (that may be in error), but over the range of many interactions with multiple nest-mates, the focal individual **and group** can arrive at a very accurate assessment of conditions. It is analogous to a statistician gaining increasing confidence in a particular null hypothesis with increasing sample sizes. These responses are tuneable - with individuals modifying their response thresholds depending upon social and environmental cues (Pratt & Sumpter, 2006). It seems that just such a process is on display in this context of nutritional state in *Temnothorax* ants. Ant foragers, like honeybees, rely on nest-mate interactions, trophallaxis-oriented cues to inform their foraging and exploitation decisions.

Nutritional state hypothesis:

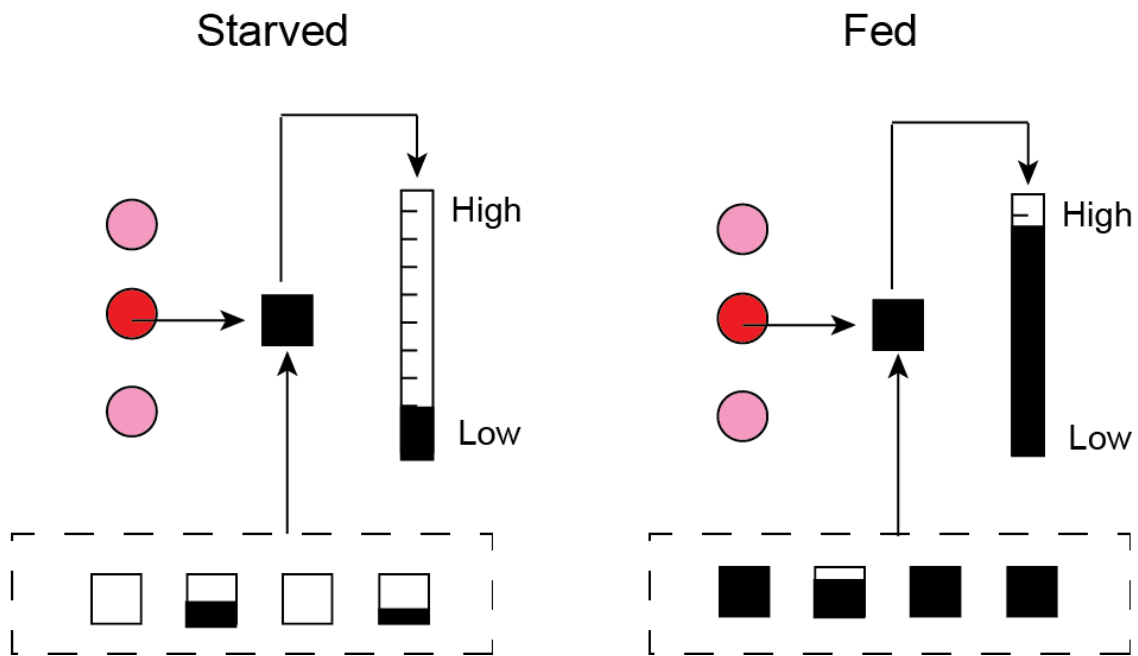


Figure 3.1. The nutritional state/ threshold hypothesis for ant social foraging. Circles represent food resources and squares represent ants. The focal ant is in black in the center. Foragers should modulate their acceptance response thresholds (for searching, exploitation, and recruitment) to match the nutritional state of the colony. This state should be communicated to the focal forager through interactions with nest-mates.

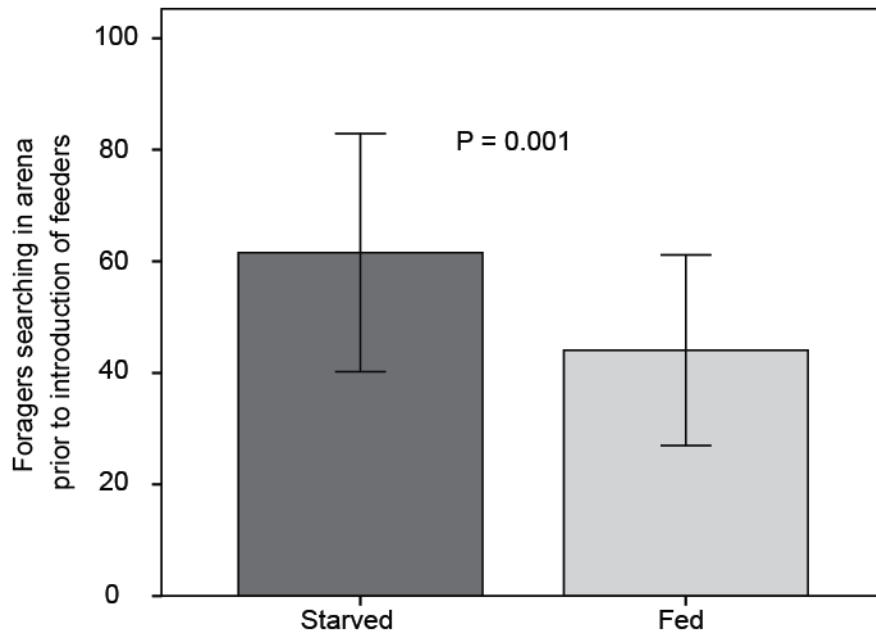


Figure 3.2. Prior to the introduction of the feeders, the number of foragers searching in the arena was counted. Significantly more ants were found in the arena when colonies were starved.

(Wilcoxon signed ranks, $Z = -3.181$, $df = 15$, $p = 0.001$)

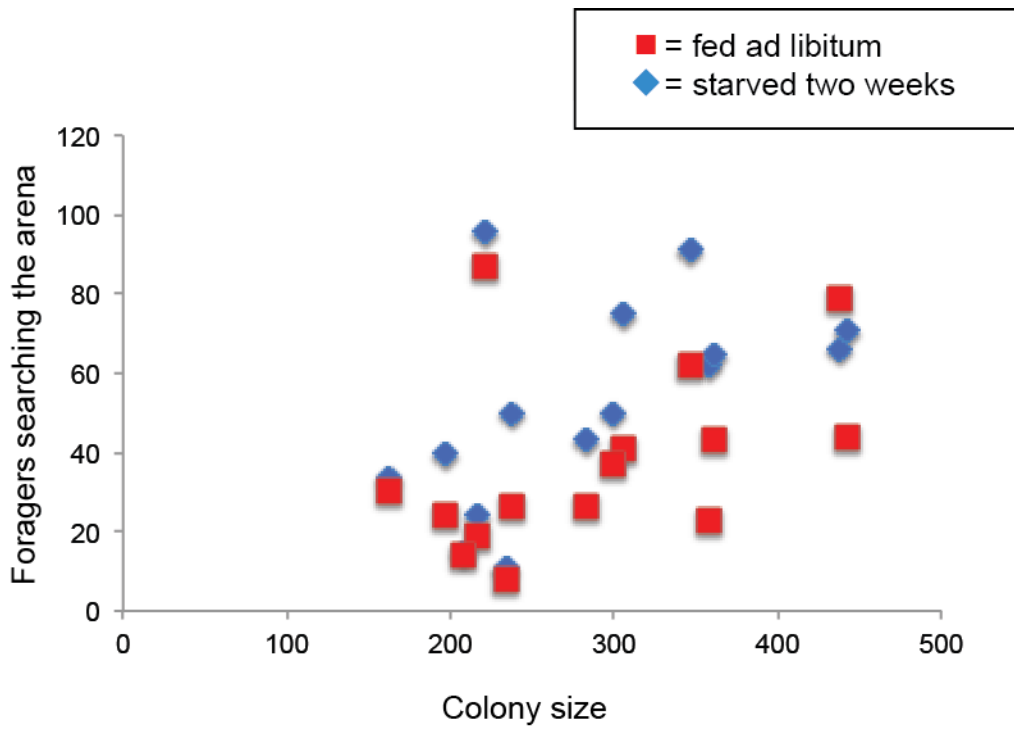


Figure 3.3. The number of exploring ants scales with colony size with starved colonies showing an increased number of explorers.

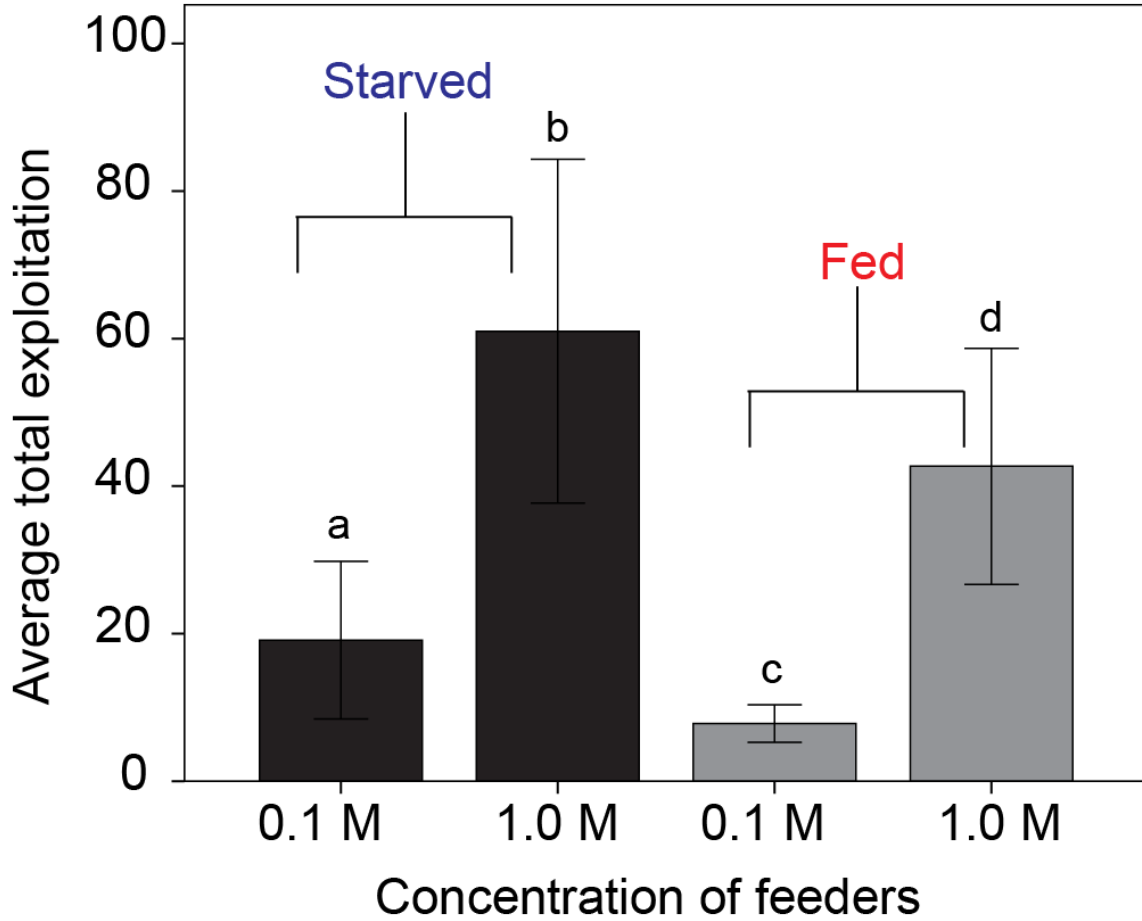


Figure 3.4. Feeding ad libitum Bhatkar diet has the effect of reducing total exploitation to both feeders. Wilcoxon signed ranks tests:

Treatment	Treatment	df	t statistic	p - value
Starved 1.0 M	Fed 1.0 M	15	2.676	0.017
Starved 0.1 M	Fed 0.1 M	15	2.274	0.038
Starved 0.1 M	Starved 1.0 M	15	-4.883	0.000
Starved 0.1 M	Fed 1.0 M	15	-3.225	0.006
Starved 1.0 M	Fed 0.1 M	15	4.778	0.000
Fed 0.1 M	Fed 1.0 M	15	-4.709	0.000

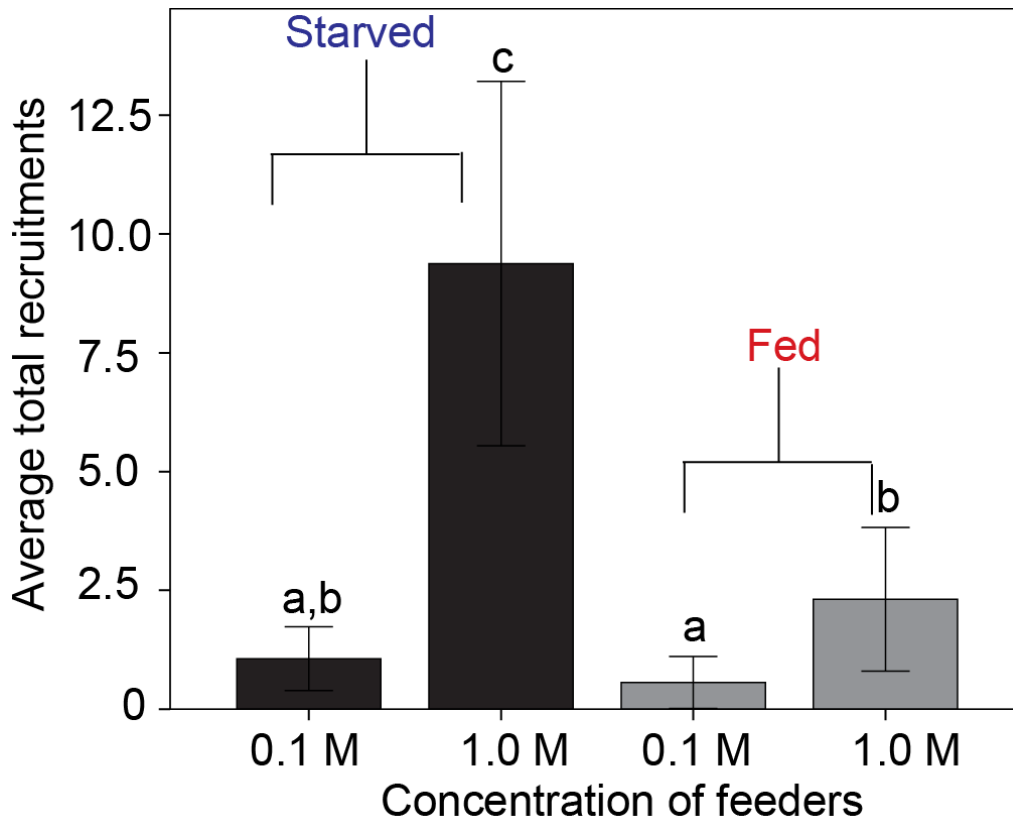


Figure 3.5. Starvation had the effect of increasing recruitment to the high feeder (though not at the low feeder). Wilcoxon signed ranks tests:

Treatment	Treatment	df	t statistic	p - value
Starved 1.0 M	Fed 1.0 M	15	3.451	0.004
Starved 0.1 M	Fed 0.1 M	15	1.581	0.135
Starved 0.1 M	Starved 1.0 M	15	-4.667	0.000
Starved 0.1 M	Fed 1.0 M	15	-1.49	0.157
Starved 1.0 M	Fed 0.1 M	15	4.51	0.000
Fed 0.1 M	Fed 1.0 M	15	-2.221	0.042

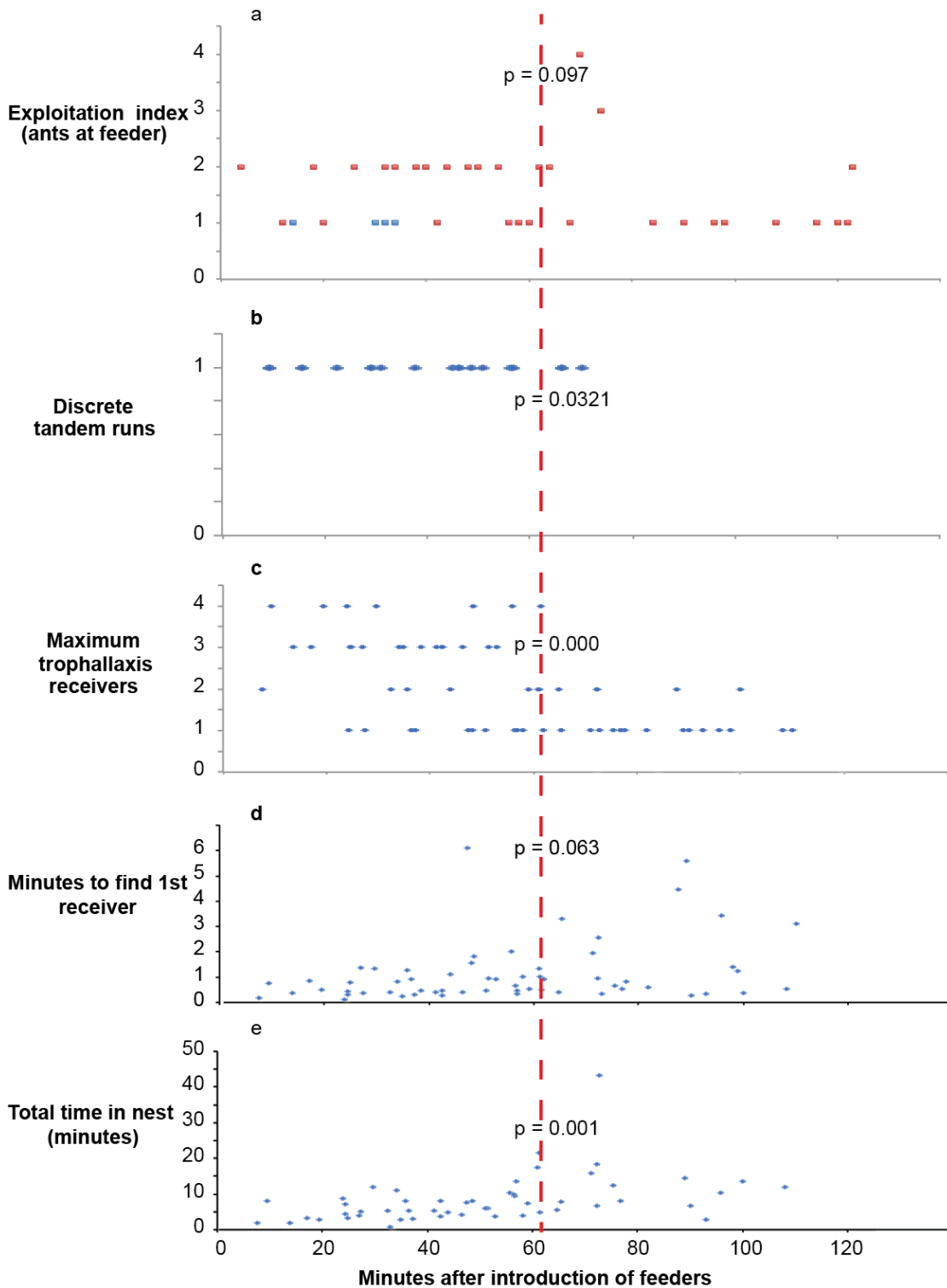


Figure 3.6. Time-matched summation of individual ant behaviors for colony A15 over the course of 120 minutes of active foraging with statistical comparisons between 1st 60 minute and 2nd 60 minute periods (the results of which are shown by the p-values in each figure). The time

demarcation is indicated by the dotted red line extending through the figures. **Figure a** shows the dynamics of feeder exploitation, an index of behavior taken by viewing the video recordings of ants exploiting the two feeders at two-minute intervals (t-test, $t = 1.684$, $df = 61$, $p = 0.097$). The intervals during which zero ants are feeding have been excluded from the figure for visual clarity. Red dots indicate exploitation of the 0.8 M feeder and blue dots indicate exploitation of the 0.1 M feeder. **Figure b** shows the number of discrete tandem runs during this time. (Pearson's chi-square test, $\chi^2 = 4.5938$, $df = 1$, $p = 0.03209$). **Figure c** shows the maximum number of concurrent trophallaxis receivers (t-test, $t = 5.384$, $df = 61$, $p = 0.000$). In **figure d**, we see the minutes required to find the first trophallaxis receiver (Log-rank Mantel Cox, $\chi^2 = 3.464$, $p = 0.063$). While the survival analysis utilized censored data, these censored values have been excluded here for clarity of presentation. **Figure e** shows the total time in the nest for returning foragers using survival analysis (Log-rank Mantel-Cox, $\chi^2 = 12.041$, $p = 0.001$). Likewise, censored values have been left out for visual clarity.

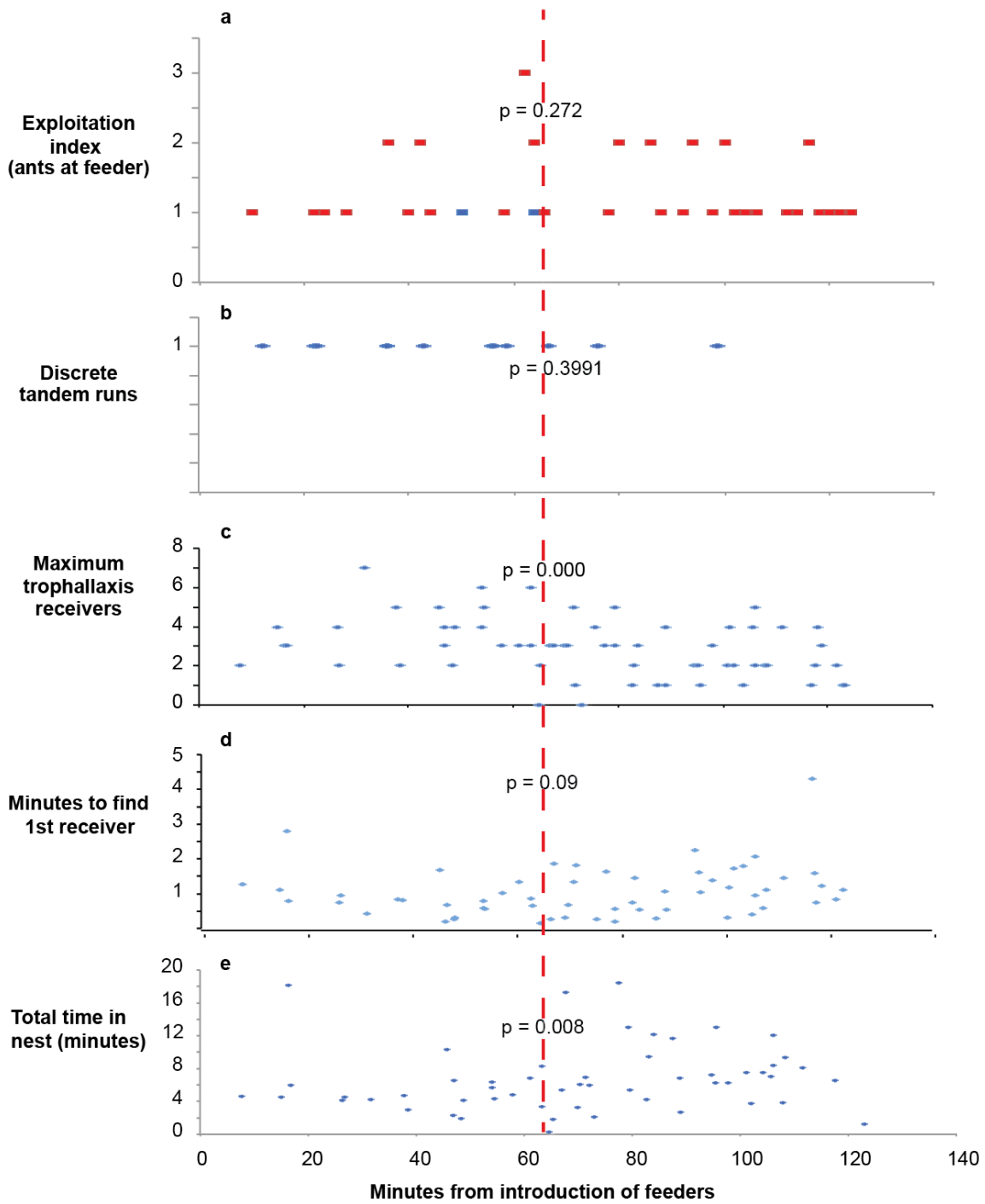


Figure 3.7. Time-matched summation of individual ant behaviors for colony A25 over the course of 120 minutes of active foraging with statistical comparisons between 1st 60 minute and 2nd 60 minute periods (the results of which are shown by the p-values in each figure). The time

demarcation is indicated by the dotted red line extending through the figures. **Figure a** shows the dynamics of feeder exploitation, an index of behavior taken by viewing the video recordings of ants exploiting the two feeders at two-minute intervals (t-test, $t = -1.109$, $df = 62$, $p = 0.272$). The intervals during which zero ants are feeding have been excluded from the figure for visual clarity. Red dots indicate exploitation of the 0.8 M feeder and blue dots indicate exploitation of the 0.1 M feeder. **Figure b** shows the number of discrete tandem runs during this time. (Pearson's chi-square test, $\chi^2 = 0.7111$, $df = 1$, $p = 0.3991$). **Figure c** shows the maximum number of concurrent trophallaxis receivers (t-test, $t = 3.746$, $df = 59$, $p = 0.000$). In **figure d**, we see the minutes required to find the first trophallaxis receiver (Log-rank Mantel Cox, $\chi^2 = 2.803$, $p = 0.094$). While the survival analysis utilized censored data, these censored values have been excluded here for clarity of presentation. **Figure e** shows the total time in the nest for returning foragers using survival analysis (Log-rank Mantel-Cox, $\chi^2 = 7.014$, $p = 0.008$). Likewise, censored values have been left out for visual clarity.

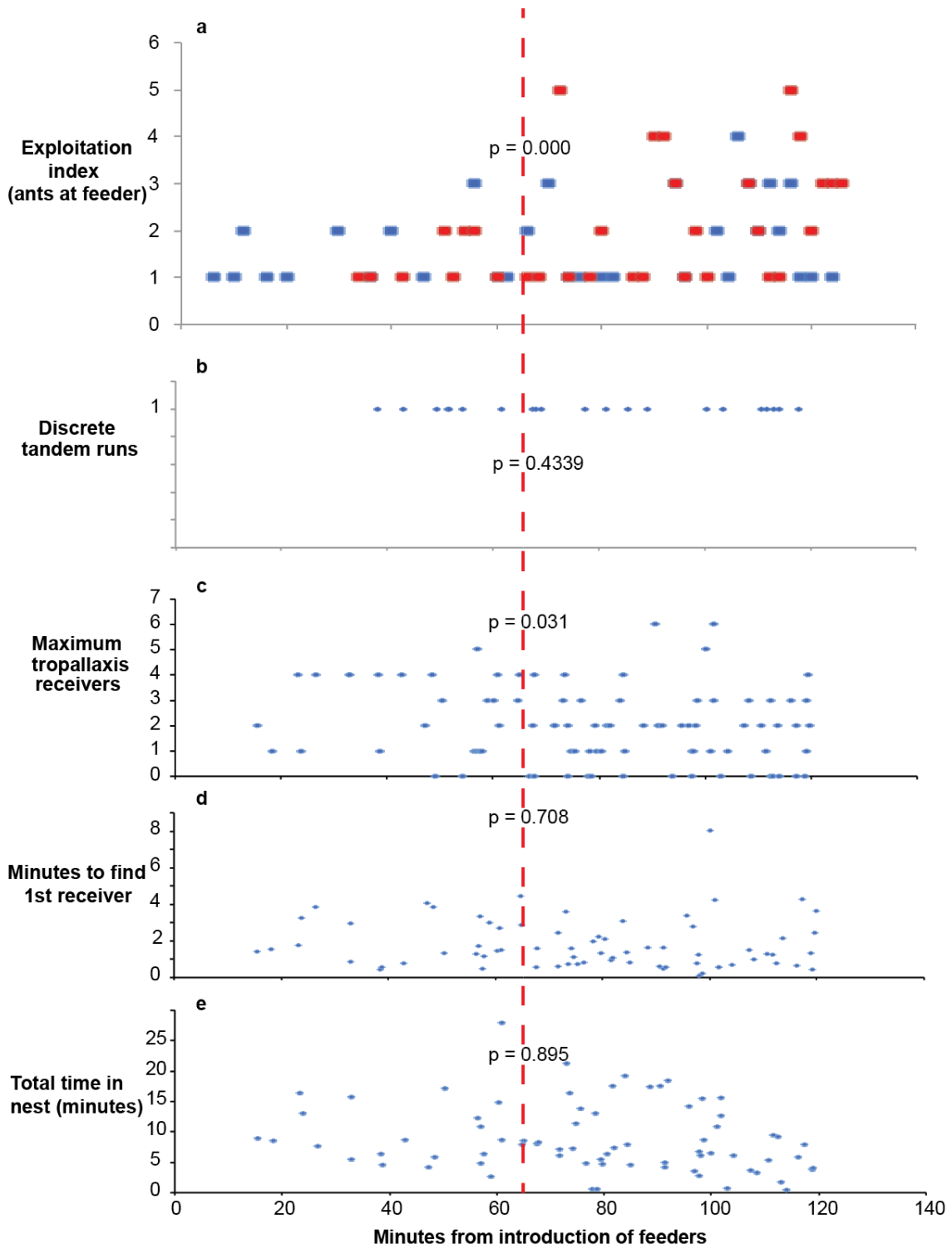


Figure 3.8. Time-matched summation of individual ant behaviors for colony A48 over the course of 120 minutes of active foraging with statistical comparisons between 1st 60 minute and 2nd 60 minute periods (the results of which are shown by the p-values in each figure). The time

demarcation is indicated by the dotted red line extending through the figures. **Figure a** shows the dynamics of feeder exploitation, an index of behavior taken by viewing the video recordings of ants exploiting the two feeders at two-minute intervals (t-test, $t = -5.55$, $df = 62$, $p = 0.000$). The intervals during which zero ants are feeding have been excluded from the figure for visual clarity. Red dots indicate exploitation of the 0.8 M feeder and blue dots indicate exploitation of the 0.1 M feeder. **Figure b** shows the number of discrete tandem runs during this time. (Pearson's chi-square test, $\chi^2 = 0.6122$, $df = 1$, $p = 0.4339$). **Figure c** shows the maximum number of concurrent trophallaxis receivers (t-test, $t = 2.193$, $df = 86$, $p = 0.031$). In **figure d**, we see the minutes required to find the first trophallaxis receiver (Log-rank Mantel Cox, $\chi^2 = 0.141$, $p = 0.708$). While the survival analysis utilized censored data, these censored values have been excluded here for clarity of presentation. **Figure e** shows the total time in the nest for returning foragers using survival analysis (Log-rank Mantel-Cox, $\chi^2 = 0.018$, $p = 0.895$). Likewise, censored values have been left out for visual clarity.

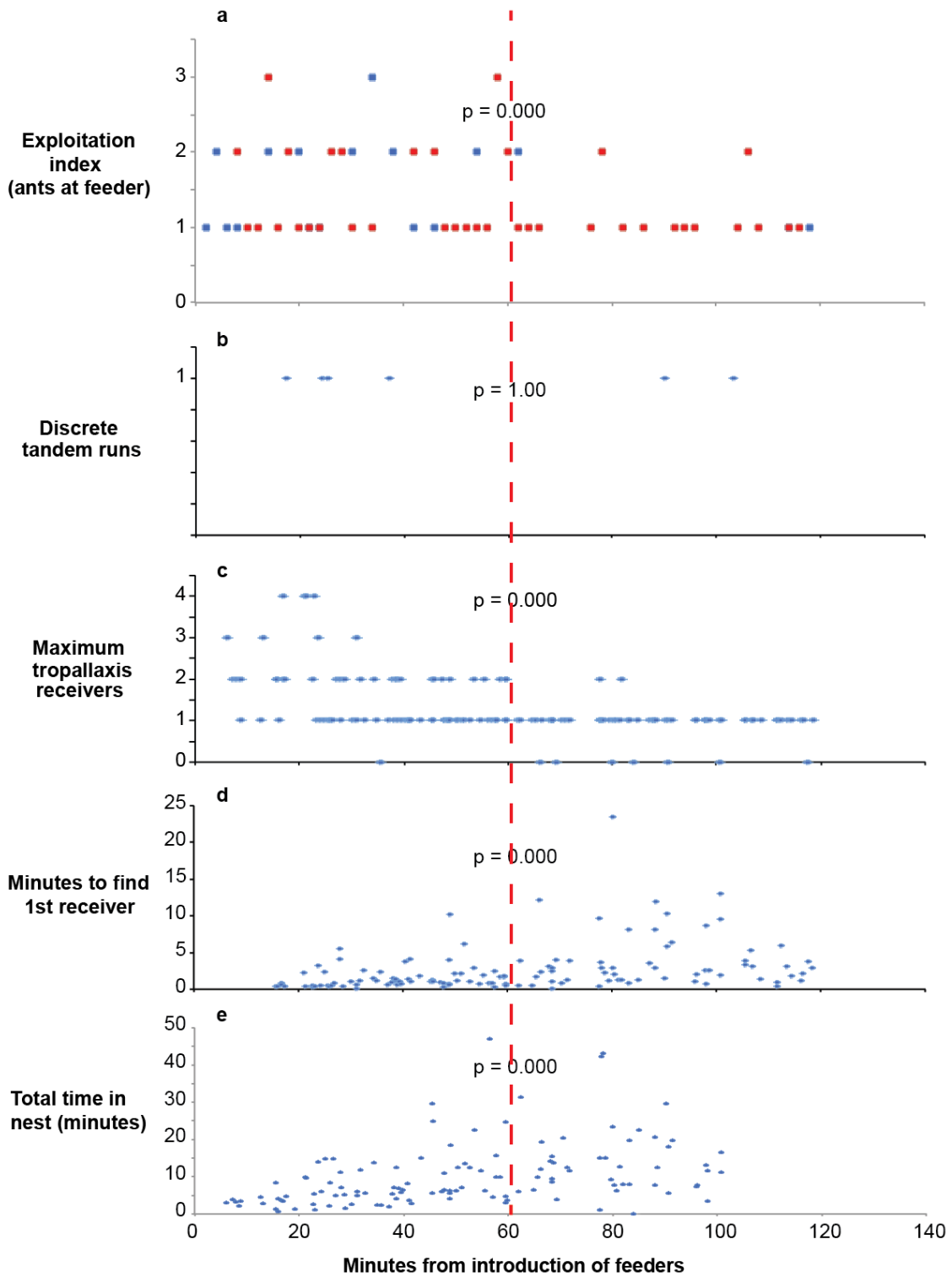


Figure 3.9. Time-matched summation of individual ant behaviors for colony A146 over the course of 120 minutes of active foraging with statistical comparisons between 1st 60 minute and 2nd 60 minute periods (the results of which are shown by the p-values in each figure). The time

demarcation is indicated by the dotted red line extending through the figures. **Figure a** shows the dynamics of feeder exploitation, an index of behavior taken by viewing the video recordings of ants exploiting the two feeders at two-minute intervals (t-test, $t = 4.111$, $df = 60$, $p = 0.000$). The intervals during which zero ants are feeding have been excluded from the figure for visual clarity. Red dots indicate exploitation of the 0.8 M feeder and blue dots indicate exploitation of the 0.1 M feeder. **Figure b** shows the number of discrete tandem runs during this time. (Pearson's chi-square test, $\chi^2 = 0.00$, $df = 1$, $p = 1.00$). **Figure c** shows the maximum number of concurrent trophallaxis receivers (t-test, $t = 5.791$, $df = 136$, $p = 0.000$). In **figure d**, we see the minutes required to find the first trophallaxis receiver (Log-rank Mantel Cox, $\chi^2 = 30.102$, $p = 0.000$). While the survival analysis utilized censored data, these censored values have been excluded here for clarity of presentation. **Figure e** shows the total time in the nest for returning foragers using survival analysis (Log-rank Mantel-Cox, $\chi^2 = 19.045$, $p = 0.000$). Likewise, censored values have been left out for visual clarity.

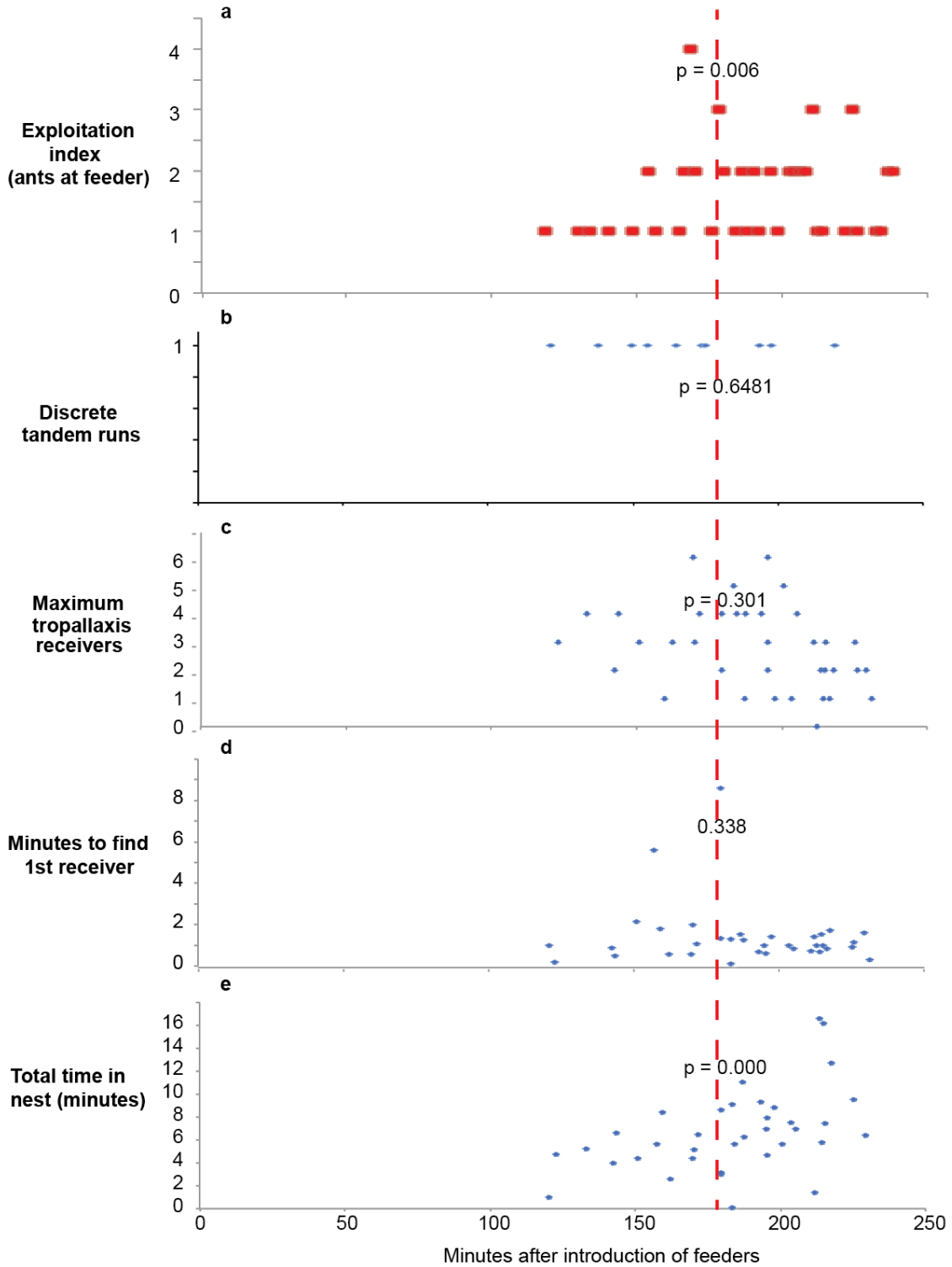


Figure 3.10. Time-matched summation of individual ant behaviors for colony A195 over the course of 120 minutes of active foraging with statistical comparisons between 1st 60 minute and 2nd 60 minute periods (the results of which are shown by the p-values in each figure). The time

demarcation is indicated by the dotted red line extending through the figures. **Figure a** shows the dynamics of feeder exploitation, an index of behavior taken by viewing the video recordings of ants exploiting the two feeders at two-minute intervals (t-test, $t = -2.825$, $df = 60$, $p = 0.006$). The intervals during which zero ants are feeding have been excluded from the figure for visual clarity. Red dots indicate exploitation of the 0.8 M feeder which was the only feeder exploited. **Figure b** shows the number of discrete tandem runs during this time. (Pearson's chi-square test, chi-square = 0.2083, $df = 1$, $p = 0.6481$). **Figure c** shows the maximum number of concurrent trophallaxis receivers (t-test, $t = 1.245$, $df = 34$, $p = 0.222$). In **figure d**, we see the minutes required to find the first trophallaxis receiver (Log-rank Mantel Cox, chi-square = 0.338, $p = 0.561$). While the survival analysis utilized censored data, these censored values have been excluded here for clarity of presentation. **Figure e** shows the total time in the nest for returning foragers using survival analysis (Log-rank Mantel-Cox, chi-square = 13.295, $p = 0.000$). Likewise, censored values have been left out for visual clarity.

CHAPTER 4

THE INFLUENCE OF RESOURCE DISTRIBUTION ON SOCIAL FORAGING

Abstract

Cooperation in nature often occurs against a backdrop of ecological uncertainty. In many species, cooperation between individuals has been shown to be an adaptation to unpredictable resources or harsh environments. Such challenging conditions may be drivers of sociality with increased group size and recruitment communication providing fitness benefits. We performed laboratory foraging experiments with colonies of the ant *Temnothorax rugatulus*, to address these questions. We presented colonies of different size with food distributions that varied in patchiness and/or ease of discovery, both in the presence and absence of recruitment communication. We found that while large colonies harvested food at a faster rate than small colonies in all circumstances, the scale of this advantage was dependent on the patchiness of resources and the presence of communication. Small colonies achieved a greater per capita foraging return than large colonies when resources were regularly distributed and easy to find. Larger groups demonstrated enhanced consistency (reduced variance in foraging success) that was exaggerated (relative to that of small colonies) when resources were harder to find. Our study reaffirms the concept that group size and communication are homeostatic social adaptations that can be tuned to ecological circumstances.

Introduction

The evolution of group size and the origins of communication are two questions central to the larger subject of sociality. Larger groups may have advantages in defense, foraging, reproduction, and physiological homeostasis. On the other hand, many cooperative groups in nature are relatively small. Members of smaller groups may benefit from an increased share of direct fitness or more efficient use of resources (excess group members could be costly in some circumstances). Why do cooperative groups vary in size and social complexity?

Emlen, in pondering the question of helpers at the nest (in birds), proposed a framework that has been influential in explaining the ultimate origins of sociality (1982). The ecological constraints

model has been invoked in systems as diverse as naked mole rats, cooperative breeding cichlids, and primates. Cooperative societies often amount to extended kin groups of 'helpers at the nest' be they birds or ants. Cooperative species tend to '...inhabit harsh, fluctuating, and highly unpredictable environments...' (Emlen, 1982). Cooperation in groups may provide benefits in these difficult environments that individual animals would be hard-pressed to match. Larger groups may also have advantages over smaller groups. In fact, positive effects of group size on survival and reproduction are well documented in such organisms as cooperatively breeding birds (Conner et al., 2004), cichlids (Brouwer, Heg, Taborsky, & Bachar, 2005), rodents (Waterman, 2006), and primates (Heymann & Soini, 1999). The benefits of group size might occur across multiple dimensions. But of these factors, an obvious subject for study is the influence of group size on foraging success.

Even in the absence of communication and without considering resource distribution, larger groups should have an advantage in terms of consistency of return per individual forager (Wenzel & Pickering, 1991). Wenzel and Pickering used the central limit theorem to show that in larger groups, individuals will experience reduced variance in foraging success relative to smaller groups. With more individuals searching the environment, larger groups will be more likely to discover rare resources that can be shared with group members. In an empirical study with a eusocial wasp, *Ropalidia marginata*, it was shown that larger groups of wasps experienced reduced variance in foraging success (Naug & Wenzel, 2006).

Wenzel and Pickering's model suggests that the benefits of group size are independent of resource distribution, but several other models have explored the issue of resource patchiness with respect to social insect foraging (Dornhaus, Klugl, Oechslein, Puppe, & Chittka, 2006; Fronhofer, Pasurka, Mitesser, & Poethke, 2011; Naug & Wenzel, 2006). Naug and Wenzel's model suggested that the benefits of group size could vary with ecological context (2006). In contrast, Dornhaus et al. found that colony size did not influence the foraging success of simulated honeybee colonies (2006). A number of related studies have explored the question of

group size and foraging success in a natural ecological context (Donaldson-Matasci et al., 2013; Naug & Wenzel, 2006). And yet, few studies have attempted to experimentally manipulate resource distribution itself (Flanagan, Letendre, Burnside, Fricke, & Moses, 2012). Flanagan et al. did not find a colony size effect in foraging for colonies of harvester ant in response to differences in seed resource distribution (2012).

Group foraging success also depends on communication, which may in turn interact with group size effects. The CLT model of Wenzel and Pickering and related empirical studies do not consider the influence of recruitment in a colony's foraging success. The paper wasp has been the primary model organism in this tradition. Paper wasps do not make use of recruitment in foraging, but are rather opportunistic solitary foragers (Richter, 2000). The ecological niche and resource distribution that a species lives in should influence the utility of recruitment communication. Wasps are typically solitary hunters, and their prey tend not to be heaped in convenient piles, but that is not true for honeybees. It has been shown that patchily distributed resources increase the benefits of recruitment for honeybees in models (Beekman & Bin Lew, 2008; Dornhaus et al., 2006) and in empirical studies (Donaldson-Matasci & Dornhaus, 2012; Dornhaus & Chittka, 2004). In contrast, resources that are regularly distributed might allow the success of smaller groups and decrease the benefits of recruitment.

In order for us to understand the ultimate origins of group size and the evolution of communication, it may be beneficial to consider the two questions together and in relation to resource distribution (Dornhaus et al., 2006). The goal of this paper was to experimentally examine the effect of group size, resource distribution and recruitment on foraging success in the ant *Temnothorax rugatulus*. This genus has become one of the leading model organisms for the study of collective decision-making (Franks et al., 2002; Pratt, 2005). It represents a good model system for foraging studies of these questions for the following reasons:

(1). Colonies occur naturally in a range of sizes (from fewer than 10 individuals to over 500). By utilizing one species with natural variation in colony size, we can examine size alone (without the confounding factors of comparing group size between species).

(2). It is possible to control for the presence or absence of recruitment. Many ant species (including *T. rugatulus*) will not recruit to insect prey small enough for a single individual to retrieve, but instead simply carry the food back to the nest (Detrain & Deneubourg, 1997). In contrast, *Temnothorax* do recruit to sugar water sources (Franks & Richardson, 2006; Möglich, Maschwitz, & Hölldobler, 1974; Shaffer et al., 2013). By changing food types it is thus possible to influence the likelihood of recruitment communication.

(3). *Temnothorax*'s tiny size and rate of movement make it possible to manipulate resource distributions and discovery rate in the laboratory and make large sample sizes feasible. It has been shown that colonies in the wild actively forage in a scale replicable in the lab (Bengston & Dornhaus, 2012).

This paper addresses four linked predictions: (i) Large colonies will demonstrate increased consistency and a greater rate of harvest relative to small colonies in all conditions, but (ii) this advantage will be exaggerated when resources are patchily distributed and difficult to find. (iii) Small colonies will be more competitive with large colonies when resources are easily found and communication is available, but (iv) in the presence of rare resources and recruitment communication, large colonies will show the greatest relative advantage over small colonies.

Materials and Methods

Experimental subjects

Queen-right colonies of *T. rugatulus* were collected from rock crevice nests in the Pinal Mountains near Globe, Arizona, (N 33° 19.000' W 110° 52.561) between March and September 2010. Colonies were censused and a cohort of eighteen colonies was selected based on population size: 9 small colonies (50 or fewer individuals) and 9 large colonies (greater than 200 individuals). Average size of the small and large colonies was 34 ± 4.4 (SE) and 327 ± 29.2 individuals, respectively. This cohort was used for Experiment 1 and one treatment of Experiment 2. For the other treatment in Experiment 2, a second cohort of 9 small and 9 large colonies was

collected from the same location between May 2011 and June 2013. In this cohort, the small colonies averaged 32.2 ± 2.7 ants and the large colonies averaged 235.4 ± 26.2 ants.

In the laboratory each colony was housed in an artificial nest made from a 2.4 mm thick balsa wood slat with a rectangular central cavity measuring 3.8 x 6.4 cm. The slat was sandwiched between 50 x 75 mm glass microscope slides that served as floor and ceiling. A 3 mm wide entrance was cut through the side of the slat. Each nest was placed in an 11 x 11 cm plastic box and kept in a Sheldon diurnal incubator at a 16:8 day:night schedule, with a daytime temperature of 23°C and a night-time temperature of 15°C. Colonies were provided weekly with water and an agar-based diet (Bhatkar and Whitcomb, 1970).

Experiment 1

In this experiment we measured the foraging success of colonies when challenged with different food distributions and in the absence of recruitment communication. To minimize recruitment, we used freeze-killed *Drosophila melanogaster* as food, because preliminary observations indicated that these flies do not elicit recruitment. Colonies were placed in arenas (measuring 74 x 60 cm) and presented with fruit flies in one of two distributions, regular or patchy (figure 4.1). In the regular treatment, 9 flies were laid out in a X by X cm grid. In the patchy treatment, 9 flies were spread loosely within a 14-cm diameter enclosure constructed from an inverted plastic Petri dish with its floor cut out to allow visibility. The enclosure was placed X cm from the nest and was accessible only through a single entrance 3 mm in width (the enclosure walls were coated with Fluon to prevent ants from climbing in or out). This barrier had the effect of reducing the discovery rate of the food.

Colonies were starved for two weeks prior to each treatment to ensure an equal nutritional state and placed into the arenas 24 hours in advance of food presentation. Flies were freeze-killed the night before presentation. Arena walls were treated with Fluon to prevent escape. The experiment began with the introduction of the fruit flies to the arena. The colonies were then observed for 8

hours continuously, with food discovery, exploitation, and any recruitment measured at 15-minute intervals. Discovery was recorded when the first ant was seen in contact with a fly. A fly was recorded as harvested when an ant had taken it back to the nest. After 24 hours, the total number of harvested flies was recorded. The patchy distribution experiment took place on June 10, 2010 and the regular distribution experiment took place on July 16, 2010. The same 9 colonies were used for both treatments.

Experiment 2

The purpose of the second experiment was to see how ease of discovery of resources influenced the foraging success of small and large colonies (Figure 4.2) in the presence of recruitment communication. We presented colonies with a single 0.8 M sucrose solution feeder in one of two treatments: a rare resource and an easily found resource. The rare resource was placed in an enclosure as described above for Experiment 1. In the 'easily found' treatment the feeder was placed 20 cm in front of the nest with no impediments to discovery. The feeder consisted of a depression well slide with a 0.1ml droplet of sugar water which was replenished at 30-minute intervals to counter evaporation. All other details were the same for this experiment as for Experiment 1. The two treatments used distinct cohorts of colonies, as noted earlier in the description of experimental subjects. For the rare treatment, these were the same colonies as used in Experiment 1. The rare treatment took place on June 25, 2010 and the easily found treatment occurred on November 20, 2014.

Statistical analysis

In SPSS, we used Mann-Whitney U tests and Wilcoxon signed ranks to compare discovery times and total harvest. Effects of treatment on variance of colony foraging success were evaluated with Levene's test. using the median as a baseline. Because we were making repeated comparisons, we utilized the Bonferroni correction when deciding upon significance. Rates of harvest in the fruit fly treatment analyzed using Kaplan Meier survival analysis in SPSS. The time of harvest of each fruit fly was determined from experimental logs. Un-harvested fruit flies were

given a duration of 480 minutes and treated as censored data. Harvest rates in the two fly treatments were compared using log-rank (Mantel-Cox) tests.

Results

We consider the results of each of our experiments using several perspectives: mean colony performance (discovery and harvest), the variance of colony performance, per capita harvest, and finally the rate of exploitation. Finally, we compare the success of colonies across treatments.

Experiment 1: foraging in the absence of recruitment communication

Discovery rate and harvest

In the patchy distribution, large colonies made the first discovery of food significantly earlier than small colonies (Mann-Whitney U test, $df = 8$, $Z = -2.341$, $p = 0.019$; Figure 4.3). In contrast, there was not a significant difference in first discovery time between large and small colonies in the regular environment (Mann-Whitney U test, $df = 8$, $Z = -0.265$, $p = 0.791$; Figure 4.3).

Small colonies harvested fewer fruit flies than large colonies regardless of distribution. After 8 hours, large colonies harvested more flies than small colonies in both the patchy treatment (Mann-Whitney U test, $df = 8$, $Z = -3.23$, $p = 0.001$; Figure 4.4) and in the regular treatment (Mann-Whitney U test, $df = 8$, $Z = -2.191$, $p = 0.028$; Figure 4.4). Large colonies in the regular environment completely harvested each fruit fly in the arena. The regular environment reduced the advantage of large colonies relative to small colonies, but the thorough harvesting by large colonies led to a mean of 9 harvested fruit flies (out of 9 present) and a variance of zero. Although small colonies harvested nearly 7 fruit flies (of the 9 present) this still was a significant difference when compared to the large colonies.

Resource distribution had a significant influence on discovery when we compare the performance of colonies within the same size class. Small colonies discovered flies sooner in the regular distribution than in the patchy distribution (Wilcoxon test, $df = 8$, $Z = -3.724$, $p = 0.000$). Similarly,

large colonies discovered flies significantly sooner in the regular treatment than in the patchy treatment (Wilcoxon test, $df = 8$, $Z = -3.725$, $p = 0.000$).

Distribution also influenced the total harvest for both size classes of ant colony. Small colonies harvested significantly more flies in the regular treatment than in the patchy treatment (Wilcoxon test, $df = 8$, $Z = -2.836$, $p = 0.005$). Large colonies harvested more flies in the regular distribution as well (Wilcoxon test, $df = 8$, $Z = -3.817$, $p = 0.000$).

Although both large and small colonies differed significantly between distribution treatments, the scale of the difference was very different (Figure 4.9). Small colonies discovered the first fruit fly an average of 39 times faster in the regular distribution relative to their first discovery in the patchy distribution. Similarly, small colonies harvested almost 5 times as many fruit flies in the regular treatment compared to the patchy treatment. In contrast, large colonies found their first fruit fly about five times faster in the regular treatment relative to their first discovery in the patchy treatment and they harvested a similar number of fruit flies regardless of distribution.

Variation in colony performance

Large colonies demonstrated enhanced consistency in food discovery times relative to small colonies. There was a significant reduction in the variance of discovery time for large colonies relative to smaller colonies in the patchy distribution ($F = 9.52$, $p = 0.007$, Figure 4.5) but not in the regular distribution ($F = 1.125$, $p = 0.304$, Figure 4.5). While large colonies experienced no change in variance in discovery time between distributions ($F = 3.174$, $p = 0.09$, Fig. 3.5), small colonies showed significantly reduced variance in discovery in the regular distribution relative to the patchy distribution ($F = 11.067$, $p = 0.004$, Fig. 3.5). While discovery times varied from 2 minutes to over 300 minutes, total harvest of flies was bounded from 0 to 9, inherently limiting the variance of outcomes. In spite of this limit, we see an effect of colony size and distribution on the variance of harvest. Large colonies differed qualitatively from small colonies (though not significantly) in the variance of harvest in the patchy environment ($F = 0.78$, $p = 0.39$, Fig. 3.6).

However, in the regular distribution large colonies differed significantly from small colonies in harvesting variance ($F = 6$, $p = 0.026$, Fig. 3.6). Small colonies did not differ in the variance of harvest between treatments (though with an obvious rightward skew in the instance of regularly distributed flies) ($F = 0.0075$, $p = 0.93$, Fig. 3.6). Likewise, large colonies did not differ in the variance of total harvest between treatments ($F = 1$, $p = 0.33$, Fig. 3.6)

Per capita harvest

In the regular distribution, small colonies actually outperformed large colonies on a per capita basis (total flies harvested/colony population) over both long and short time periods. Over 8 hours of foraging, small colonies harvested significantly more flies per capita than large colonies (Mann-Whitney U, $df = 8$, $Z = -3.468$, $p = 0.000$). The per capita advantage of small colonies was also true for the 1st hour of foraging (Mann-Whitney U, $df = 8$, $Z = -2.606$, $p = 0.008$). It must be noted that this per capita advantage may be due to the finite supply of fruit flies provided. If flies had been replaced as they were harvested, the per capita advantage may have shifted to favor the large colonies. Even with finite resources, the relative advantage of small colonies in per capita foraging success disappeared when the resources were patchily distributed. Over 8 hours there was no significant difference between large and small colonies in per capita foraging success in the patchy distribution (Mann-Whitney U, $df = 8$, $Z = -0.928$, $p = 0.387$) and in the first hour of foraging, large colonies demonstrated higher per capita harvest than small colonies (Mann-Whitney U, $df = 8$, $Z = -2.514$, $p = 0.05$).

Rate of harvest

Large colonies harvested fruit flies at a faster rate than small colonies in the regular distribution (Log rank (Mantel-Cox), $\chi^2 = 108.161$, $p < 0.001$; Fig. 3.10a) and in the patchy distribution (Log rank (Mantel-Cox) $\chi^2 = 91.087$, $p < 0.001$; Fig. 3.10b), however this relative advantage seemed to be exaggerated in the patchy distribution. Comparing rates between treatments (by size class) revealed a significant influence of distribution on the rate of harvest with the regularly distributed

fruit flies being harvested faster for both size classes. Finally, it should be noted that there were no observed tandem runs in either distribution of fruit flies.

Experiment 2: foraging in the presence of recruitment communication

Discovery rate and harvest

Large colonies discovered the rare sugar water feeder significantly earlier than small colonies (Mann-Whitney U, $df = 8$, $Z = -2.92$, $p = 0.002$; Fig. 3.7) a result mirroring the discovery of the patchily distributed fruit flies in experiment 1. Similarly, large colonies also found the easily discovered feeder earlier than small colonies, but the scale of this advantage was reduced (relative to the case of the rare feeder) (Mann-Whitney U, $df = 8$, $Z = -2.782$, $p = 0.005$; Fig. 3.7). In addition, large colonies exploited the rare feeder more than small colonies with a significantly greater number of ant feeding events (Mann-Whitney U, $df = 8$, $Z = -3.58$, $p = 0.000$; figure 4.8). Large colonies also showed greater exploitation than small colonies when the feeder was easily found, but the scale of this advantage was again reduced (relative to the case of the rare feeder) (Mann-Whitney U, $df = 8$, $Z = -3.585$, $p = 0.000$; figure 4.8)

Variation in colony performance

Large colonies demonstrated reduced variance in discovery time of the sugar water feeder relative to small colonies when the feeder was rare (Levene's test, $Z = 15.82$, $p = 0.001$) and also when the feeder was easily discovered (Levene's test, $Z = 7.573$, $p = 0.014$). Large colonies showed reduced variance in total exploitation of the sugar water compared to small colonies when the feeder was rare (Levene's test, $Z = 13.764$, $p = 0.0019$), but when the feeder was easily found large colonies actually showed greater variance than small colonies (Levene's test, $Z = 5.38$, $p = 0.034$).

Per-capita harvest

When the feeder was rare (difficult to find) large colonies showed a much greater per capita return on foraging. While there was not a significant difference between size classes in the first

hour of foraging (Mann-Whitney U, $df = 8$, $Z = -1.463$, $p = 0.19$), over 8 hours large colonies demonstrated significantly greater per capita harvest than small colonies (Mann-Whitney U, $df = 8$, $Z = -2.964$, $p = 0.002$). In contrast, when the feeder was easily found, there was no significant difference in per capita exploitation between size classes during 8 hours of foraging (Mann-Whitney U, $df = 8$, $Z = -1.457$, $p = 0.145$).

Rate of harvest

Because the supply of sugar water was, in effect, unlimited we did not perform rate analysis similar to the earlier experiment. Instead, we plotted the average exploitation over time. When the feeder was rare, large colonies showed much greater discovery and exploitation than small colonies (Figure 4.10). In fact, three of the nine total small colonies failed to discover the rare feeder within the 480 minute time limit. When the feeder was easily found, small and large colony exploitation curves matched one another (in terms of the times of peak exploitation and apparent satiation; Figure 4.10). While there was robust recruitment by large colonies to both the easily found and rare feeders, small colonies performed very little recruitment (even to the easily found feeder). Only three tandem runs were observed from small colonies to the easily found feeder in the course of all nine trials. In spite of this lack of recruitment, the small colonies were apparently able to meet their nutritional needs (as evidenced by the satiation curve and matching per capita harvest) when the resource was easily found.

Comparing the success of colonies across treatments

What is the relative influence of resource distribution and communication on colony foraging within each size class? We first consider the case of foraging without communication: By taking the ratio of discovery times and total harvest in the two treatments (patchy and regular fruit flies), we can clearly see that resource distribution has a greater influence on small colonies. We performed a test on the ratios of discovery time for small and large colonies (patchy discovery time/ regular discovery time). Resource distribution showed a greater, but not significant, influence on small colonies' first discovery time compared to large colonies (Mann Whitney U, $Z =$

- 1.902, $df = 8$, $p = 0.063$; Fig. 3.9). The same metric can be used to look at the relative influence of resource distribution on total harvest (averaging the ratios of regular total harvest: patchy total harvest). In order to deal with the problem of dividing with zeroes, for colonies that harvested no flies, the 'zero' was changed to a 'one' for this analysis. Only small colonies harvested zero flies after 8 hours, so this was an inherently conservative alteration of the data. Even with this adjustment, small colonies were more strongly influenced by resource distribution than large colonies in total harvest (Mann-Whitney U, $Z = -2.935$, $df = 8$, $p = 0.004$; Fig. 3.9). Because variance was bounded in total harvest, the difference in average harvest is clearer than the more inherently variable average discovery time. If we compare variances of discovery time ratios and harvest ratios, the data again show that small colonies are more inherently erratic than large colonies. Small colonies showed a strong but not significant trend towards greater variance in discovery time ratio (Levene's test using median as center, $df = 16$, $F = 1.34$, $p = 0.26$; Fig. 3.9). Small colonies showed a significantly greater harvest ratio variance than large colonies (Levene's test using median as center, $df = 16$, $F = 4.31$, $p = 0.054$; Fig. 3.9). This calculation likewise involved changing 'zero' harvested flies to 'one'.

Because we used different colonies for the sugar water experiments, we could not calculate ratios of discovery or exploitation for comparison as we did for the drosophila experiments.

Discussion

Our study illustrates that resource distribution, communication, and group size interact to influence the success of ant colony foraging. As we predicted, large colonies showed greater consistency and a faster rate of harvest relative to small colonies in all conditions, but this advantage was increased when resources were rare or patchily distributed. Small colonies presented with regularly distributed fruit flies or an easily discovered sugar water feeder performed competitively with large colonies in a number of ways: in the case of the regularly distributed fruit flies, small colonies found the first fruit fly as quickly (and with similar variance) as large colonies. In terms of per-capita harvest, small colonies outperformed large colonies in the

regular distribution (though this could be due to the finite supply of fruit flies provided). Small colonies were able to meet their nutritional needs as easily as large colonies when a single easily found sucrose solution feeder was present (as evidenced by the time-matched exploitation curves). But when the sucrose solution feeder was difficult to find, many small colonies failed to discover the food source within the 8-hour observational period. In contrast, large colonies showed robust recruitment and exploitation of this hidden feeder (although with a delayed exploitation peak relative to large colonies in the 'easily found' treatment). In general, large colonies showed greater robustness to resource distribution conditions. Regularly distributed or patchy, easily found or rare – large colonies were more consistent in their discovery and harvest than small colonies.

While we show clear effects stemming from colony size we must admit our dearth of understanding of the real world behavioral ecology of *Temnothorax* ants. What are the actual nutritional needs of colonies? It should be noted that these ants have shown extraordinary resistance to starvation (Rueppell & Kirkman, 2005). While our study showed context-specific advantages to larger colony sizes in our arena experiments, we can only make limited claims concerning the effect this might have on the fitness of actual colonies. Food resources in nature are very often ephemeral (especially nutrient-rich resources such as nectar), and it isn't unreasonable to suggest that the colony-size effects we documented in the lab might have very real fitness consequences for colonies in their natural context. But as we pointed out earlier, *Temnothorax* colonies occur across a wide range of colony sizes in their natural environment, and further studies are necessary to test for any potential fitness consequences across this size range.

Our study is the first to experimentally manipulate resource distribution and communication to demonstrate the context-dependent variance reduction and colony size effects predicted by many models (Beekman & Bin Lew, 2008; Dornhaus et al., 2006; Fronhofer et al., 2011; Naug & Wenzel, 2006). It could be very difficult to tractably alter resource distribution in a natural

environment in an effective way for such wide-ranging species as *Apis mellifera*. Our lab-based experimental manipulation of resource distribution and communication serves as a complement to experiments performed in the field. For example, Dornhaus and Chitka showed that elimination of honeybee waggle dance recruitment did not influence colony foraging success in a temperate European setting but did influence colony success in Indian dry tropical forest (2004). Though they did not experimentally alter resource distribution, they were able to film colonies and use the bees' own dances to make a map of resource locations. The results suggested that the European environment had a more regular distribution of food resources than the much patchier Indian dry forest. For honeybees, communication appeared to be beneficial in the patchy environment but not the regular environment.

Why might the reduction of variance be an important factor influencing the evolution of group size? In order to survive, living things must maintain equilibrium in the many requirements of life: water balance, food intake, and temperature (among others). Much of the benefit of group size amounts to a diminution of the extremes experienced by individual organisms. Tent caterpillars, for example, are able to maintain slightly higher temperatures within their canopy than without during cold spells (Ruf & Fiedler, 2000). Honeybees are able to keep their hive within a temperature optimum by collectively fanning their wings (Stabentheiner, Kovac, & Brodschneider, 2010). Our study demonstrates that larger groups of foraging social insects reduce the variance of foraging success relative to smaller groups, and that this advantage is mediated by resource distribution and communication. Consistency in the provisioning of resources may be a key component in the success of larger social groups. Our study has emphasized the point that small groups are more inherently erratic in their foraging success. This inconsistency would likely influence the production of offspring. In a study of productivity in the ant, *Leptothorax allardycei*, Cole found that small colonies were more variable in their brood production than large colonies (Cole, 1986). Similarly, Bouwma and associates found that small colonies of a social wasp showed increased variability in productivity relative to larger colonies (Bouwma, Nordheim, & Jeanne, 2006). Likewise, larger colony size in a social allodopine bee correlated with a reduction

in variance in brood production, relative to small colonies (Stevens, Hogendoorn, & Schwarz, 2007). Thus, we see that group size influences the variance of foraging success in many other species of social insect and that this has consequences for production.

In the social hymenoptera, it has been suggested that progressive provisioning is the essential pre-adaptation for the evolution of eusociality. (Hölldobler & Wilson, 2009) Progressive provisioning is hardly limited to the social hymenoptera. Other groups requiring an ongoing feeding regime and extended parental care for their young include many birds and mammals (e.g. meerkats, chimpanzees, dolphins, and *Homo sapiens*). A case could be made for extended care and progressive provisioning being the key pre-adaptation for the evolution of cooperation throughout the natural world. Developing young in such species require consistent provisioning with inconsistent feeding regimes resulting in developmental delays, mortality, or reduced fecundity (Dmitriew & Rowe, 2011). Environmental constraints theory suggests that cooperation most often evolves in challenging or unpredictable environments (Emlen, 1982). Larger foraging and care-providing groups may be able to more consistently provide for offspring, an advantage over smaller groups that is magnified in unpredictable environments. Thus, the evolution of sociality may result from an inter-play between environmental constraint and life history pre-adaptation. Organisms which have slow-developing young or a constant 'crop' of brood to attend to will be more likely to evolve cooperative foraging than taxa which do not progressively provision their young.

Once cooperative foraging evolves, selection may favor larger groups and increased communication, particularly in environments that are unpredictable. In terms of foraging, the advantage of larger groups may not be greater per capita exploitation of resources but rather greater consistency in harvest of food resources. The CLT model of Wenzel and Pickering makes this point clearly (1991). As group size increases, other forms of social homeostasis must develop, such as a regulation of reproduction. Ant colonies exemplify this with seasonally-tuned production of workers and sexuals (Kwapich & Tschinkel, 2013). Viewing ant colonies as super-

organisms, it may be useful to judge many aspects of colony phenotype as homeostatic adaptations – including collective foraging behaviors.

While our study showed the general benefits of larger group size, we also found that smaller colonies outperformed large colonies on a per-capita basis when food was regularly distributed and easy to find. In a highly predictable environment, larger group size may be maladaptive or at least less beneficial – with more mouths to feed and less return on the investment. Thus, while our study emphasizes the advantages of large (and more coordinated) social groups, this advantage is dependent upon ecological circumstances. In many social taxa, stable group sizes are relatively small. The societies of many wasps and social bees may not need to be large or have sophisticated recruitment to address the challenges of survival in their particular environments. Our study lends support to the view that the group size and communication can best be viewed as context-dependent adaptations to ecological conditions and biological niche.

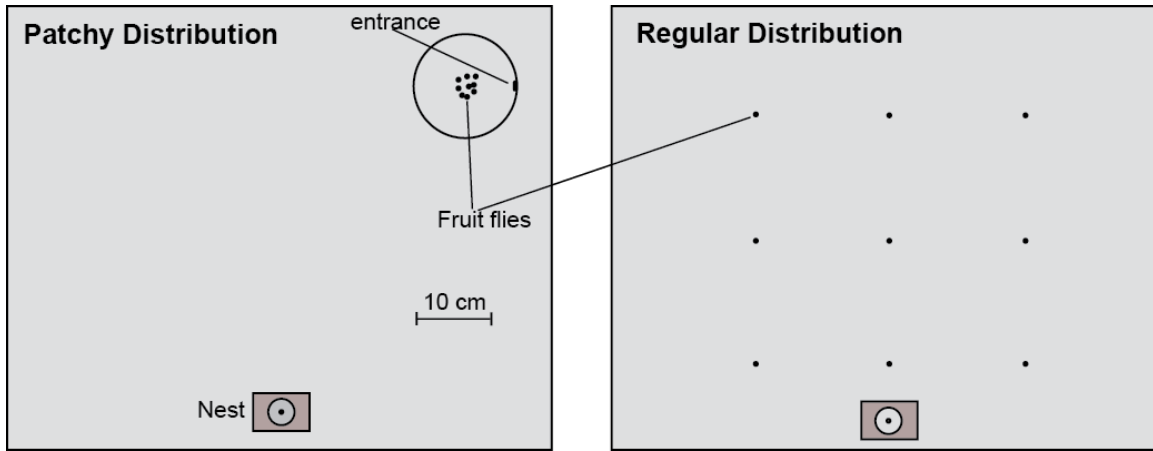


Figure 4.1. Experiment 1 design (foraging in the absence of recruitment communication): the food source was a loose pile of nine fruit flies inside a walled area (patchy distribution treatment) or the same number of fruit flies distributed in regular pattern (regular distribution treatment).

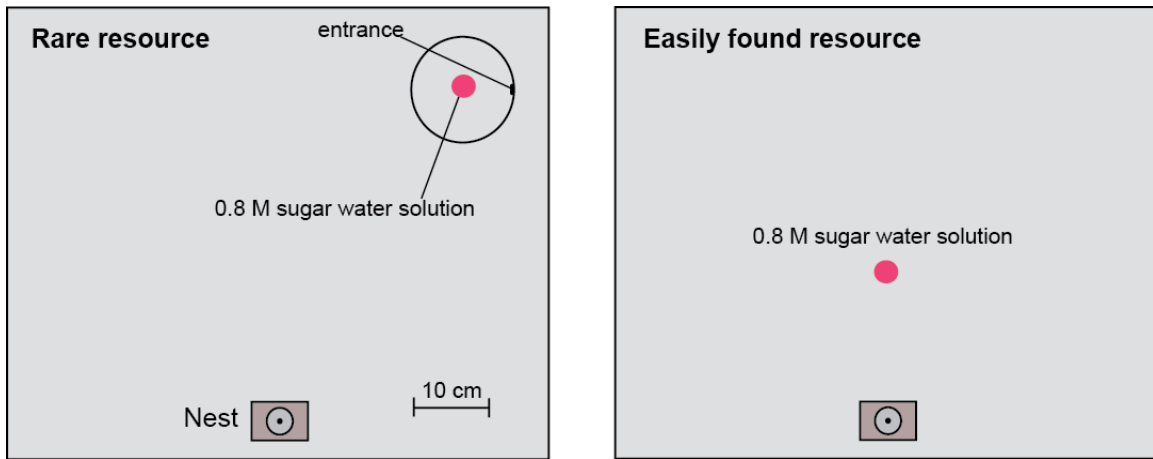


Figure 4.2. Experiment 2 design (foraging in the presence of recruitment communication): the food source was a droplet of 0.8 M sucrose solution inside a walled area (rare resource treatment) or a droplet of the same concentration 20 cm in front of the nest (easily found resource treatment).

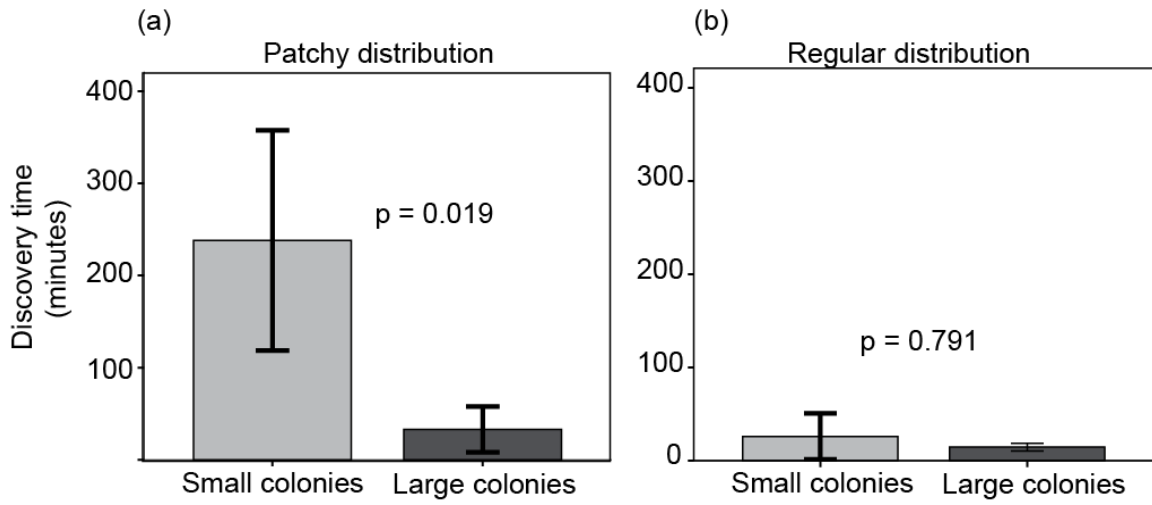


Figure 4.3. Initial discovery times (drosophila). Large colonies discover the first drosophila sooner than small colonies in the patchy distribution (a) but not in the regular distribution (b).

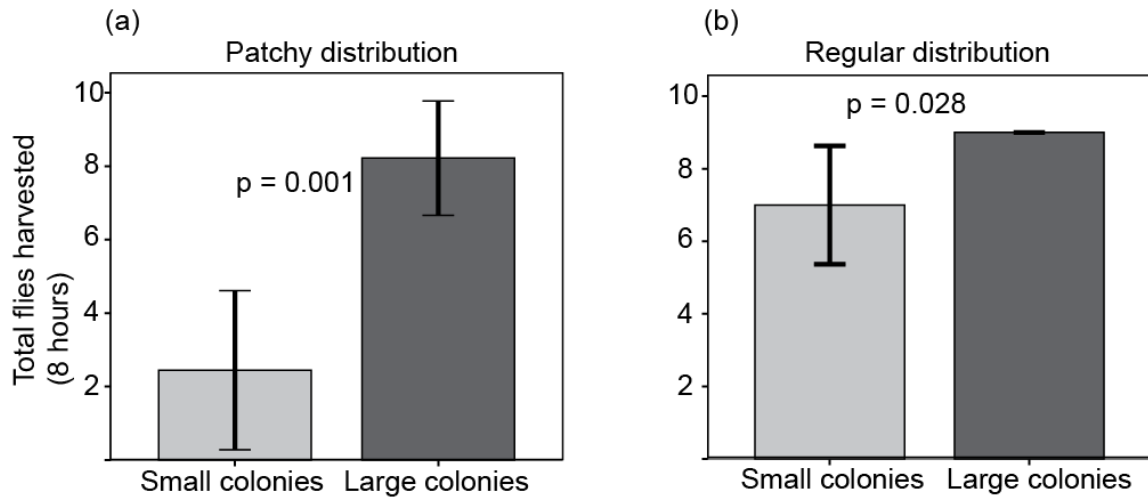


Figure 4.4. Large colonies harvest more flies than smaller colonies in both the patchy (a) and regular (b) distributions.

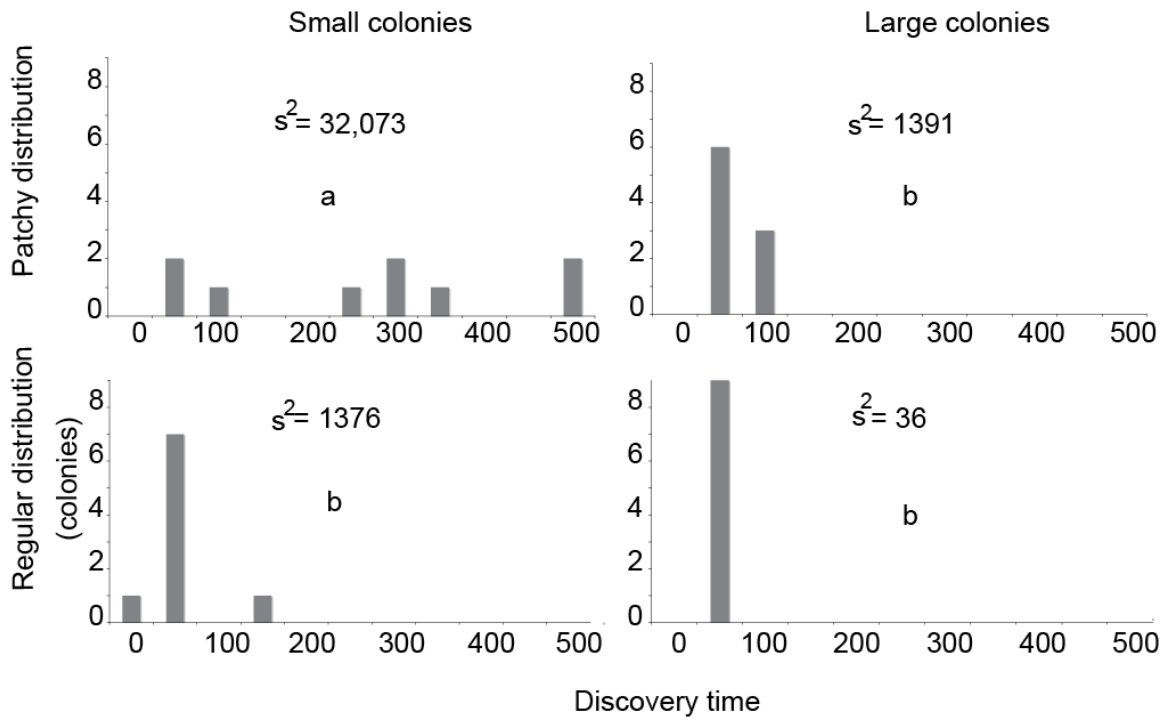


Figure 4.5. Resource distribution and colony size influence the variance of fruit fly 1st discovery times. The letters, **a** or **b** indicate Levene's test outcomes for differences in variance.

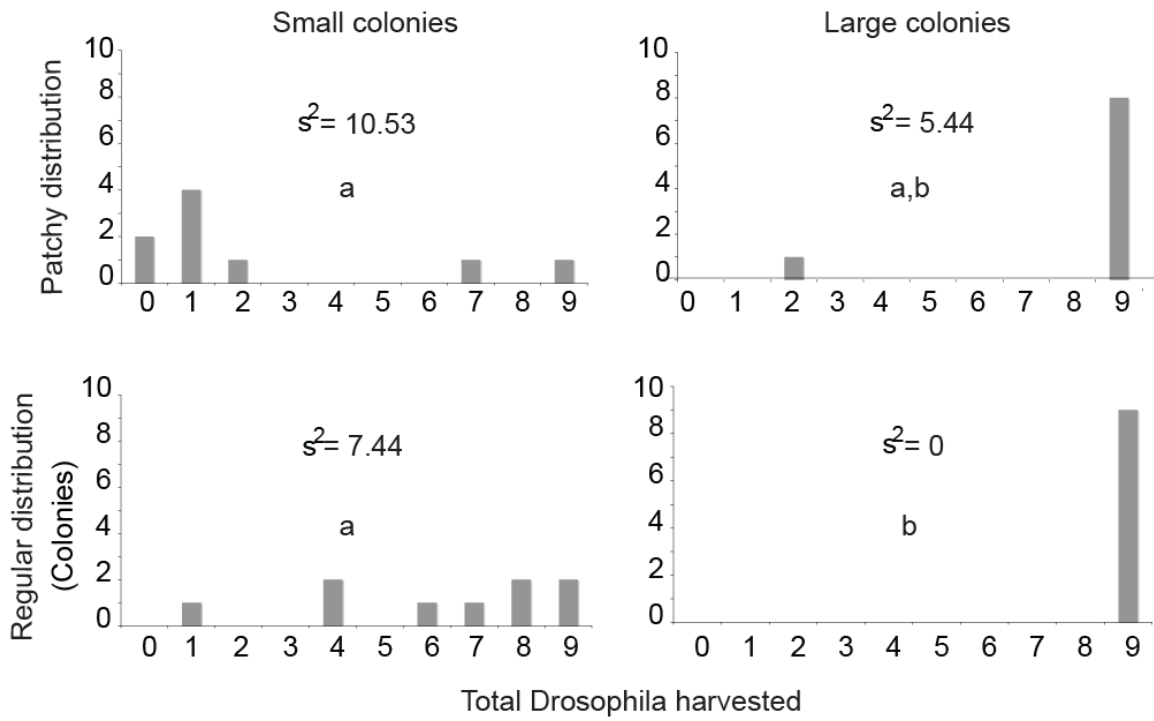


Figure 4.6. Resource distribution and colony size influence the variance of total fruit fly harvest.

The letters **a** or **b** represent Levene's test outcomes comparing variance.

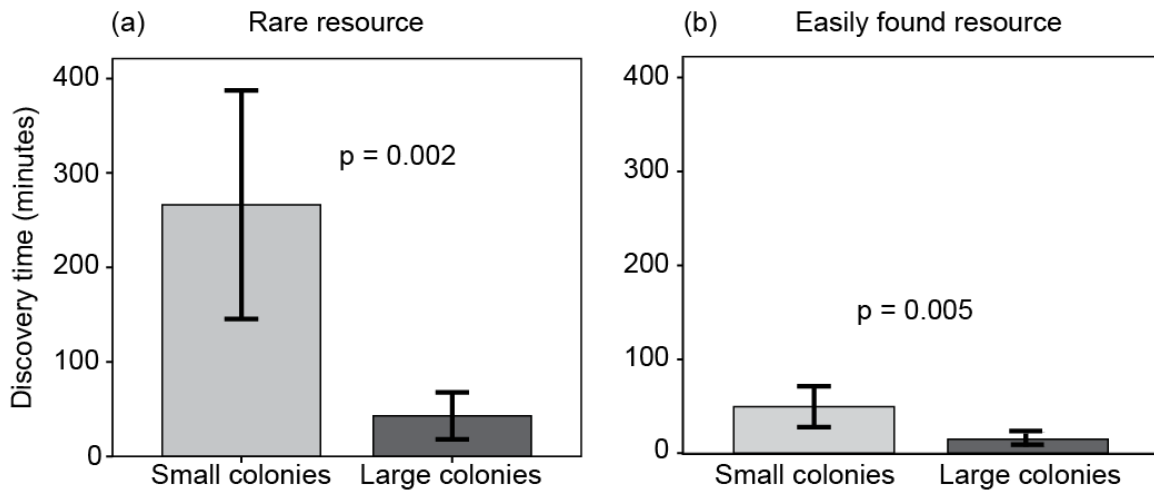


Figure 4.7. Large colonies discover the sugar water feeder sooner than small colonies in both treatments: (a) when the resource is rare (difficult to find) and (b) when the resource is easily found.

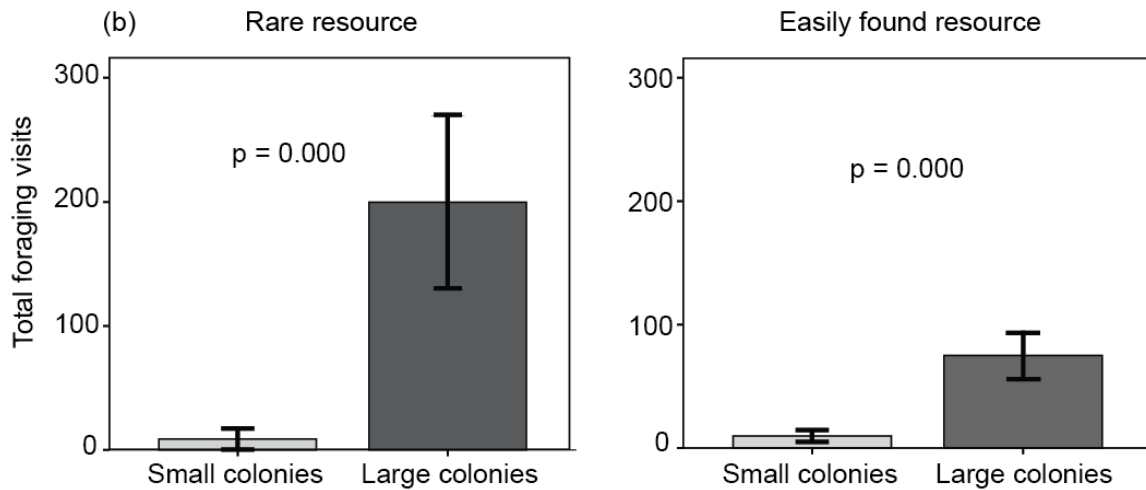


Figure 4.8. Large colonies showed greater total exploitation of the sugar water feeder in both treatments: in (a) the patchy distribution and in (b) the regular distribution. It should be noted that a different cohort of colonies was used between treatments. The large colonies in the second cohort were smaller in average size than the large colonies in the first cohort and this may have influenced total exploitation.

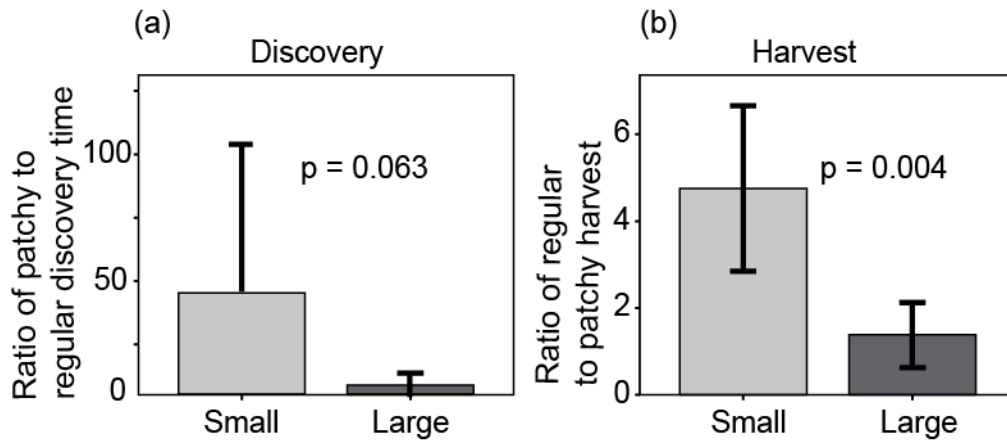


Figure 4.9. In the absence of communication, resource distribution has a greater impact on the foraging success of small colonies than that of large colonies. The plot on the left (a) shows the ratio of initial discovery times of drosophila for colonies (patchy: regular) within a size class. The plot on the right (b) shows the ratio of total harvest of drosophila (regular: patchy) within a size class. Compared to themselves, small colonies experience a greater shift in discovery time and harvest than large colonies between environments.

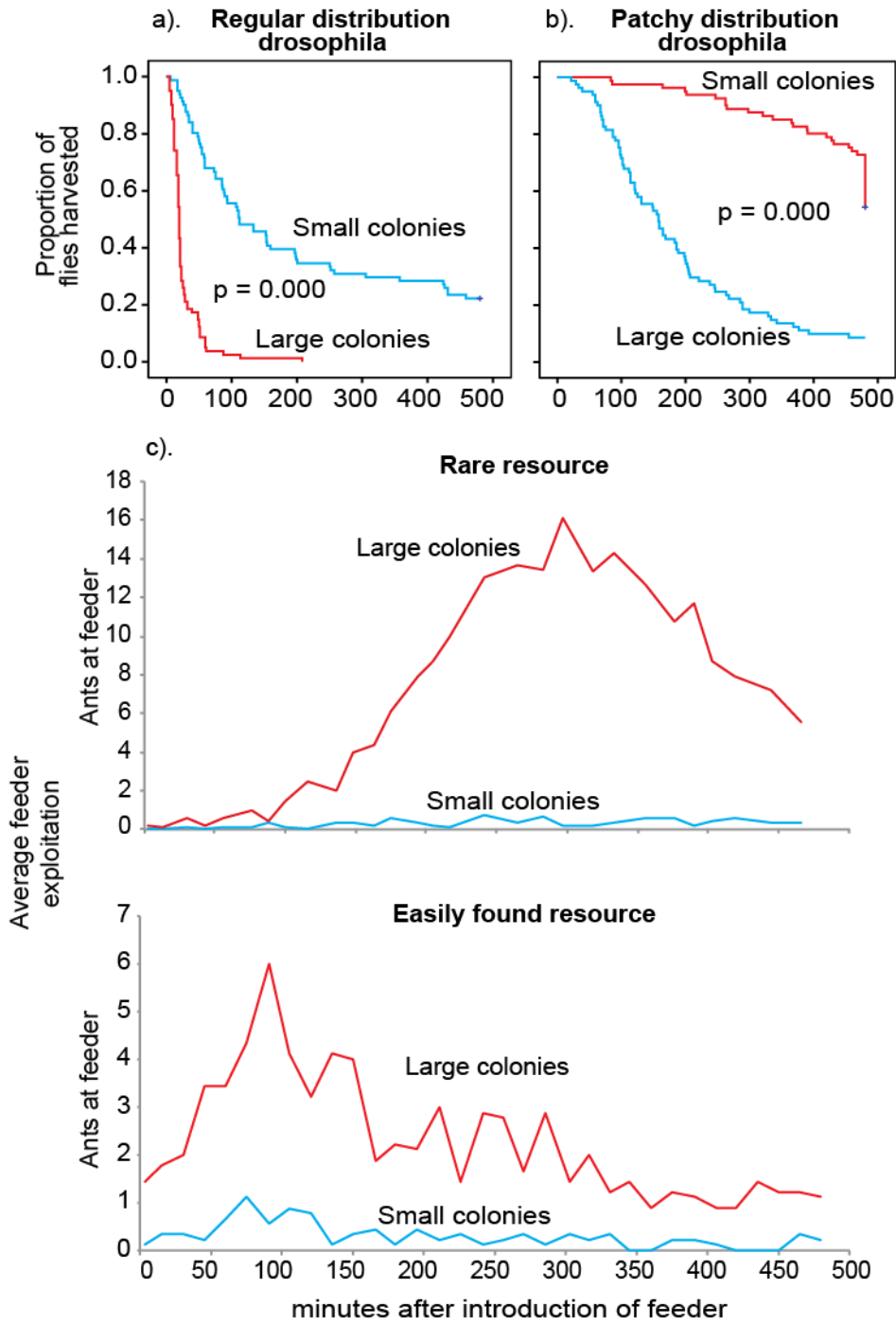


Figure 4.10. Large colonies have an increased rate of harvest relative to small colonies in all treatments: a) Survival analysis showing the relative harvest rate between large and small colonies when presented with a regular distribution of fruit flies. b). Survival analysis showing the rates of harvest when colonies were presented with a patchy distribution of fruit flies, and c)

average exploitation over time of a rare (c) and easily discovered (d) 0.8 M sucrose solution feeder.

CHAPTER 5

CONCLUSION

This dissertation investigated proximate and ultimate questions surrounding the organization of social foraging in *Temnothorax* ants. At the proximate level, I documented the collective foraging behavior of colonies and investigated how this group phenotype can emerge from the actions of individual ants. Building and validating a model of collective choice, I found that group attributes of allocation and flexibility could be predicted on the basis of the mathematically linear form of recruitment (the tandem run) and two individual behaviors: quality-based recruitment and quality-based attrition (Appendix A). This work was the first thorough investigation of the consequences of linear positive feedback for group behavior and validated certain theoretical predictions of the non-linearity hypothesis, an idea originally proposed by Jean-Louis Deneubourg and colleagues (Detrain & Deneubourg, 2008; D. Sumpter, 2010). My studies confirmed that a purely emergent framework for collective choice is used by the small societies of *Temnothorax* ants during social foraging (Chapter 2). While some individual ants had the opportunity to make direct comparisons of feeder quality – they did not use this potential information to alter the colony-level foraging response. Instead (and similar to the process that occurs in the large societies of honeybees and mass-recruiting ants), colonies make collective foraging choices through a form of parallel processing where colony choice is an emergent property and the result of differential rates of flow resulting from quality-based changes in behavior at the level of individual ants. I next tested the responsiveness of colonies and individuals to changes in nutritional state (Chapter 3). In starved colonies, there was an increase in exploration behavior, exploitation, and recruitment. By observing individually paint-marked foragers, I found that recruitment and exploitation behavior correlated with the reception foragers receive in the nest. Thus, similar again to a process documented in honeybees, *Temnothorax* ants appear to modulate their foraging behavior in response to both environmental and social information (Lindauer, 1949; Seeley, 1989; 1995). Finally, I investigated ultimate questions regarding the utility of group size and communication in different ecological contexts (Chapter 4). Larger colonies showed increased consistency and a greater rate of harvest than smaller colonies, but this advantage was mediated by the distribution

of resources. While patchy or rare food sources exaggerated the relative success of large colonies, regularly (or easily found) distributions leveled the playing field for smaller colonies. Large colonies made greater use of recruitment communication, and this was a particular advantage when resources were difficult to find.

The study of foraging communication in the social insects (and especially the ants) has a long history. Many folk sources (including the Bible) make reference to the behavior of ants (whose foraging trails are an everyday and yet charismatic phenomenon). The modern science of myrmecology (including the investigation of foraging) has its roots in the natural philosophers (most of them amateurs) in the late 1800's. John Lubbock (The Baron Avebury) showed that ants make use of odor trails in their social foraging (Lubbock, 1888). Eric Wasmann proposed a 'sensile feeler language' by which ants communicated generally (including recognizing nestmates and in foraging) (Wasmann, 1905). At the time such ideas might have seemed outlandish, but Karl von Frisch's landmark description of the honeybee dance language validated a view that social insects use a chemical and tactile language when communicating the location of food resources (Frisch, 1967).

Many later innovations in the study of foraging communication were anticipated by the work of EO Wilson in the early 1960's studying fire ants (Wilson, 1962a; 1962b). Wilson showed that ant pheromone trails provide modulated positive feedback and organized foraging at the colony level. This work preceded the field of self-organization that would develop two decades later. With an emphasis on mathematical modeling and computer simulation, the field of self-organization took the concept of the ant pheromone trail and used it as a guiding metaphor for the new 'complexity' paradigm. In this view, the pheromone trail epitomized the larger concept of biological complexity where a global behavior or phenotype emerges from the local interactions of simple agents. This new paradigm took a reductionist approach that perhaps undervalued the complicated details of living insect behavior. At the same time, another tradition in social insect foraging also followed Wilson's lead (helped by the expanding field of chemical ecology). In this tradition, the

mechanisms of communication were carefully explored: the actions, glands, and exact chemical compounds responsible for social insect behavior. This tradition grew out of the field of ethology and emphasized proximate detail – with a greater respect for the nuanced details of social insect communication (Hölldobler & Wilson, 1990).

Now, in the early 20th century, the complexity paradigm (and related concept of self-organization) has become, arguably, the dominant paradigm in the sciences (Wolfram, 2002). The two strands of research in social insect foraging communication (the self-organization and mechanistic perspectives) are reintegrating. Modelers are rediscovering the value of social insect behavioral complexity and signal diversity as they attempt to make sense of the collective behavior of colonies (Seeley, Visscher, Schlegel, & Hogan, 2012). For example, *Temnothorax* ants have long been an inspiration for modelers of self-organization (particularly in regard to house-hunting). Recent evidence (from both an emergent and mechanistic perspective) points to the importance of context-dependent nature of alarm signals in *Temnothorax* ants (Robinson, Jackson, Holcombe, & Ratnieks, 2005) (Takao Sasaki, personal communication). The field of self-organization will need to expand to take into account both signal diversity and the sophistication of individual social insects. It is my hope that this work respects both of these traditions from the history of the study of social insect foraging communication.

The goal of this thesis was to gain a better understanding of the proximate mechanisms and ultimate context shaping social foraging in *Temnothorax* ants. The title, ‘Wisdom of the acorn’ is intended as an homage to works such as Seeley’s ‘Wisdom of the Hive’ (1995) and Page’s ‘Spirit of the Hive’ (Page, 2013) which both hearken back to the earlier poetical works of amateur entomologists including Maeterlinck (Maeterlinck, 1906). *Temnothorax* ants are cavity dwellers and many species make their home in acorns or rock cavities. What are the lessons we can take from the rock-cavity dwellers? The main lesson is that, even while we should respect the potential for behavioral complexity in individual ants, these small societies rely on an emergent framework for making collective foraging decisions (similar to the larger societies of mass-recruiting ants and

honeybees). We learn from them the importance of the mode of communication (and the form of positive feedback) in shaping a group behavior. A clear understanding of their collective behavior can only come from an appreciation for the ultimate context in which that behavior occurs. Finally, we see in their social foraging a clear example of a super-organism at work: where individual foraging behavior is but one manifestation of the homeostatic processes in an integrated collective being. The real task of documenting the wisdom of the acorn is still a work in progress. When that book gets written, the story of *Temnothorax* social foraging will no doubt reflect lessons from similar systems (such as honeybees and mass-recruiting ants) but perhaps also provide inspiration for the larger task of understanding biological complexity more generally.

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APPENDIX A

LINEAR RECRUITMENT LEADS TO ALLOCATION AND FLEXIBILITY IN COLLECTIVE
FORAGING BY ANTS



Contents lists available at ScienceDirect

Animal Behaviour

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

Linear recruitment leads to allocation and flexibility in collective foraging by ants



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ARTICLE INFO

Article history:

Received 28 April 2013

Initial acceptance 6 June 2013

Final acceptance 20 August 2013

Available online 26 September 2013

MS. number: A13-00366

Keywords:

ant

collective foraging

communication

cooperation

decision making

linear recruitment

tandem running

Temnothorax rugatulus

Positive feedback in communication is central to the emergence of collective behaviour in animal groups. The nonlinearity hypothesis predicts that group behaviour will be defined in large part by the degree of cooperative interaction in this feedback. Strong interactions mean that the effectiveness of communication grows nonlinearly with signal quantity, leading to robust spatial patterning and consensus decision making. These predictions have been supported in many experimental systems, but the corresponding behaviour of linear systems, where signal effectiveness is proportional to signal quantity, has not been well explored. We examined the consequences of tandem running, a linear method of recruitment, for collective decision making by foraging colonies of the ant *Temnothorax rugatulus*. We found that colonies collectively chose the better of two unequal feeders, but they allocated foragers equally when feeders were identical. This result accords with theoretical predictions and contrasts with symmetry breaking seen in species with highly nonlinear trail pheromone recruitment. Colonies were also able to reallocate foragers when the quality of two unequal feeders was switched, again in accord with theoretical expectations. We built a model based upon the behaviour of individual ants and found that colony-level qualities of choice, allocation and flexibility can be predicted by two behaviours: quality-dependent linear recruitment and quality-dependent attrition. These experiments are the first thorough investigation of the consequences of linear recruitment for collective animal behaviour.

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The collective behaviour of insect societies is a leading example of emergence in the biological sciences. Without leaders or central control, colonies build complex nest structures, regulate their internal environment, allocate workers across multiple tasks and respond adaptively to environmental changes (Seeley 1995; Hölldobler & Wilson 2008). The underlying mechanisms are not well understood, but some insights have been gained for collective decision making, when colonies choose the best among several nest sites or food sources. These decisions are not merely summations of individual choices, but complex outcomes of interactions among colony members, none of which possess global information about every option (Seeley 1986; Bonabeau et al. 1997; Sumpter 2010).

The central mechanism for decision making is positive feedback through recruitment (Detrain & Deneubourg 2008). Recruitment includes any behaviour by which a worker summons nestmates to a place of interest, such as a food source or nest site. It takes a variety of forms across the social insects, but one of the best-studied

examples is mass recruitment by pheromone trails (Hölldobler & Wilson 2008). When a *Lasius niger* colony is presented with two unequal sources, scouts that find the better one have a higher likelihood of laying a trail than do scouts that find the weaker one (Beckers et al. 1993). Recruits further reinforce the trails, also with a quality-dependent probability. Over time this differential positive feedback generates more effective trails, and stronger exploitation, at the better source (Beckers et al. 1990, 1993).

While positive feedback can yield differences in exploitation, it does not necessarily result in true consensus, with all individuals choosing a single source. Consensus is vividly illustrated when a colony is given a choice between two identical options. Rather than exploit both equally, mass-recruiting ants tend to concentrate all of their foragers on only one of them, a phenomenon known as symmetry breaking (Beckers et al. 1990; Sumpter & Beekman 2003). The 'nonlinearity hypothesis' suggests that this outcome requires not only positive feedback but also a strongly nonlinear relationship between the effectiveness of recruitment and the number of recruiters (Detrain & Deneubourg 2008; Sumpter 2010). The idea can be represented in a simple model of an ant choosing between two trails to food sources A and B. The probability that she chooses the trail to A (P_A) is expressed as follows:

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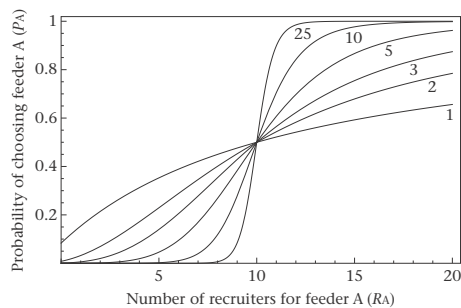


Figure 1. The effect of nonlinear recruitment on individual decision making between two options A and B, illustrated with plots of equation (1). Each plot gives the probability of an individual choosing A as a function of R_A , the number of nestmates already recruiting to A. In all plots $R_B = 20$ and $k = 1$, but each plot has a different value of the exponent n (shown under each plot). For low values, the relationship is nearly linear. Higher values yield an increasingly step-like function, leading to a clear preference for the option with the greater number of recruiters, even when the difference in recruitment is very small.

$$P_A = \frac{(k + R_A)^n}{(k + R_A)^n + (k + R_B)^n} \quad (1)$$

where R_A and R_B are the number of individuals recruiting to feeders A and B, respectively. The parameter n determines how steeply (or nonlinearly) recruitment effectiveness rises with recruiter number, while k determines the attractiveness of an option in the absence of any trail (Fig. 1). For sufficiently large n (roughly 1 or greater), colonies are predicted to make consensus choices even when presented with identical options. This is because a strongly nonlinear relationship magnifies small chance differences in exploitation, driving the ants toward exclusive use of only one option. Thus, even a tiny difference in pheromone concentration on competing trails leads to a clear preference for the stronger trail, at least for colonies of sufficient size (Beekman et al. 2001). Mass recruitment by ants exemplifies this consensus-building process, but many other examples are found in a variety of social animals including humans and bacteria (Saffre et al. 1999; Crespi 2001; Jeanson et al. 2005; Ame et al. 2006; Visscher & Seeley 2007; Ward et al. 2008; Clotuche et al. 2011; Kameda et al. 2012). The specific behavioural mechanisms vary widely, but they all have one feature in common: as the number of individuals supporting a given option increases, their per capita effectiveness at drawing new adherents grows.

Besides its role in consensus formation, nonlinear recruitment is also expected to yield some loss of decision-making flexibility. For mass-recruiting ants, models predict that a colony exploiting a food source will have difficulty switching to a better source that becomes available later (Detrain & Deneubourg 2008; Sumpter 2010). This is because positive feedback from the established trail overwhelms any nascent trail laid by discoverers of the better source. Experimental evidence supports this prediction in mass-recruiting ants (Beckers et al. 1990) and stingless bees (Schmidt et al. 2006).

Not all recruitment is nonlinear, and very different outcomes are predicted for colonies using linear methods (Sumpter & Beekman 2003; Detrain & Deneubourg 2008; Sumpter 2010; Lanan et al. 2012). For example, honeybees recruit using the waggle dance, in which a successful forager advertises a food source using a series of dance circuits, with the number of circuits encoding food quality

(Seeley 1986; Couvillon 2012). Waggle dancing is linear because the effectiveness of an additional circuit is the same regardless of how many dancers are active (Seeley et al. 1991). A potential recruit attends to a single dance at a time, and other dances do not reinforce the recruitment signal (Seeley & Towne 1992). With linear recruitment, models predict that colonies offered two sources of different quality exploit both, but allocate more foragers to the better one (Camazine & Sneyd 1991). If the sources are identical, colonies are not expected to reach consensus, but instead to divide their foraging effort equally (Camazine & Sneyd 1991; Sumpter & Beekman 2003; Lanan et al. 2012). Finally, these colonies should flexibly redistribute foraging effort in response to changes in resource quality. Linear recruitment has not received much study, but the limited evidence is consistent with these predictions. Honeybee colonies allocate foragers according to feeder quality, and they quickly reallocate effort when relative feeder quality is altered (Seeley et al. 1991; Granovskiy et al. 2012). However, the most revealing test of the nonlinearity hypothesis (i.e. whether groups show symmetry breaking when given two equal options) has not been carried out with honeybees or any other species showing linear recruitment.

This study investigates the collective consequences of linear recruitment in an exceptionally tractable system: the ant *Temnothorax rugatulus*. *Temnothorax* is a Holarctic genus whose small colony size and robustness to laboratory conditions has made it a model system for collective decision making, especially nest site selection (Franks et al. 2002; Pratt et al. 2002). Colonies typically live in rock crevices, hollow nuts or other pre-formed cavities, and are adept at choosing the better of two or more sites, distinguishing them on the basis of cavity volume, interior dimness, entrance size and other features (Franks et al. 2003; Visscher & Seeley 2007). Decisions depend on active scouts that find and assess candidate sites, and then recruit to them with a probability that depends on site quality (Mallon et al. 2001). Recruitment is not by pheromone trails, but instead by tandem runs, in which a leader ant releasing an ephemeral pheromone directly guides a nestmate to the candidate site (Moglich et al. 1974; Franks & Richardson 2006; Franklin et al. 2011). Like the honeybee waggle dance, the effectiveness of tandem runs is linear in recruitment effort: as long as there is a pool of potential recruits at the old nest, each additional tandem run is expected to increase the arrival rate of new ants by the same amount (Pratt et al. 2002).

Temnothorax also use tandem runs when foraging, and this is the context that we examine here. Although their foraging behaviour in nature has only begun to be documented (Bengston & Dornhaus 2013), laboratory studies show that they recruit to sugary liquids (Franks & Richardson 2006), which they exploit and share through complex food exchange networks (Sendova-Franks et al. 2010). Unlike nest site selection, where tandem running is combined with a nonlinear quorum rule (Pratt et al. 2002), the simpler process of foraging appears to rely purely on tandem runs. This makes it a suitable context in which to test the effects of linear recruitment on collective decision making. Our approach was to present *T. rugatulus* colonies with three basic decision challenges as follows. (1) Can they discriminate between sucrose feeders of different concentration, allocating more foraging effort to the stronger feeder? (2) When given identical feeders, do colonies reach consensus on a single one, or do they instead allocate foragers equally to both? (3) If relative feeder quality is changed midway through a foraging bout, can colonies flexibly alter their foraging effort to track the better source? To link these colony-level properties to individual recruitment actions, we also developed a simple model of forager behaviour and tested whether it predicted the patterns of choice, allocation and flexibility demonstrated in our experiments.

METHODS

Experimental Subjects

Colonies of *T. rugatulus* were collected from rock crevice nests in the Pinal Mountains near Globe, Arizona, U.S.A. (33°19.00'N, 110°52.561'W) between April and June 2010. In the laboratory each colony was housed in an artificial nest made from a 2.4 mm thick balsa wood slat with a rectangular central cavity measuring 3.8 × 6.4 cm. The slat was sandwiched between 50 × 75 mm glass microscope slides that served as floor and ceiling. A 3 mm wide entrance was cut through the side of the slat. Each nest was placed in an 11 × 11 cm plastic box and kept in a Sheldon diurnal incubator on a 16:8 h day:night schedule, with a daytime temperature of 23 °C and a nighttime temperature of 15 °C. Colonies were provided weekly with water and an agar-based diet (Bhatkar & Whitcomb 1970). On completion of the study, colonies were maintained in similar conditions for use in other behavioural experiments. Colonies typically decline after 1–2 years and are then killed by placing them in a –20 °C freezer. No permits or licenses were required for this study.

Choosing between Unequal Feeders

In this experiment colonies were presented with two sucrose solution feeders of unequal quality. The 'good' (0.8 M) and the 'poor' (0.1 M) sources consisted of 0.1 ml droplets on the arena floor. Twelve replicates were run simultaneously, with observers rotating between arenas to collect data at approximately 5 min intervals for 2 h. At each observation we recorded recruitment (the number of tandem runs being led in the direction of each feeder) and exploitation (the number of ants drinking from each droplet). After the experiment, we calculated foraging effort in two ways: (1) we took the average number of exploiters at each feeder over all observations; (2) we took the number of exploiters at each feeder at the point of maximum exploitation (i.e. when the sum of ants at both feeders had reached its greatest value). We also measured recruitment effort as the average number of tandem runs to each feeder over all observations. We watched each tandem run until we could make a reasonable conclusion about which feeder it was aiming for. Because the exact number of observations varied between experiments, we performed most analyses on average recruitment and exploitation (per observation) to allow comparison between experiments.

To ensure strong motivation to forage, ants were starved for 14 days prior to the experiment. Colonies were placed into arenas (75 × 60 cm) 24 h before the start of the experiment. Feeders were placed 30 cm from the nest and 40 cm from each other. They were replenished once every 30 min with an additional 0.1 ml of sugar water. All colonies contained brood, and average colony population was 382 ± 40 (this and all other measures of variability are given as standard errors). This experiment was performed on 15 September 2010.

Choosing between Equal Feeders

In this experiment colonies were given a choice between two feeders of equal quality (0.8 M sucrose). Methods were otherwise the same as in the unequal choice experiment described above. We tested 14 colonies with an average population of 401 ± 46; all colonies contained brood. This experiment was performed on 29 August 2010.

Responding to a Switch in Feeder Quality

In these trials, colonies were initially presented with one poor and one good feeder (0.1 M and 0.8 M). Colonies were observed for

just under 1 h, and then the quality of the two feeders was switched. To facilitate this change, the droplets were placed into depression well slides rather than directly on the arena floor. At the switch, each old slide was removed and immediately replaced with a fresh slide containing the new solution. The switch was made 50–57 min after initial introduction of the feeders. Average and peak exploitation, as well as average recruitment, were calculated both before and after the switch. All other details were the same as in the prior experiments. We tested 20 colonies with an average population size of 311 ± 33 individuals. This experiment was performed over 2 days (10 colonies per day) on 20 October and 22 October 2010.

Individual Behavioural Rules

To determine the individual behaviour leading to a colony-level decision, and to estimate parameter values for a mathematical model of the process, we replicated the unequal choice experiment with marked colonies of ants. In each of four colonies, every worker received a unique combination of paint dots on the head, thorax and abdomen using Pactra R/C Car Lacquer paint. With separate cameras trained on the nest, each feeder and the entire arena, the colonies were recorded for 4 h as they foraged.

From these videos, three behaviours were quantified: food discovery, recruitment and attrition. For discovery, we determined the number and identity of all ants that exploited any feeder at any time during the experiment. The independent discovery rate α was estimated via survival analysis of the discovery times of each of these active foragers. Specifically, we fitted an exponential distribution to the latencies between the start of the experiment and each forager's first appearance at a feeder, and estimated α as the parameter of this distribution. We restricted this analysis to the first 15 min of the experiment, before any tandem runs were observed, so that we could be certain that recruitment did not influence the rate of discovery. If a forager found neither feeder in this period, she was assigned a latency of 15 min and treated as censored data. We first calculated α separately for each feeder, but found no effect of feeder quality. We therefore estimated a common value for both feeders.

Attrition was defined as the rate λ_i at which exploiters of feeder i abandoned it. We used survival analysis to fit an exponential distribution to the intervals between each ant's discovery of a feeder and her abandonment of it. We did this separately for the good and poor feeders and estimated λ_{good} and λ_{poor} as the parameters of the fitted distributions. An ant was considered to have abandoned a feeder if she went back to the nest and never returned to that feeder, or if she switched to exploiting the other feeder. To minimize effects of colony satiation on attrition, data were analysed for only the first 60 min of the experiment. If an ant was still exploiting a feeder at the 60 min mark, she was treated as censored data and assigned an interval of 60 min minus her discovery time.

Recruitment to feeder i was quantified by the rate β_i at which ants exploiting the feeder led successful tandem runs to it. A run was deemed successful if the follower reached the feeder within 5 min of the run's breakup. To minimize effects of colony satiation, we analysed only the first 60 min of each trial. For each feeder, we calculated β as the total number of successful tandem runs to that feeder divided by the total number of ant-minutes of exploitation at that feeder. The latter was calculated by summing the total exploitation time of each ant that foraged at the feeder during the 60 min interval. Estimates of β and the other parameters were calculated using the statistical computing environment R (R Foundation for Statistical Computing, Vienna, Austria).

Only a fraction of each colony's workers forage. To estimate this fraction, we counted the number of workers that exploited a feeder

at least once in the first 2 h after introduction of sugar water. We then counted the entire colony population with the aid of still images from video recordings of the nest interior. The results showed that an average of $24 \pm 8\%$ of the total worker population foraged.

RESULTS AND MODEL

Choosing between Unequal Feeders

For 12 colonies presented with unequal feeders, the better feeder had a significantly higher average number of feeding visits per observation (Wilcoxon signed-ranks test: $Z = -3.1$, $N = 12$, $P = 0.002$; Fig. 2a). This preferential exploitation was mirrored by a greater average recruitment effort to the better feeder ($Z = -2.9$, $N = 12$, $P = 0.003$; Fig. 2b). At peak exploitation, most colonies allocated the majority of foragers to the better feeder (Fig. 2c).

Choosing between Equal Feeders

When 14 colonies were presented with two equal feeders (0.8 M sucrose) there was no significant difference in average number of feeders per observation (Wilcoxon signed-ranks test: $Z = -1.7$, $N = 14$, $P = 0.084$; Fig. 3a) or average recruitment per observation ($Z = -1.8$, $N = 14$, $P = 0.065$; Fig. 3b). At peak feeding, colonies showed equal allocation to both feeders, with few colonies making a clear choice in favour of one feeder (Fig. 3c).

Responding to a Switch in Feeder Quality

When 20 colonies were initially presented with unequal feeders they allocated more foragers to the better one, just as in the first experiment. Both the average number of ants feeding (Wilcoxon signed-ranks test: $Z = -2.240$, $N = 20$, $P = 0.025$; Fig. 4a) and the average recruitment effort ($Z = 3.052$, $N = 20$, $P = 0.001$; Fig. 4b) were significantly greater at the better feeder, and it had the larger number of foragers at peak exploitation (Fig. 4c). When feeder quality was switched at about the 50 min mark, recruitment and exploitation at the formerly good feeder initially remained high. Within 20–30 min, however, tandem runs faded there and began towards the other, now superior feeder (Fig. 5). Exploitation similarly shifted, so that the average number of ants feeding at the newly good feeder exceeded that at the newly poor one ($Z = -3.472$, $N = 20$, $P = 0.001$; Fig. 4d).

On the other hand, there was no significant difference in average recruitment effort between the two feeders after the switch (Wilcoxon signed-ranks test: $Z = -0.616$, $N = 20$, $P = 0.538$; Fig. 4e). This was due to the initial continuation of tandem runs to the

formerly good feeder, including runs that were initiated before the switch. These faded as their leaders arrived at the feeder and discovered its reduced quality. When we excluded observations during this 30 min transitional phase from the analysis, average recruitment in the remaining post-switch period was significantly greater to the newly good feeder than to the newly poor feeder ($Z = -3.000$, $N = 20$, $P = 0.003$). In spite of the lag in rerouting of recruitment, most colonies were ultimately able to allocate the majority of their foragers to the new location of the better feeder, as measured by the allocation of foragers at the time of peak feeding after the switch (Fig. 4f).

Model of Foraging Dynamics

How do colonies of *Temnothorax* achieve the decision making, allocation and flexibility demonstrated in our experiments? To answer this, we developed a simple mathematical model of a choice between two feeders, A and B. The model assumes three possible behavioural states for each ant: uncommitted, committed to feeder A, or committed to feeder B (Fig. 6). The model assumes that the numbers of ants committed to each feeder (X_A and X_B) depend on random discoveries, recruitment and attrition. The rates of change of X_A and X_B can be represented by the following ordinary differential equations:

$$\frac{dX_A}{dt} = \alpha(N - X_A - X_B) + \beta_A X_A(N - X_A - X_B) - \lambda_A X_A \quad (2)$$

$$\frac{dX_B}{dt} = \alpha(N - X_A - X_B) + \beta_B X_B(N - X_A - X_B) - \lambda_B X_B \quad (3)$$

This model has four parameters: N is the total number of potential foragers, α is the rate at which uncommitted ants independently discover a feeder, β_A and β_B are the recruitment rates to each feeder, and λ_A and λ_B are the attrition rates at each feeder. Ants that abandon a source become uncommitted and can make further discoveries or be recruited. From our data on individually marked ants, we estimated the following parameter values: α equals 0.0125 discoveries per ant per minute and does not depend on feeder quality, β equals 0.015 and 0.006 recruits per committed ant per minute for good and poor feeders, respectively; λ equals 0.009 and 0.038 for the good and poor feeders, respectively. We assumed N equals 100 foragers, based on a colony size of 400 ants.

The model was integrated numerically, using MatLab's ode45 function (MatLab, Mathworks, Natick, MA, U.S.A.), to describe the expected time course of feeder exploitation for the three experiments. For each experiment, the model reproduced the basic pattern seen in the data: clear preference for the better of two unequal feeders (Fig. 7), equal allocation to two equivalent feeders

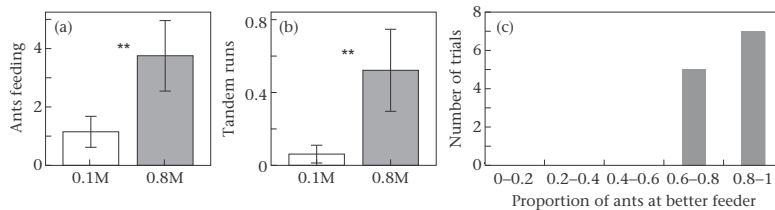


Figure 2. Collective choice by 12 ant colonies presented with unequal sucrose feeders. (a) Mean \pm SE number of ants feeding per observation at a 0.1 M feeder (white) and a 0.8 M feeder (grey). (b) Mean \pm SE number of tandem runs per observation. ** $P < 0.01$. (c) Forager distribution at peak feeding time, defined as the observation with the highest total number of foragers at both feeders. Bars show the number of colonies with a given proportion of foragers at the better feeder. The skewed distribution shows strong preference for the better feeder.

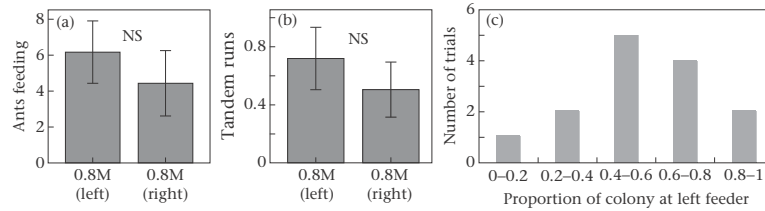


Figure 3. Collective choice by 14 ant colonies presented with identical 0.8 M sucrose feeders: (a) number of ants exploiting each feeder; (b) number of tandem runs to each feeder. (c) Forager distribution at the time of peak feeding. The single mode near 0.5 shows that colonies tended to allocate foragers equally between the feeders, rather than concentrating on only one of them.

(Fig. 8) and flexible reallocation when resources changed in quality (Fig. 9).

Because ordinary differential equation (ODE) models do not account for stochasticity and the discrete nature of individual ants, they might produce misleading results when forager number is low. To confirm the model's predictions, we therefore re-implemented it as an agent-based simulation, using the program NetLogo (Wilensky 1999). The model tracked 100 foragers, each of which could occupy the same three behavioural states used in the ODE. Transitions between states were governed by the same discovery, recruitment and attrition rules described above, implemented probabilistically at time steps representing 120 min. The results (not shown) closely matched those of the ODE model, except for the added noise expected in a stochastic simulation. The details and full implementation of this model are available in the OpenABM model library (<http://www.openabm.org/models>).

Recruitment Effort

The experimental results above showed more recruitment to good than to poor feeders, but they did not give actual numbers of tandem runs. This is because arenas were sampled intermittently, meaning that long runs might be counted more than once and short ones missed altogether. For accurate counts, we turned to the four

trials with marked colonies used to estimate model parameters. During the first 60 min after food discovery, scouts initiated an average of 10.0 ± 2.7 tandem runs to the better feeder and 1.5 ± 0.9 tandem runs to the poor feeder. Tandem runs were usually initiated near the nest entrance, where the would-be recruiter stood still, apparently releasing a pheromone signal that attracted one or more interested followers. Often several ants would jostle to follow this leader, so that several short tandem run events might occur in a brief period. Usually, only a single follower would persist, but leaders sometimes led two ants concurrently to a feeder. Many tandem runs broke up before reaching their destination, but their followers often found the feeder soon after on their own. We therefore counted a run as successful if the follower discovered the feeder within 5 min of breakup. In the first 60 min after food discovery, we saw an average of 5.0 ± 2.7 successful runs to the better feeder and only 0.5 ± 0.5 successful runs to the poor feeder. This difference matches the pattern seen in the main experiments using a cruder measure of recruitment effort.

DISCUSSION

We have shown three important features of collective foraging behaviour by *T. rugatulus* colonies. First, they are effective decision-makers, readily choosing the better of two sucrose feeders. Second,

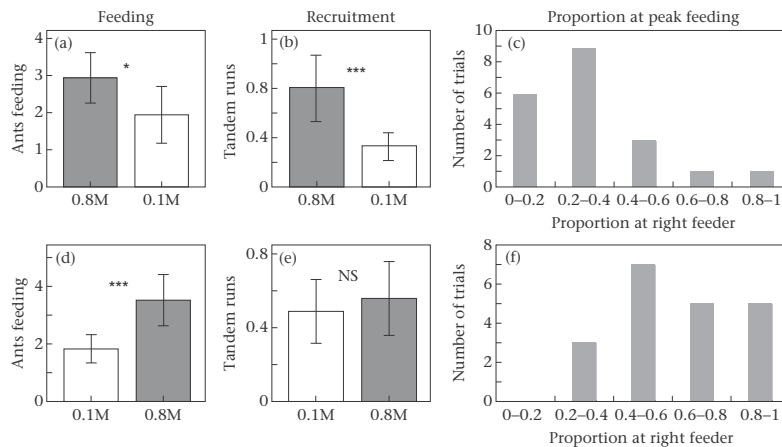


Figure 4. Response of 20 ant colonies to a change in relative quality of two feeders before and 60 min after the quality of the two feeders was switched, respectively: (a, d) numbers of ants feeding at each feeder; (b, e) number of tandem runs to each feeder; (c, f) forager distribution at the time of peak feeding. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

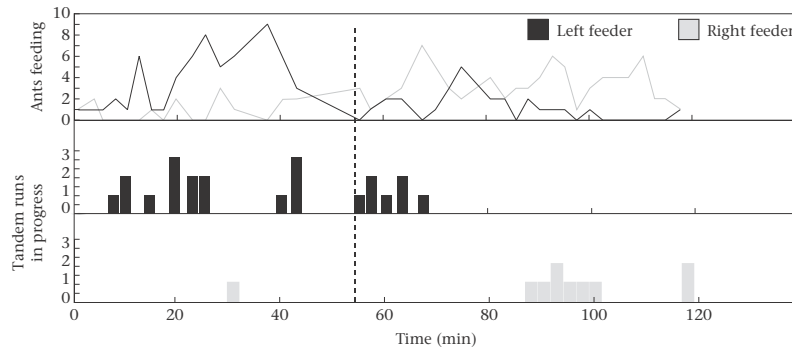


Figure 5. Detailed time course of feeder exploitation and recruitment by a single ant colony from the experiment presented in Fig. 4. The left feeder was initially better than the right one. When their quality was switched (dotted line), the relative number of ants exploiting each feeder was reversed. At first, tandem recruitment continued towards the left feeder, but was eventually replaced by recruitment to the newly superior right feeder.

they allocate foragers equally when presented with feeders of equal quality. Third, they flexibly reallocate exploitation following a change in feeder quality. Analysis of individual behaviour, combined with a mathematical model, shows that these features can be explained as an emergent property of simple individual rules. Foragers encounter feeders randomly and then make quality-dependent decisions about whether to exploit them and whether to recruit nestmates. By recruiting to good feeders with higher probability, and abandoning them with lower probability, they can collectively choose the better of two feeders, even without individual ants visiting both feeders and comparing them.

Our results confirm predictions of the nonlinearity hypothesis, which relates a group's collective behaviour to the mathematical relationship between recruitment effort and recruitment effectiveness. This hypothesis has yielded insights about the effects of highly nonlinear relationships, such as those found in mass

recruitment by pheromone trails (Beckers et al. 1990; Nicolis & Deneubourg 1999; Camazine et al. 2001; Detrain & Deneubourg 2008). Nonlinearity is expected to create consensus even when options do not vary, a prediction that has been confirmed many times (Beckers et al. 1990; Millor et al. 1999; Saffre et al. 1999; Sumpter & Beekman 2003). Our study expands this research by offering the first thorough analysis of a species with linear recruitment. Tandem runs summon recruits with an effectiveness that is directly proportional to recruitment effort. As a result, they should not create the strong information cascades that drive consensus in nonlinear systems. Our results confirm this prediction, finding equal allocation of foraging effort between equal options, rather than consensus on one. We further confirm two other predictions: (1) linear recruitment leads to differential exploitation of feeders of different quality, rather than exclusive focus on the better option; (2) linear recruitment allows colonies to track changes in relative option quality, rather than getting locked into an initial choice. The latter is a risk for species with highly nonlinear recruitment (Beckers et al. 1990; Detrain & Deneubourg 2008), although they may have other behavioural means to escape these traps (de Biseau et al. 1991; Dussutour et al. 2009a, b; Gruter et al. 2012; Latty & Beekman 2013).

Our results are consistent with previous work on honeybees, where waggle dance effectiveness also shows a linear relationship to recruitment effort (Seeley & Towne 1992; Sumpter 2010). Honeybees have not been subjected to a choice between two equal feeders, the most revealing test of the nonlinearity hypothesis. However, they do show both flexibility to changing conditions and forager allocation according to quality (Seeley et al. 1991; Seeley 1995). Both of these features have been argued to have adaptive value by allowing colonies to track patchy and ephemeral flower patches (Seeley 1995; Dornhaus & Chittka 2004; Donaldson-Matasci & Dornhaus 2012). The lack of strong consensus means that colonies can direct the bulk of their foragers to the best patches while continuing to monitor poorer ones. If these patches later improve, the colony can rapidly redirect foraging effort accordingly. Flower patches can have brief periods of maximum nectar productivity, making speedy reallocation particularly important to bees (Seeley 1985).

Temnothorax ants conceivably derive similar benefits, but too little is known of their foraging ecology to be sure. Alternatively, the emergent properties described above may not be selective drivers for the ants' reliance on linear recruitment. That is, they may use

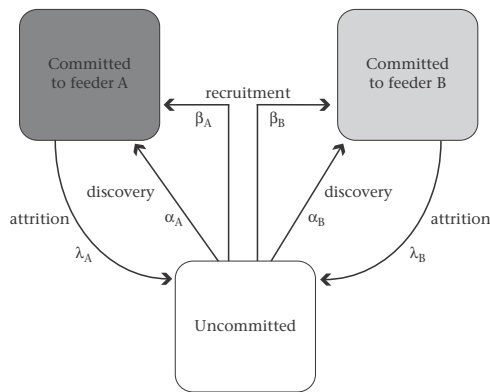


Figure 6. Model of ant foraging behaviour in the presence of two feeders. Boxes represent the three behavioural states that a forager can occupy: uncommitted, committed to feeder A, or committed to feeder B. Arrows indicate the ways that a forager can change state: uncommitted ants can discover a feeder at rate α , or be recruited by an already committed ant at rate β . Committed ants abandon their feeder at rate λ , returning to the uncommitted state. Both β and λ depend on feeder quality, but α does not.

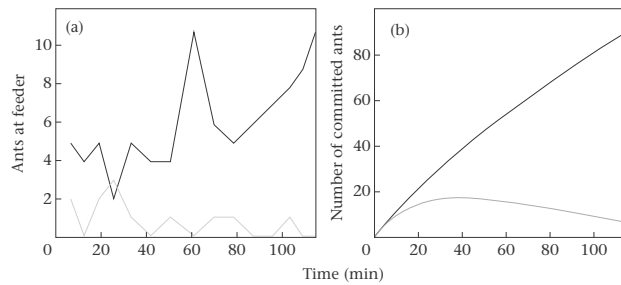


Figure 7. A simple behavioural model predicts colonies' choice of the better of two feeders. (a) Results for a single experimental colony presented with a good (0.8 M) and poor (0.1 M) sucrose feeder. Black and grey lines show numbers of ants feeding at the good and poor feeders, respectively. (b) An ordinary differential equation (ODE) implementation of the model in Fig. 6 predicted a similar predominance of exploitation at the better feeder. Absolute exploitation levels are higher for the model, because it measures the total number of ants committed to each feeder, while the data show only those ants that were currently at the feeder.

tandem runs not because of their linearity, but simply because they are an effective communication method for small colonies (Hölldobler & Wilson 2008; Dornhaus et al. 2012). Mass recruitment by pheromone trails can only be sustained with large populations (Beekman et al. 2001; Dornhaus et al. 2012), but *Temnothorax* colonies rarely number more than a few hundred workers, making tandem running a better way to share information about food sources. If tandem recruitment is primarily a result of small colony size, then the consequences of its linearity, including flexible reallocation of foragers, may be an epiphenomenon rather than an adaptation to unstable food resources.

Even for honeybees, allocation across multiple options is not always a desirable outcome. When a swarm chooses a site to build a new nest, consensus on a single option is vital to ensure an adequate workforce (Seeley 2010; Rangel & Seeley 2012). Like honeybees, *Temnothorax* are also cavity-nesters and face a similar need for consensus during house-hunting (Franks et al. 2002; Pratt et al. 2002; Visscher & Seeley 2007). Both groups have converged on a similar strategy to achieve consensus despite their linear recruitment: they add a nonlinear component in the form of a quorum rule (Pratt et al. 2002; Seeley & Visscher 2004). Scouts use waggle dances or tandem runs to attract more scouts to candidate sites. Once a site achieves a minimum number of adherents, its scouts switch to rapid movement of the passive bulk of the colony. This threshold effect mirrors the step-like growth in trail

attractiveness seen in mass-recruiting ants, and it has a similar consensus-building effect (Sumpter & Pratt 2009). The facultative use of a quorum may allow colonies to optimize their collective behaviour according to circumstances. Similar tunability is found in *L. niger*, where ants can adjust the degree of symmetry breaking by modulating the intensity of pheromone deposition (Portha et al. 2004).

The emergence of complex group patterns from nonlinear communication is one of the more revealing insights into collective behaviour of recent decades (Camazine et al. 2001; Sumpter 2010). A combination of theory and experiments has shown how nonlinear interactions between colony members are at the heart of self-organized phenomena including aggregation, collective decision making, nest construction and pattern formation (Camazine et al. 2001; Buhl et al. 2005; Jeanson et al. 2005; Detrain & Deneubourg 2008). Nonlinearity can generate rapid consensus on a single option, which may be advantageous when speed and unanimity are important, as in nest site selection by an exposed and vulnerable group. In other contexts, however, strong consensus may not be the most adaptive outcome. When, for example, a colony monitors many rapidly changing food sources, the greater flexibility and broader allocation given by linear interactions may be more beneficial. Even within the same society, linear and nonlinear recruitment can complement one another, each one adaptive in the appropriate circumstances.

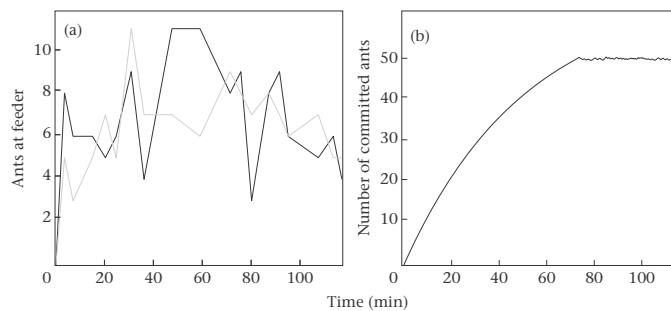


Figure 8. The behavioural model predicted equal forager allocation to feeders of equal quality. (a) Exploitation of two identical 0.8 M feeders by an experimental colony. (b) The ordinary differential equation (ODE) model predicted the observed pattern of equal exploitation (the two lines were identical and thus lie on top of one another).

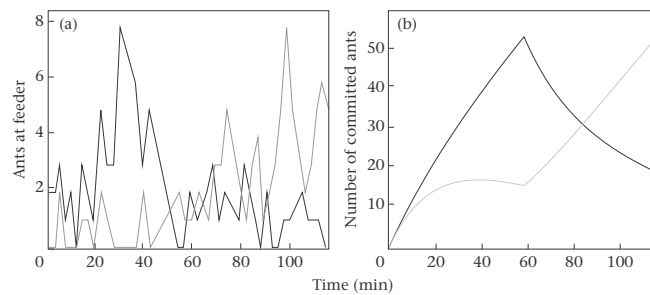


Figure 9. The behavioural model predicted the ability of ant colonies to track changes in resource quality. (a) Feeder exploitation by an experimental colony initially presented with a good feeder at the left (black line) and a poor one at the right (grey line). After the feeder qualities were reversed at 55 min, exploitation levels also reversed. (b) The ordinary differential equation (ODE) model predicted a similar switch.

Acknowledgments

This work was supported by the National Science Foundation (grants 1012029 and 0959514). We thank Gage Schaper and Savannah Christy for assistance in data collection, and Jean-Louis Deneubourg for valuable guidance on the nonlinearity hypothesis.

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APPENDIX B

NOTES ON THE NATURAL FORAGING HABITS OF TEMNOTHORAX ANTS

Colonies of *Temnothorax* ants mount a robust response (in terms of recruitment and exploitation) to concentrated solutions of sugar water (Franks & Richardson, 2006; Möglich et al., 1974; Shaffer et al., 2013). But what are the natural habits of *Temnothorax* ants? The discipline of behavioral ecology attempts to explain the behavior of animals within the natural context they live in: their ecology, evolutionary history, and natural habits. While my experiments detail the colony and individual-level exploitation of sugar water, the picture is incomplete without some evidence for the use of nectar as a food source in nature.

Records of the foraging behavior of *Temnothorax* ants (also formerly known as *Leptothorax*) are difficult to find. In part this is due to their tiny size and lack of conspicuous mass- recruitment (displayed by many other genera of ants). Accounts emphasize that they tend to be solitary foragers, scavenging for insect prey, and cryptic in their habits. William Morton Wheeler (writing more than a century ago) provided what can still be considered the most extensive record of the foraging habits of Leptothoracine ants (Wheeler, 1910). In his 1910 book, 'Ants Their Structure, Development, and Behavior' (page 341) he writes, 'Some ants (*Leptothorax* sp.) obtain the honey-dew merely by licking the surface of leaves and stems on which it has fallen...' In the same book, Wheeler also describes a remarkable source of carbohydrate nutriment obtained by a particular species of Leptothoracine, *Leptothorax emersoni*. This species, a boreal ant, described in mountains of New England lives in xenobiotic (commensal) relationship with another ant species, *Myrmica canadensis* (a variety of *M. brevinodis*). Wheeler describes their relationship as such, '*The Leptothorax, though consorting freely with the Myrmica workers in their galleries, resents any intrusion of these ants into its own chambers. Theinquilines do not leave the nest to forage but obtain all their food, in a very interesting manner, from their hosts. Both in the natural and artificial nests the Leptothorax are seen to mount the backs of Myrmicas and to lick or shampoo their surfaces with a kind of feverish excitement. This shampooing has a two-fold object: to obtain the oleaginous salivary excretion with which the Myrmicas cover their bodies when they clean one another, and to induce these ants to regurgitate the liquid food stored in their crops. The Leptothorax devote most of their time to licking the heads and clypei of their nest mates, stopping from time to time to imbibe the liquid food from their lips. Whenever the Myrmica*

workers return to the nest after visiting the aphids on neighboring plants, they are intercepted by the Leptothorax and compelled to pay toll in this comical manner. (pp 434 – 435). This behavior was also documented by Henry C. McCook in his book, 'Ant Communities And How They Are Governed: A Study in Natural Civics' (pp 250 – 253) as well as several other natural history accounts of the era (McCook, 1909). More recently, 'A Field Guide to the Ants of New England' described the foraging habits of the species, *Temnothorax ambiguus* as: 'Feeds on Honey dew, plant nectar, tiny insects...' (p. 326) (Ellison, Gotelli, Farnsworth, & Alpert, 2012). Another recent study suggested that *Temnothorax* 'ants forage in the leaf litter presumably for small arthropod prey' (Bengston & Dornhaus, 2012). Like the ants themselves foraging in nature, accounts of *Temnothorax* exploitation of honey dew is cryptic in the literature. Unlike *Lasius* ants or *Myrmica*, it appears that *Temnothorax* (or *Leptothorax*) do not actively tend aphid herds, but are instead opportunistic feeders on the sugary secretions they encounter in their scavenging. Still, the accounts of Wheeler (and the obvious robust exploitation we see in lab experiments) imply that nectar is one component of the feeding habits of these ants.