COLLECTIVE DECISION-MAKING AND FORAGING IN A COMMUNITY OF DESERT ANTS

By

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A Dissertation Submitted to the Faculty of the

GRADUATE INTERDISCIPLINARY PROGRAM IN ENTOMOLOGY AND INSECT SCIENCE

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College
THE UNIVERSITY OF ARIZONA

2010
THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

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ACKNOWLEDGEMENTS

I would like to thank my advisor, Judie Bronstein, for all of the inspiration and guidance she provided during my graduate work, and for fostering such an exciting, diverse academic community in her lab. She has made me the ecologist I am today. I am also indebted to all of the obligate, facultative, inquiline, transient and permanent members of the Bronstein lab, who have shared ideas, participated in lively discussions, and provided essential feedback over the years.

I would also like to thank my committee members for their feedback and support. Dan Papaj was particularly helpful in helping me learn about experimental design for behavior studies, and Molly Hunter provided not only feedback on my work, but also wisdom on presentation skills and navigating the academic world. Anna Dornhaus and her lab group deserve special thanks; meeting Anna in my second year changed not only the direction of my dissertation work, but my perspective on social insects in general.

Sharon Richards and Nick Strausfeld of the Center for Insect Science always went above and beyond in helping the IDP students, no matter now difficult the problem. I must also thank Teresa Kundra and Rachael Mattul, who assisted me in preparing for the defense.

Finally, I must thank Andrew Waser, my husband who provided not only moral but scientific support during my time at the University of Arizona.
DEDICATION

This work is dedicated to everyone who is a naturalist at heart.
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ABSTRACT

Ant colonies are often considered to be a superorganism, exhibiting complex collective behaviors, reproducing at the colony level, and dividing functional roles among groups of workers. For this reason, it is often appropriate to study ant behavior at the colony, rather than the individual, level. In this study, I investigated decision-making and foraging behavior in colonies of several species belonging to the ant community of Sonoran Desert scrub habitat. First, I used laboratory experiments to examine how the spatial structure of *Crematogaster torosa* colonies changes in response to the availability of temporally stable food sources. I found that in this polydomous species the formation of nests is associated with foraging, but that colonies will build broodless structures called “oustations” regardless of food presence. Next, I examined colony spatial structure of a related polydomous species, *Crematogaster opuntiae*, in the field. I found that colonies used large foraging territories up to three hectares in size, containing up to twenty nest entrances interconnected by a network of trails. Nest location appeared to be related to foraging, with nests located close to extrafloral nectar-secreting cacti (*Ferocactus wislizeni*) and a negative relationship between cactus density and territory size. Within colonies, forager behavior on neighboring cacti was not independent at short distances, suggesting that separate plants in this system cannot be treated as independent replicates. In the third chapter, I used an individual-based simulation model to investigate the effects of individual worker behavior on the ability of pheromone-recruiting ant colonies to maintain trails to multiple food sources simultaneously. Interestingly, small changes in the behavior rules used by individuals led to large-scale changes in emergent
behaviors at the colony level. Lastly, I used field experiments to relate the ability of colonies of three ant species to maintain multiple trails to their ranking in the community competitive dominance hierarchy. I found that the most dominant species tended to forage asymmetrically, whereas the least dominant species exhibited more symmetrical patterns of foraging. The ability of ant colonies to collectively maintain multiple trails may therefore be an adaptive trait linked to the foraging ecology of species.
INTRODUCTION

An explanation of the problem and a review of the literature

“Superorganism” is a term used to refer to groups of organisms that collectively behave like a single, unified organism. Ant colonies fit this description, with groups of workers that play various functional roles, reproduction that occurs at the colony level (Wilson and Sober 1989), and nests and other structures that play a physiological role in maintaining the colony (Turner 1999). Collectively, ant colonies can also exhibit emergent group behaviors that are difficult to predict through observations of individual workers (Franks et al. 2009). For this reason, ants are among the most often cited examples of self-organized complex systems. The interaction of individual workers following various behavioral rules can lead to a variety of complex colony-level behaviors, including group foraging raids (Franks et al. 1991), traffic lane formation (Couzin and Franks 2003), and construction of nests (Bonabeau et al. 1998).

The ant colony, rather than the individual worker, may therefore be the more appropriate scale at which to study the behavior and ecology of ants. However, due to the difficulty of determining the spatial extent of colonies in the field, few studies have considered ants at this scale. The goal of this dissertation work was to investigate decision-making and foraging behavior at the scale of the whole ant colony. The study took place in Sonoran Desert scrub habitat, a community in which several ant species are unusually amenable to delineation of colony boundaries. Specifically, I considered the effects of the temporal and spatial availability of food resources on colony behaviors, including nest and trail formation and use of foraging territories. I also investigated the
relationship between the competitive ability of species and collective foraging strategies of colonies, and the ways in which individual worker behavior can influence colony-level patterns of foraging.

An explanation of the dissertation format

In this dissertation, I present the results of a broad investigation of collective decision-making and foraging behavior. The work includes detailed work on several Sonoran Desert ant species, as well as studies encompassing many species at the scale of a community at one well-studied Sonoran Desert site. Similarly, I employed a wide range of methods, including computer simulations and experiments in both the field and laboratory.

Appendix A, “The function of polydomy: the ant Crematogaster torosa preferentially forms new nests near food sources and fortifies outstations” examines the effect of stable food sources on nest-building behaviors of a common North American desert ant species, under laboratory conditions. In Appendix B, “An ants-eye view of an ant-plant protection mutualism,” I then examine how the spatial distribution of stable food resources (extrafloral nectar-secreting cacti) affects the spatial arrangement of nests, trails, and territories in a second Sonoran Desert ant species, Crematogaster opuntiae. Appendix C, “Contrasting models of ant colony foraging behavior: individual decision-making and symmetry breaking” presents the results of a computer modeling study designed to investigate the effects of individual worker behavior on the ability of ant
colonies to maintain trails to multiple food sources simultaneously. In Appendix D, “Foraging strategy and symmetry breaking in desert ants,” I then use field experiments in Sonoran Desert habitat to relate the ability of colonies of three ant species to maintain multiple trails to their ranking in the community competitive dominance hierarchy.
PRESENT STUDY

I present the methods, data, analyses, and conclusions of this study in the four appended manuscripts. Below, I briefly summarize the main conclusions from each of these studies.

In Appendix A, I investigated whether colonies of *Crematogaster torosa*, an ant found in Sonoran Desert Scrub, form new polydomous nests and broodless residences called outstations in order to better exploit temporally stable food resources. Laboratory-reared colonies were connected to a new foraging arena containing potential nest sites with or without food for four months. When food was present, most colonies formed polydomous nests nearby and the remainder formed outstations. When food was absent, the behavior of colonies was significantly different: ants frequently formed outstations but never polydomous nests. Workers often fortified the entrances to both outstations and polydomous nests, and used the chambers for storage of dried insect tissue (“jerky”). In an investigation of spatial fidelity, we found that workers on the between-nest trail were associated with the original nest, whereas workers collecting food were more likely to be associated with the new nest or outstation. We conclude that because polydomous nests only formed near food resources, polydomy has a foraging function in this species.

In Appendix B, I report on a three-year study that employed a combination of behavioral aggression assays, baiting, and observation to describe the spatial distribution of nests and trails of the Sonoran Desert ant *Crematogaster opuntiae* (Buren) in relation to the spatial arrangement of stable food sources (the extrafloral nectar-secreting cactus *Ferocactus wislizeni*). I found that territories of *C. opuntiae* colonies are large, covering
areas of up to three hectares, and that colonies are highly polydomous, with up to twenty
nest entrances interconnected by trails and dispersed throughout the territory. The
behavior of ant workers from the same colony visiting separate cacti was non-
independent at distances less than 5m, highlighting the importance of considering ant
colonies rather than individuals as the unit with which plants interact.

In Appendix C I report on a computer simulation model that examined symmetry
breaking, an emergent pattern exhibited by some social insects in which colonies with
access to two or more identical food sources allocate foragers among them
asymmetrically. When symmetry breaking occurs, colonies are unable to maintain trails
to multiple food sources simultaneously. Symmetry breaking may be an epiphenomenon
that occurs in all ant species that use pheromone trail recruitment, or it may be an
adaptive behavior related to specific ecological features of an ant species. I investigated
whether the symmetry breaking phenomenon changed when I varied behavior of
individual ants. Specifically, I varied the decision rule used by individuals to choose
between trails, and the ability of individuals to discriminate between trails with similar
amounts of pheromone. Although asymmetry was greater than our null expectation in all
simulations, some rules produced patterns of foraging that were much more asymmetrical
than others. The relationship between forager group size and asymmetry also varied
among simulations using different rules. It is therefore likely that asymmetrical foraging
is not an epiphenomenon of pheromone trail recruitment, and that symmetry breaking
may vary among ant species based on their foraging ecology.
Finally, in Appendix D I explore the ecological and evolutionary significance of symmetry breaking. I do this by comparing the occurrence of symmetry breaking across species in a Sonoran Desert ant community with a clear dominance hierarchy and evidence of a trade-off between numerical dominance and discovery ability. Competitive dominance was strongly correlated with how asymmetrically colonies foraged, with the highest-ranking species in the dominance hierarchy exhibiting the most asymmetry. This suggests that in a community structured by a trade-off between numerical dominance and discovery ability, symmetry breaking may enable dominant species to recruit large numbers of workers to resources. Species lower in the hierarchy may need to avoid symmetry breaking in order to rapidly explore their environment and discover new food sources.
REFERENCES


APPENDIX A- THE FUNCTION OF POLYDOMY: THE ANT
CREMATOGASTER TOROSA PREFERENTIALLY FORMS NEW NESTS
NEAR FOOD SOURCES AND FORTIFIES OUTSTATIONS
The function of polydomy: the ant *Crematogaster torosa* preferentially forms new nests near food sources and fortifies outstations

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Key Words: ants, polydomy, outstations, social insects, collective behavior, foraging
Abstract

Many ant species are polydomous, forming multiple spatially segregated nests that exchange workers and brood. However, why polydomy occurs is still uncertain. We investigated whether colonies of *Crematogaster torosa* form new polydomous nests to better exploit temporally stable food resources. Specifically, we tested the effect of food presence or absence and distance on the likelihood that colonies would form a new nest. Because this species also forms little-known structures that house only workers without brood (outstations), we also compared the function of this structure with true nests.

Laboratory-reared colonies were connected to a new foraging arena containing potential nest sites with or without food for four months. When food was present, most colonies formed polydomous nests nearby and the remainder formed outstations. When food was absent, the behavior of colonies differed significantly, frequently forming outstations but never polydomous nests. Distance had no effect on the type of structure formed, but when food was present a larger proportion of the workforce moved shorter distances. Workers often fortified the entrances to both structures and used them for storage of dried insect tissue (“jerky”). In an investigation of spatial fidelity, we found that workers on the between-nest trail were associated with the original nest, whereas workers collecting food were more likely to be associated with the new nest or outstation. *Crematogaster torosa* appears to have a flexible colony structure, forming both outstations and polydomous nests. Polydomous nests in this species were associated with foraging and were only formed near food resources.
Introduction

Social insect colonies are frequently described as superorganisms, in which groups of workers play various functional roles, and reproduction occurs at the colony level (Wilson and Sober 1989). In ecological studies, it is therefore often useful to consider colonies as single organisms, because the emergent properties of the group are more relevant than the individual behaviors. For instance, through the actions of many workers (both foragers and nest workers), leafcutter ant colonies function as herbivores, consuming quantities of vegetation comparable to large ungulates (Herz et al. 2007). The collective ability of colonies to rapidly recruit workers also makes them particularly effective at protecting mutualistic plant and insect partners (Del Val and Dirzo 2004). The nests and other structures produced by ant colonies can be considered extensions of the superorganism, serving important physiological roles such as thermoregulation and nutrient storage (Turner 1999). Ant colonies can also significantly modify the space surrounding their nests by altering soil chemistry and the plant community (MacMahon et al. 2000). Determining how individual colonies of social insects are distributed in space is thus critical for understanding the foraging strategies of ants, as well as the spatial scale of their interactions with prey, mutualistic partners, and their environment (Anderson and McShea 2001; Lach et al. 2010).

Unlike wasp and bee colonies that typically build a single nest, many ant species build multiple nests, as well as other structures in their territory (Anderson and McShea 2001; Debout et al. 2007). A colony is considered polydomous if it has two or more spatially segregated nests that house both workers and brood, and if there is a regular
exchange of workers among nests (Debout et al. 2007). Polydomy is common, occurring in at least 166 species in 49 genera of ants (Debout et al. 2007). Polydomous colonies can be either polygynous (having multiple queens) or monogynous (single queen), and although true nests always contain brood, they do not necessarily contain a queen. Nests may be clumped or dispersed within the colony territory, and are often interconnected by a network of trails.

A variety of hypotheses have been proposed for the adaptive benefits of polydomous organization. One possibility is that polydomy is an adaptation for increased foraging efficiency, especially in species that exploit stable, patchy resources such as extrafloral nectar and insect honeydew (Pfeiffer and Linsenmair 1998). Polydomous organization decentralizes the ants’ foraging network, spreading the work force among multiple nests and reducing the distance that foragers need to travel to patches (Holway and Case 2000). This foraging strategy, dispersed central place foraging, has been described in many ants (Pfeiffer and Linsenmair 1998; Holway and Case 2000; Cerda et al. 2002; Dillier and Wehner 2004). To efficiently collect stable, patchy resources, colonies may need to position foragers near many plants simultaneously over long periods of time (Mody and Linsenmair 2003). When groups of foragers show fidelity to a particular area around a polydomous nest and there is a dedicated subset of workers moving on the between-nest trails, polydomous nests can also function as transfer stations for food stockpiles (Pfeiffer and Linsenmair 1998).

Although polydomous nests may improve foraging efficiency in some species, their dual function as brood-rearing chambers makes it difficult to tease apart the factors
leading to their formation. For instance, colonies may create polydomous nests as a thermoregulatory strategy for brood-rearing (Pedersen and Boomsma 1999), or segregate brood in a new nest in order to avoid reproductive conflicts with the queen (Banschbach and Herbers 1996). It is therefore difficult to determine the relative importance of colony foraging strategy in their formation.

However, there is also a second nest-like structure that colonies can create that lacks this brood-rearing function. “Outstations” contain workers but not brood, are spatially segregated from other colony nests, and are connected to the rest of the colony by regular worker traffic (Anderson and McShea 2001). Colonies may create outstations either in pre-existing cavities or in newly constructed chambers. Of the five ant species reviewed by Anderson and McShea (2001) that construct outstation chambers, at least three are also polydomous [Formica pratensis, Lasius niger, and Oecophylla sp. (Debout et al. 2007)]. These outstations may confer the same foraging benefits as polydomous nests by stationing workers closer to resources, but lack the brood-rearing function of true nests.

Nothing is currently known about how species that use both polydomous nests and outstations determine when to construct a new structure, or what type of structure to create. In this study, we asked whether polydomous nests and outstations play similar roles in colony foraging, and what conditions lead to their formation. We hypothesized that colonies would be most likely to form new polydomous nests near stable food sources, in order to more efficiently exploit them. We used laboratory colonies of the species Crematogaster torosa (Formicidae: Myrmicinae), an ant that frequently forages
on extrafloral nectar-secreting cacti in the Sonoran Desert (Lanan, pers. obs.). Our study addresses four questions. First, does the presence or absence of food affect the likelihood that a colony will build a new nest or outstation in a particular location? Investigating the effect of food presence serves to test the function of nests and outstations in a foraging context. Second, how does distance to food from the original nest affect colony response (i.e., foraging from a central nest over long distances, constructing an outstation, constructing a polydomous nest, or moving the entire colony)? Third, are outstations and polydomous nests used differently, or do they serve similar functions? Finally, do foragers show spatial fidelity to either the polydomous nests or outstations? If so, do workers on the between-nest trail also exhibit spatial fidelity?

**Methods**

**Study species**

*Crematogaster torosa* is a common ant species that ranges throughout the neotropics and the southwestern United States (Longino 2003). In Costa Rica, they nest in plant cavities, forming large, polydomous colonies. They restrict the entrances of their nests by building baffles from a mixture of soil, debris, and fibers, and in some cases may also construct pavilions to house honeydew-secreting insects (Longino 2003). In southeastern Arizona, this species (previously named *Crematogaster arizonensis*) commonly forages at the extrafloral nectaries of the barrel cactus *Ferocactus wislizeni* in Sonoran Desert scrub habitat, where they also appear to form polydomous colonies. We have observed *C. torosa* nests in the soil under the bases of the cacti, as well as inside cavities in the plant tissue; the ants frequently build partial coverings made from soil and
plant fibers over the extrafloral nectaries (Lanan, pers. obs). Although the size and foraging range of *C. torosa* colonies in the Sonoran Desert are not yet known, a related nectar-collecting species, *Crematogaster opuntiae*, maintains large territories up to a half-hectare in area, with many widely distributed polydomous nests and outstations (Lanan 2010). The distances chosen for this experiment are based on the range of nest to food distances we observed in *C. opuntiae*.

**Nest set-up and experimental design**

We collected founding queens at the Santa Rita Experimental Range south of Tucson, Arizona on the evening of 21 July 2007, using a portable UV lamp. We housed each colony in the laboratory in an open 17.8x7x7.6 cm acrylic arena coated on the sides with Fluon to prevent escape. Inside the arena, colonies were provided with a small pile of artificial nest chambers. These chambers consisted of two test tubes partly filled with water, plugged with cotton (a standard ant-housing method, see Hölldobler and Wilson 1990), and partially closed with a thin wall of oil-based modeling clay (Van Aken Plastalina, Rancho Cucamonga, CA) on the end, as well as empty test tubes partially closed with clay. The pile of tubes was covered with a piece of black paper; colonies were provided with a surplus of tubes so that they were not space-limited. Each colony was fed chopped freeze-killed cockroaches and a 1:10 mixture of honey and water twice a week, and was provided with additional water in cotton-stoppered test tubes. Colonies were kept on an approximately 9:15 light:dark cycle.

For the experimental treatment, we connected each queenright arena to an empty, identical arena using clear, flexible 0.6 cm plastic tubing (Fig. 1), so that the ants could
move between them. We removed all traces of food from the queenright arena. The ants were subsequently fed exclusively in the new arena (hereafter, “feeding arena”). In the feeding arena, we placed a potential nest structure. This structure was similar to the one inhabited by the ants in the queenright arena, consisting of two water tubes, two empty test tubes partly covered with clay on the end, and two test tubes partly filled with water, stoppered with cotton, and partially covered with modeling clay on the end. This structure was also covered with a piece of black paper. We randomly allocated each of the 21 *Crematogaster torosa* colonies to one of three groups: seven colonies were connected to the feeding arena by a 30 cm length of plastic tubing, seven were connected by a 152 cm tube, and seven were connected by a 304 cm tube. Colony worker numbers did not vary significantly among the treatment groups (ANOVA, $F_{4,34} = 0.40, P=0.80$, Table 1). All colonies began the experiment with one queen. We continued to feed the ants diluted honey and cockroaches twice a week in the feeding arena for the duration of the experiment. We allowed the ants to move freely between the arenas for four months.

We made monthly observations of the number of ants in each structure and the conditions of the structures. We also recorded the presence or absence of brood in each structure. We were unable to count brood due to their fragility, clumped arrangement, and the constricted nest entrances.

We followed this experimental treatment with a control treatment six months later, in which the same colonies were again connected to identical chambers, but continued to be fed in the queenright arena. For the control we used only two distance treatments, 30cm and 304cm. Seven colonies were randomly chosen for each distance.
Colony sizes were similar and all details of the control experiment were identical to those described for the experimental treatment, except that the food was only available in the queenright arena.

**Site fidelity experiment**

At the end of the four-month experimental treatments we tested whether foragers (at the food source) were associated with the original nest or the nest in the feeding arena. Specifically, we examined whether ants collecting food in the feeding arena and ants walking in the trail tube between nests showed fidelity to that area or to a particular nest. Twenty minutes after adding the food, we marked the gasters of the first 30 ants found entering or leaving the trail tube at the point where it connected to the queenright arena, using green acrylic paint (Testors brand). An additional 30 ants were marked with red paint as they collected food in the feeding arena. Seven colonies did not have high enough activity levels to allow marking 60 ants. For those colonies, we marked a smaller but equal number of ants per group (between 6 and 17). Although workers initially responded to paint marking by waving their sting in the air, they quickly returned to typical worker behaviors. Twenty-four hours later, we recorded the number of marked ants in the queenright nest, the queenright arena, the trail tube, the feeding arena, and the new nest structure.

Stored food and building materials from the nests were examined under a microscope to determine the type of material. We analyzed all of our data using JMP 5.0, STATISTICA 8, and MINITAB. Figures were converted to vector format using CorelDRAW X3.
Results

Effect of food presence

When food was present in the new arena, fourteen colonies (67%) formed polydomous nests, while the remaining seven (33%) formed outstations by moving only workers. In contrast, when no food was available in the new arena, two colonies (14%) moved no workers and the remaining 12 colonies (86%) formed outstations. None of the colonies formed polydomous nests. There was a significant difference between the number of polydomous nests formed in the food and no-food treatments (Fisher’s Exact Test, $P<0.001$), but there was no difference between the two treatments in how many colonies moved at least some workers (Fisher’s Exact Test, $P=0.153$, observed power $=0.568$). No colonies in any treatment moved all workers, and only one colony in the treatment where food was present moved the queen to the new nest (Table 1). During the food treatment in a second polydomous colony, the queen died during the last two weeks of the experiment and the workers began rearing reproductive brood in both nests. Also, some structures that began as outstations became polydomous nests over the four-month period. Five colonies (24%) recorded as having outstations during the first month’s observation became polydomous by the end of the experiment (i.e., brood was moved only after use of the new structure was well-established). In contrast, in only one case did a colony initially recorded as polydomous revert to having only an outstation at the end of the four months. In most cases colonies in both treatments formed outstations or polydomous nests within the first month, but in one case, a colony in the food treatment only established a polydomous nest after three months.
There was no relationship between total colony size and the proportion of ants that moved into the new structure in either the food treatment (Spearman Rank Correlation, $\rho = -0.123; P = 0.594$; Fig. 2) or no-food treatment (Spearman Rank Correlation, $\rho = 0.229; P = 0.431$; Fig. 2).

**Effect of distance**

In the food treatment, the proportion of the total work force that moved into the new nest decreased as distance between arenas increased from 30cm to 304cm (Spearman Rank Correlation, $\rho = -0.453; P = 0.039$; Fig. 3). However, there was no difference between the 30cm and 304cm distance treatments in the experiment without food (Spearman Rank Correlation, $\rho = -0.408; P = 0.147$). There was also no observed effect of distance on whether colonies in the food experiment formed polydomous nests or outstations (Chi-Square Test, $X^2 = 1.20; P = 0.273$, observed power $=0.394$).

**Use of the polydomous nests and outstations**

We observed three distinct behaviors of the ants as they utilized the new structures. *Brood moving* led to the shift from an outstation to a polydomous nest and took place only in the treatment with food present. *Food storage* and *Entrance modification* were associated with both outstations and nests and took place in both treatments.

Entrance modification occurred in fifteen colonies during the food treatment (71%) and nine colonies in the treatment without food (64%), and we did not observe a difference between the two treatments (Fisher’s Exact Test, $P = 0.721$, observed power $=0.082$). Workers modified the clay plugs in their tubes to create small, round entrances.
less than 2 mm in diameter. The ants used bits of clay, cotton, and cockroach exoskeleton to build these coverings of the tube entrances. At least one worker was always observed guarding this entrance after construction was complete. These modified entrances were more common in polydomous nests (92.9%) compared to outstations (63.2%), although this difference was non-significant (Fisher’s Exact Test, $P=0.098$, observed power $=0.331$).

Food storage occurred when colonies used the new chambers to store small pieces of dried cockroach muscle tissue, or insect “jerky” (Gayahan and Tschinkel 2008). The jerky was always placed in large piles in the dry tubes, or in small piles close to the entrance of the tubes with water behind a plug. Food storage occurred in twelve colonies of the treatment with food (57%), and four colonies of the treatment without food (29%), a non-significant difference between treatments (Fisher’s Exact Test, $P=0.166$, observed power $=0.510$). All colonies in all treatments also stored at least a small amount of jerky in the queenright nest. Food storage was more common in polydomous nests (78.6%) compared to outstations (26.3%, Fisher’s Exact Test, $P=0.005$).

We related the occurrence of these three behaviors to the number of workers occupying the new structures in the treatments with and without food. Brood-moving only occurred in the treatment with food. This behavior was strongly related to the number of workers in the new structure. Colonies were more likely than not to move brood once 56 workers or more occupied the new structure (Logistic Regression, $X^2=18.505; P<0.0001$, Fig. 4a). Brood was never moved in the treatment without food, even though up to 46 workers (12.9%) moved to the new structure. Workers were more
likely than not to modify the entrances once 32 workers occupied the new structure in the feeding treatment, and once 37 workers occupied the new structure in the treatment without food (Logistic Regression, $X^2=6.374; P=0.0116$, $X^2=6.773; P=0.0093$, Fig. 4b). The number of workers associated with the food storage behavior differed between the two treatments. In the treatment with food, the probability that colonies would store food in the new structure passed 0.5 once 73 workers were in the new structure (Logistic Regression, $X^2=24.178; P<0.0001$, Fig. 4c). However, in the treatment without food the probability passed 0.5 once only 18 workers were in the new structure (Logistic Regression, $X^2=11.089; P=0.0009$, Fig. 4c).

**Spatial fidelity of workers**

Ants moving between the nests and ants collecting the food were two separate groups that showed fidelity to different locations. The distribution of ants marked while moving between arenas and while collecting food in the feeding arena differed significantly on the day following marking (Chi-Square Test, $X^2=61.8; P<0.0001$). Ants marked while entering or leaving the between-arena tube were more common in the queenright nest and tube (Fig. 5a, 5b), whereas ants marked in the feeding arena were more common in the feeding arena and new nest (Fig. 5c, 5d).

The distribution of ants marked on the between-nest trail did not differ between polydomous colonies and colonies with outstations (Chi-Square Test, $X^2=7.84; P=0.098$, observed power = 0.951). Nor was there a difference between polydomous colonies and outstation colonies in the distribution of ants marked while collecting food (Chi-Square Test, $X^2=8.87; P=0.064$, observed power = 0.972; Fig. 5).
Discussion

Effect of food presence

When Crematogaster torosa colonies were given access to a new stable food patch, colonies always formed either an outstation or a polydomous nest nearby. However, when colonies encountered a new site that lacked food on the other hand, none of the colonies formed a new nest there, and some colonies failed to move any workers to it at all. This result is consistent with the hypothesis that polydomy enables ants to efficiently exploit stable food resources. Such a nesting strategy may particularly benefit species like C. torosa that forage on patchy, persistent food sources such as plant extrafloral nectaries. In many ways, this colony-level foraging strategy resembles that of clonal plants, which have a modular organization and can quickly establish new ramets in rich patches of soil (Slade and Hutchings 1987; Holway and Case 2000). A modular colony structure can be rapidly expanded to include new resource patches within the colony territory. Furthermore, the tendency to form small outstations regardless of the presence of food may enable colonies to establish a presence in areas that could be useful in the future if the resource distribution changes. Outstations may then quickly transition to become large polydomous nests when food is present. This is similar to the trail-laying and home range marking behaviors of the ant L. niger. The presence of these markings prompt workers to behave at food sources as they would at closer distances to the nest, essentially creating an extension of the nest over larger areas (Devigne and Detrain 2006).
The most striking result from these experiments was how readily the colonies occupied and modified new potential nest sites. In almost all cases, when presented with a suitable nest site with or without a nearby food source, *C. torosa* workers quickly occupied it, often modifying the entrance. When food was present nearby, many structures that began as outstations quickly became polydomous nests housing large amounts of brood. When food was not present nearby, the structures persisted as outstations, housing smaller numbers of workers for the duration of the experiment.

**Effect of distance**

The proportion of the workforce that moved to the new structure decreased as distance increased in the treatment with food present, suggesting that distance can influence the size of new structures that form and the number of workers that utilize them. This pattern may indicate that colonies allocate fewer workers to structures that are distant from the original nest, or that workers move to distant structures at a slower rate. However, there was no effect of distance on the proportion of the workforce that moved in the treatments without food. Similarly, there was no effect of distance on whether colonies formed polydomous nests or outstations in either treatment. Devigne and Detrain (2006) observed a similar pattern in the short-term foraging behavior of *Lasius niger*, with more intense recruitment to new food resources occurring at shorter distances to the nest, and changes in scout foraging and recruitment behavior depending on food distance. Our results show that distance can also have an effect on the more long-term process of recruitment to new polydomous nests when food is present.

**Use of polydomous nests and outstations**
Although outstations differ from polydomous nests in that they are not used for brood-rearing, ants using the two structures in this study had several other behaviors in common. Upon occupying the new structure, workers frequently modified the entrance to create a smaller, more defensible passage. These entrances were always guarded regardless of the amount of workers and brood inside, and regardless of whether food was present nearby. Occupying all available nest sites regardless of food availability may carry important benefits for ant colonies. *Pheidole desertorum* ants are reported to maintain “backup nests” (i.e., outstations) to which they can retreat in the event of an army ant attack (Droual 1984). Occupying multiple sites may also protect the colony’s territory from incursion by competitors, especially when nest sites are limited. Ants in the new structure would be able to respond quickly to defend that area of the territory from invaders, as well as to rapidly exploit any new food sources that appeared there. Occupying an outstation with even a few workers may also preserve the space for later use in case the colony grows larger. By fortifying the entrances, *C. torosa* workers made the new chambers more defensible, even if only a few workers remain inside. Use of broodless outstations could be one factor affecting the success of invasive ants, which often feed on nectar and honeydew spread throughout their foraging range and typically have polydomous or unicolonial nest structures (Tsutsui and Suarez 2003). Like the colonies in our experiment, unicolonial species like *Linepithema humile* might use outstations as place-holders to exclude native ants from potential nest sites while colonies grow. However, data on the frequency of outstations in most polydomous species is lacking, due to the lack of nest excavation studies in ants (Laskis and Tschinkel 2009).
Without excavating colonies, researchers are unlikely to be able to distinguish between outstations and true nests in the field.

Both the outstations and polydomous nests formed in this study were frequently used for food storage. Similar to the insect jerky reported in *Solenopsis invicta* (Gayahan and Tschinkel 2008), *C. torosa* colonies stored small pieces of cockroach tissue in the driest parts of their chambers. This is only the second record of this remarkable food-processing and storing activity, which can be added to the impressive list of complex behaviors found in ants, including agriculture (Mueller et al. 2005), tool use (Banschbach et al. 2006), and a variety of learning behaviors (Dornhaus and Franks 2008). Although creation of insect jerky is unknown for *C. torosa* in the wild, the co-occurring desert species *Crematogaster opuntiae* often have piles of dried insect tissue in their nests (Lanan, pers. obs). Gayahan and Tschinkel (2008) speculated that this behavior may be common among desert ants, where the habitat is dry enough to prevent spoilage. Since the range of *C. torosa* includes much of the neotropics, it would be interesting to investigate whether this behavior occurs in wetter climates as well.

The stored jerky must serve to feed the ant larvae, because adult ants can only feed on liquid foods. This raises the question: what is the adaptive significance of storing food that only larvae can eat, in an outstation that contains no larvae? Storing food away from the nest may be a bet-hedging strategy, keeping excess food in a separate location to prevent destruction or the spread of spoilage. Alternatively, appropriate storage space may be limited, prompting the ants to use all available dry chambers regardless of distance from the brood.
Why make an outstation, and not a nest? Although the two structures are otherwise similar, outstations lack the risk associated with moving brood and are not subject to the same environmental limitations as brood chambers. The brood must be carried from the queenright nest to the new polydomous nest, during which time it is vulnerable to predation and desiccation. Furthermore, not all chambers that are suitable for housing workers are suitable for rearing brood, and their suitability may change seasonally. Colonies of many species move brood up and down in the nest (Penick and Tschinkel 2008) or between nests seasonally in order to maintain the optimal temperature for brood rearing (Pedersen and Boomsma 1999).

**Spatial fidelity of workers**

Our study provides evidence that both polydomous nests and outstations serve as distribution centers, with some workers foraging and depositing food, and others transporting food between nests. Despite differences in worker number and the presence of brood, our site fidelity experiment revealed that there was no difference between outstations and polydomous nests in the frequency of worker exchange with the home nest. The majority of the workers we marked foraging near the new nest or outstation showed fidelity to that structure, remaining inside 24 hours later. The majority of the workers marked on the trail between the structures, on the other hand, were found in the original queenright nest the next day. This is similar to the behavior of *Lasius fuliginosus* described in 1966 by Dobrzanska (as described in Anderson and McShea 2001), as well as of the polydomous species described by Pfeiffer and Linsenmair (1998). Constant movement of workers on the trail between structures may allow for rapid information
transfer, and may enable a colony to respond to a changing environment by shifting the
distribution of resources or the workforce. At the same time, colonies may also benefit
from specialized workers that show fidelity to the outstations or polydomous nests,
improving efficiency through shorter foraging distances and division of labor. Both the
outstations and polydomous nests may function as distribution hubs, with local foragers
stashing insect jerky for later transport to the home nest. Similar distinctions between
foragers that collect food and workers that transport food has been described for several
other species of ants (Fewell et al. 1992; Ratnieks and Anderson 1999; Detrain and Tasse
2000).

A potential mechanism for the formation of polydomous nests, outstations, and other
structures

What mechanism determines the type of structure that the ants create? We
propose that true polydomous nests and certain intermediate structures such as outstations
exist along a continuum of complexity in construction, and that a set of simple behavioral
rules may account for the formation of a variety of such structures among social insects.
In both Temnothorax ants and honeybees, colonies searching for a new home are able to
choose a suitable nest site using quorum sensing (Mallon et al. 2001). Scouts investigate
potential nest sites, and recruit new scouts with varying levels of intensity based on their
assessment of site quality. At potential nest sites, scouts use their direct encounter rate
with other workers to sense a quorum (Pratt 2005; Seeley et al. 2006). After this critical
number is achieved, the workers switch to colony-moving behaviors (piping in bees,
carrying in Temnothorax), and the colony quickly moves to the new nest. We suggest that
a simple adaptation of the quorum sensing method could account for the formation of both polydomous nests and outstations. We found that the colonies were likely to move brood once 56 workers were in the new nest, and to modify the doorways once 32 or 37 workers were in the new structure. The number of workers above which food storage occurred differed greatly between the treatments, however, suggesting that other mechanisms may influence this behavior. In a scenario in which workers had different quorum thresholds for various nest-making behaviors such as construction and brood moving, we could easily envision the formation of a variety of nest-like structures along areas of high traffic in a colony’s foraging range. Polydomous nests would tend to form in the highest-traffic areas, while simple outstations would form in lower-traffic areas. This mechanism is particularly likely for trail-making ants that rely on patchy, persistent resources such as extrafloral nectar and insect exudates, because these ants tend to visit the same locations over long periods of time. For instance, in field populations of *C. torosa* we have observed the ants constructing coverings over extrafloral nectaries on barrel cacti (Lanan, *pers. obs.*). These coverings are built in a similar manner to the modified entrances observed in this study, and the nectaries are locations that receive low but consistent ant traffic. However, the fact that the food storage behavior was associated with a much lower worker threshold in the treatment without food indicates that some behaviors may be context-dependent. Future work on *C. torosa*’s nest-building strategy may yield insight into versatility of quorum sensing as a decision-making strategy, which has been reported in a variety of systems, including microorganisms (Crespi 2001).
Further investigation into the formation of polydomous nests and other non-nest structures may yield new insights into collective decision-making in social insects, as well as complex systems in general. Intermediate stages of polydomy such as outstations add physical complexity to the structures observed in nature, yet could be the result of very simple rules. Although outstations and nests exist along a continuum of behaviors including construction, worker movement, food storage, and brood movement, an outstation does not need to progress to a nest. Furthermore, shifts in the type of structure may not be unidirectional, and a nest could revert to an outstation depending on colony needs. Although this study showed no difference between nests and outstations in worker traffic or storage function, further study is needed to determine whether these structures serve different functions in the field. In the future, the many non-nest structures that ants use to modify their environment may prove to be a rich source of insight into the processes shaping the behavior of social insects and other complex systems.

Acknowledgements

We thank Mary Price, Emily Jones, Jenny Jandt, Tuan Cao, Nhi Duong, Kim Franklin, Anne Estes, Margaret Couvillon, Aimee Dunlap, Martha Hunter, Dan Papaj, Cédric Devigne, and two anonymous reviewers for comments on this manuscript, and Emily Kaleugher for laboratory assistance. We would also like to thank the Center for Insect Science at the University of Arizona and National Science Foundation grant no. IOS 0841756 for funding this research.

Declaration of integrity

These experiments comply with the current laws of the United States of America.
The authors declare that they have no conflict of interest.
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Detrain C, Tasse O (2000) Seed drops and caches by the harvester ant Messor barbarus: do they contribute to seed dispersal in Mediterranean grasslands? Naturwissenschaften 87:373-376


### Tables

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colonies that made outstation s</th>
<th>Colonies that became polydomous</th>
<th>Colonies that stored food</th>
<th>Colonies that modified entrance s</th>
<th>Mean percentage of ants in new nest</th>
<th>Mean percentage of ants in old nest</th>
<th>Mean colony size</th>
</tr>
</thead>
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<tr>
<td>30 cm no food</td>
<td>7/7</td>
<td>0/7</td>
<td>3/7</td>
<td>4/7</td>
<td>6.2%</td>
<td>93.8%</td>
<td>233.7 +/- 34.6</td>
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<tr>
<td>30 cm food</td>
<td>1/7</td>
<td>6/7</td>
<td>5/7</td>
<td>5/7</td>
<td>47.6%</td>
<td>52.4%</td>
<td>277.7 +/- 54.4</td>
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<tr>
<td>152 cm food</td>
<td>3/7</td>
<td>4/7</td>
<td>3/7</td>
<td>5/7</td>
<td>34.0%</td>
<td>66.0%</td>
<td>295.6 +/- 79.8</td>
</tr>
<tr>
<td>304 cm no food</td>
<td>5/7</td>
<td>0/7</td>
<td>1/7</td>
<td>5/7</td>
<td>2.8%</td>
<td>97.1%</td>
<td>229.7 +/- 34.2</td>
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<tr>
<td>304 cm food</td>
<td>3/7</td>
<td>4/7</td>
<td>4/7</td>
<td>5/7</td>
<td>24.2%</td>
<td>75.7%</td>
<td>290.4 +/- 23.9</td>
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</table>

**Table 1**
Figure Legends

Table 1 Summary of colony responses to the treatments with and without food present. The number of colonies out of seven that formed outstations, became polydomous, stored food, and modified entrances, as well as the mean percentage of workers found in the nest structures at the end of the experiment and the mean colony size +/- standard error for each group are shown for the three distance groups, 30 cm, 152 cm, and 304 cm.

Fig. 1 Experimental setup. The queenright nest (a) was placed inside the queenright arena (b), which was connected to the feeding arena (d) and new chamber (e) by a trail tube made from a length of plastic tubing (c). Colonies were randomly assigned to three distance groups, at 1. 30 cm, 2. 152 cm, and 3. 304 cm. In the control treatment, food was placed in the queenright arena (b), and only the 30 cm and 304 cm treatments were used.

Fig. 2 The relationship between the total colony size and the proportion of ants that moved into the new chamber. The three distance groups are shown as triangles (30 cm), squares (152 cm), and circles (304 cm). The treatment with food present is represented by open shapes, while the treatment without food is represented by filled shapes.

Fig. 3 The proportion of ants that moved to the new structure in the three distance classes in the food treatment. Data are shown as points, and box plots indicate the means, 25% and 75% quartiles, minimum, and maximum data points.
Fig. 4 The probability of moving brood (a), modifying doorways (b), and storing food (c) as a function of the number of workers in the new structure. Data from the treatment with food is shown as black circles, while the logistic regression functions are shown as black lines. Data from the treatment without food is shown as open circles, with logistic regression functions shown with dashed lines. Logistic regression functions for the treatment with food are $Y = 1/(1+e^{(4.634-0.083x)})$ ($X^2 = 18.505; P < .0001$) for brood moving, $Y = 1/(1+e^{(0.922-0.0305x)})$ ($X^2 = 6.374; P = 0.0116$) for entrance modification, and $Y = 1/(1+e^{(10.49-0.141x)})$ ($X^2 = 24.178; P < .0001$) for food storage. In the treatment without food, logistic regression functions are $Y = 1/(1+e^{(2.227-0.302x)})$ ($X^2 = 6.773; P = 0.0093$) for entrance modification, and $Y = 1/(1+e^{(0.4.455-0.256x)})$ ($X^2 = 11.089; P = 0.0009$) for food storage. (Brood moving never occurred during the treatment without food).

Fig. 5 The location and percentage of marked ants recaptured on the day following marking, for ants marked while feeding in the new arena (a) and ants marked while walking in the trail tube (b). The ants marked entering and leaving the trail tube were more commonly found in the queenright nest and arena, while the ants marked while feeding were more commonly found in the feeding arena and new nest or outstation.
Figures

1. 

2. 

3. 

Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
APPENDIX B - AN ANTS-EYE VIEW OF AN ANT-PLANT PROTECTION MUTUALISM
An ants-eye view of an ant-plant protection mutualism

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Key Words: ants, mutualism, social insects, polydomy, foraging, extrafloral nectar
Abstract

Ant protection of extrafloral nectar-secreting plants is a common form of mutualism found in most habitats around the world. However, very few studies have taken a whole-colony perspective, investigating the spatial structure and nest arrangement of the ant colonies that visit these plants. Here, we describe the spatial distribution of nests and trails of the Sonoran Desert ant *Crematogaster opuntiae* (Buren) in relation to the extrafloral nectar-secreting cactus *Ferocactus wislizeni* (Englem). To determine the size and position of ant colonies we used a combination of behavioral aggression assays, baiting, and observation of trails between nests from 2007 to 2009. We found that territories of *C. opuntiae* colonies are large, covering areas of up to three hectares. These ants appear to be highly polydomous, with up to twenty nest entrances interconnected by trails and dispersed throughout the territory. Conspecific ant workers from neighboring territories were hostile, and attacked each other upon contact. Each territory included many extrafloral nectar-secreting barrel cacti, and within each territory we observed workers visiting between five and thirty-four plants. The behavior of ant workers from the same colony visiting separate cacti was non-independent at distances less than 5m. This result highlights the importance of considering ant colonies rather than individuals as the unit with which plants interact.
Introduction

Ant protection of extrafloral nectar (EFN)-secreting plants is a common form of mutualism found in most habitats around the world. In these interactions, ants visit the plants for food, then act aggressively towards other plant consumers, often resulting in reduced levels of herbivory. These interactions are accessible, easy to manipulate, and range from obligate to facultative, making them a useful model system for the study of mutualism (Bronstein 1998; Heil and McKey 2003).

Most studies of ant-plant mutualisms have taken a plant-centered approach. For instance, recent studies of facultative ant-plant protection mutualisms have focused on the benefits of ant visitation to plants in terms of fitness (e.g., (Oliveira et al. 1999; Cuautle and Rico-Gray 2003) herbivore deterrence (e.g. (Oliveira et al. 1999; Dutra et al. 2006; Ness et al. 2006), the costs of attracting ants (e.g., (Brouat and McKey 2001; Hatada et al. 2002), plant strategies to attract the most beneficial ants (e.g., (Federle et al. 1997), and plant strategies to avoid being cheated by ants (e.g., (Izzo and Vasconcelos 2002). Our understanding of these interactions from the ant perspective is much less developed. Studies have shown that EFN is an important dietary resource for many ants (Davidson 1997; Bluthgen et al. 2004), comprising up to 90% of the food collected by some species (Tillberg and Breed 2004). Recently, Byk and Del-Claro (2010) presented some of the first experimental evidence that EFN has a significant positive effect on ant colony fitness (Byk and Del-Claro 2010). The availability of EFN as an easily accessible, nearly unlimited source of carbohydrates can also change the behavior of ants, making them more aggressive towards insect herbivores (Ness et al. 2009). Beyond these few studies,
however, we know very little about the consequences for ants of participating in facultative protection mutualisms.

Most studies of facultative ant plant protection mutualisms only consider the subset of worker ants that visit the plants, rather than the colony as a whole. Ant species that participate in facultative EFN mutualisms tend to be ground-nesting, and the location and foraging range of the colony is generally not included in studies of these systems. However, in an ecological sense, it is useful to think of the entire colony as an individual organism. Ant colonies are often large in both size and number of workers; they are usually sessile, long-lived, and reproduce at the colony level through production of alates (Hölldobler and Wilson 1990). A single ant colony can have a significant impact on its environment; for instance, leafcutter ant colonies can consume quantities of vegetation comparable to ungulate herbivores (Herz et al. 2007). The wide variety of behaviors, cues and signals used by ants enable information sharing and coordination of collective actions at the colony level, including decisions about where to forage. Thus, although only a few individual workers may be observed interacting with a nectar-secreting plant, it may not be appropriate to treat them as independent individuals as one would solitary insects. Even the behavior of workers visiting separate plants can be influenced by the same, potentially unseen factors operating at the colony level. It may therefore be inappropriate to treat the fates of neighboring, reward-producing plants as independent from each other in the likely case that they are tended by ants from the same colony. Despite the potential problem that this poses for researchers using plants as replicates in experimental studies of ant-plant mutualisms, we know of no direct examination of this phenomenon.
For an ant colony that feeds upon EFN, the spatial and temporal arrangement of this resource poses unique challenges. Many other foods collected by ants, including insect prey, carrion, and seeds, vary in spatial distribution over time. Colonies often collect these ephemeral resources using rapid pheromone recruitment or foraging trunk trails leading from a central nest entrance (Hölldobler and Wilson 1990). In contrast, EFN can be secreted by plants for months or years, thus persisting in the same location for long periods of time. Because these resource patches do not move, ant colonies that collect EFN may need to position foragers near many plants simultaneously over long periods of time.

One way colonies might deal with patchily distributed, temporally persistent resources is through the spatial arrangement of their nests (Holway and Case 2000). Many ant species are polydomous, a condition in which the colony is distributed among multiple, spatially segregated nests that regularly exchange workers (Debout et al. 2007). One hypothesis for why polydomy occurs is that it enables colonies to position foragers closer to patches and reducing forager travel distance (Holway and Case 2000). For instance, the Malaysian giant ant *Camponotus gigas* tends colonies of honeydew-secreting insects on trees. (Like extrafloral nectar-secreting plants, honeydew-secreting insects are both patchy and temporally stable.) *C. gigas* has numerous polydomous nests distributed throughout the colony territory at the bases of trees interconnected by a network of trails (Pfeiffer and Linsenmair 1998). We hypothesize that dispersed nest distribution may be a common strategy for efficient foraging when resources are both
patchy and temporally persistent, and we therefore expect to find this nest organization in ground-nesting ant species that tend EFN-secreting plants.

The relationship between ant colony spatial organization and resource distribution is interesting from an ant foraging perspective, but also has important implications for understanding ant-plant mutualisms. The quality of protection a particular plant receives is dependent on both ant abundance and recruitment rate (Ness et al. 2006). Nest distance can affect both of these factors, with more workers visiting closer plants (Inouye and Taylor 1979) and recruitment occurring faster over shorter distances (Morales 2000). Plants that are tended by colonies with a dispersed, polydomous nest arrangement may therefore be better protected compared to plants tended by colonies with one central nest. The quality of protection a particular plant receives may also be strongly influenced by its location in relation to ant colony features such as nests and territory boundaries, factors rarely considered in studies of facultative EFN mutualisms (but see Dreisig 2000; Cogni et al. 2003; Lenoir 2003).

In this study, we investigated the distribution of territories, trails, and nest entrances for individual colonies of *Crematogaster opuntiae* foraging among a landscape of extrafloral nectar-secreting plants in the Sonoran Desert of southeastern Arizona, USA. This ant species is one of the most common visitors to nectar-secreting plants in this habitat (Ness 2006; Ness et al. 2006), and extrafloral nectar is an important component of its diet (Ness et al. 2009). We then used the data we collected on colony spatial arrangement to investigate how proximity of individual extrafloral nectar-secreting barrel cacti (*Ferocactus wislizeni*) to colony features such as nest entrances and
territory boundaries affected worker visitation and recruitment to the plants. Specifically, our study addresses these questions:

1. How are *C. opuntiae* colonies organized spatially in relation to the landscape of extrafloral nectar-bearing *F. wislizeni*?

2. Do barrel cacti vary in how frequently they are tended by *C. opuntiae*, based on where they occur in relation to territory boundaries or nest entrances?

3. Is ant behavior on different barrel cactus individuals independent, or do changes in recruitment to one cactus also affect recruitment to neighboring cacti?

**Methods**

*Study species and location*

The fishhook barrel cactus *Ferocactus wislizeni* (Cactaceae) is commonly found in deserts and grasslands with summer rainfall from southern Arizona and southeastern California to northern Sonora, Mexico (Benson 1981). It secretes extrafloral nectar (hereafter EFN) from approximately 50-200 modified spines at the crown of the plant. Although it co-occurs in the Sonoran Desert with other EFN-secreting plants, including saguaro (*Carnegiea gigantea*), cholla and prickly pear (*Opuntia* spp.), senna (*Senna covesii*), and white-thorn acacia (*Acacia constricta*), *F. wislizeni* is the only one that secretes significant amounts of extrafloral nectar and attracts ants year-round (Ness et al. 2009). At least twenty-five species of ants are associated with *F. wislizeni* across southern Arizona (M. C. Lanan, *unpublished data*). Censuses and experimental studies of this ant-plant association have been conducted since 2003 in Sonoran Desert scrub habitat at the Desert Laboratory in Tucson, Arizona, USA (32°13’11”N; 111°00’14”W) (Morris
et al. 2005, Ness 2006, Ness et al. 2006, 2009). At this location each barrel cactus individual is typically tended by one of four common native ant species, *Crematogaster opuntiae*, *Solenopsis auraea*, *Solenopsis xyloni*, and *Forelius pruinosus*. These ant species vary in the quality of defense that they provide to the cactus against insect herbivores (Ness 2006; Ness et al. 2006) and exhibit differing seasonal patterns of abundance (Morris et al. 2005). Multiple ant species rarely co-occur on a single barrel cactus, and one species of ant typically dominates a plant for weeks or months at a time (M. C. Lanan, W. F. Morris, J. H. Ness, J. L. Bronstein, unpublished data).

In this study, we focused on the behavior of *Crematogaster opuntiae*, a common ant species in the southwestern U. S. that frequently visits extrafloral nectaries on cacti (Pickett and Clark 1979; Miller 2007). Although it is often active throughout the day, this species is most active in the early mornings, evenings, and at night (M. C. Lanan, personal observation), and is a frequent visitor to barrel cacti and other extrafloral nectar-secreting plants (Sullender 1998; Ness et al. 2006). Previous reports suggest that this species can be polydomous, with multiple nest entrances spread throughout its territory (Sullender 1998).

The research presented here was conducted at three study sites at the Desert Laboratory where previous research on these interactions has been conducted (Morris et al. 2005; Ness 2006; Ness et al. 2006; Ness et al. 2009). Site 1, a 3.2-hectare plot, is located on relatively flat alluvial soil at 739 m elevation and contains 186 *F. wislizeni*. Sites 2 and 3 are located on rocky, sloped terrain at 850-860m elevation. Site 2 is one hectare in area and contains 107 *F. wislizeni*, while Site 3 is 1.5 hectares in area and
contains 58 plants. Site 2 is bounded on the western edge by a cliff face and Site 3 is bounded on the southern edge by a road. All other site boundaries were arbitrary, with cacti growing both inside and outside the borders. Because of the importance of accurate spatial data for portions of this study, we created detailed maps of the locations of all _F. wislizeni_ on these three sites using a combination of GPS and surveying techniques. In addition, a 5m grid was marked out on Site 3 using steel stakes in order to make more accurate measurements.

1. **How are _C. opuntiae_ colonies organized spatially in relation to the landscape of EFN-bearing plants?**

   **Ant colony territories**

   Our previous observations of both laboratory and field colonies suggested that _C. opuntiae_ is highly territorial, and that workers will quickly respond to a foreign conspecific worker (i.e., one from a different colony) by attacking her. In highly territorial ants, pairwise confrontation tests can be used as a method to determine colony membership and deduce territory boundaries (Dejean et al. 2010). We conducted confrontation tests by placing pairs of worker ants inside clean, dry plastic vials and observing their behavior for 5 min. Behaviors were classified as either non-threatening (grooming, trophyllaxis, spending time in close proximity to the other ant) or threatening (agitated running, biting, exuding liquid on the sting, and dismemberment of the opponent). As a control, we conducted these confrontation tests on 100 pairs of ants collected from the same nest entrance, as well as 82 pairs of ants from two nest entrances >100m apart, known from our preliminary work to be entrances to different colonies. We
observed consistently threatening behaviors in 0% of the known within-colony confrontations, but within 90% of the known between-colony matchups. These data suggest that confrontation tests are, in the absence of genetic data, a reliable indicator of colony identity for this species. Based on this evidence, in the experiments described below, we rejected the hypothesis that ants belonged to the same colony when they engaged in constantly threatening behaviors for 5 min. This is a conservative test of colony boundaries (i.e., we may have erroneously concluded that some pairs belonged to the same colony if they were slightly less aggressive to each other).

To determine the size and location of *C. opuntiae* colony territories at each study site, we used the same confrontation test protocol as above. Because foraging *C. opuntiae* workers in this habitat are found almost exclusively on barrel cacti and workers are rarely observed on the surface of the ground or around nest entrances (M. C. Lanan, *unpublished data*), we assessed the spatial extent of territories based on which barrel cacti individual colonies occupied. For the confrontation tests, we collected 3-10 ants from each *F. wislizeni* using an aspirator, and brought them to the laboratory in plastic vials. All confrontation tests were conducted within 3 hr of worker collection, and 97.5% of the pairwise tests yielded clear results. In the remaining 2.5% of cases, the first confrontation test was not conclusive (for example, we observed biting followed by grooming rather than dismemberment); in these few cases, we repeated the test with a new pair of ants. We did not test every possible pairing of ants between the cacti; rather we selected pairings that would enable us to deduce boundaries, such that all aggressive and non-aggressive pairs were supported by at least two trials. The results of the experiments were
overlaid on site maps to deduce ant colony territories and territorial boundaries. The experiment was conducted three times at each study site in November 2007, January 2008, and January 2009.

*Nest and trail locations*

One colony (Colony G, Fig. 1) at Site 2 was used for a further investigation of the spatial arrangement of *C. opuntiae* nest entrances and trails. We selected Colony G because its territory boundary was completely contained within the site, and because the relatively flat terrain made it possible to locate more of its cryptic nest entrances. Although several more colonies also occurred at Site 2, the difficulty in finding nest entrances and the mainly nocturnal foraging behavior of this species required us to focus on only one colony. We used two methods to determine whether Colony G was polydomous, as well as to determine the location and number of nests. To locate nest entrances, at the onset of the summer rainy season in July 2007 and July 2008 we made observations of winged alates as they emerged before the mating flight. We located the remainder of the nest entrances in July-August 2007, 2008, and 2009 by placing bait (ground-up Pecan Sandies cookies, Kellogg Company) mixed with UV-reflective, fluorescent yellow or red powder (Dayglo Color Corporation) at the crown of each barrel cactus within the territory. After dark, using a hand-held UV light, we followed workers returning with these baits to their nests. Because alates do not travel outside the nest before their mating flight and must therefore be reared in situ as brood, we assumed that all entrances where alates were observed led to true nests containing both workers and brood. Entrances that were found using the baiting method were checked for the
presence of brood by carefully lifting and replacing rocks. Outstations (nest-like structures containing only workers, not brood (Anderson and McShea 2001; Lanan 2010), were noted but not included in this study. In order to relate nest location to EFN availability, we counted the number of nectaries on all plants within the boundaries of Colony G.

We also used the baiting method to locate trails that workers in Colony G followed between barrel cacti and nest entrances, by marking the routes along which ants carried the bait and checking for workers on subsequent days. We mapped the location of nests and trails for this colony in July and August of 2007, 2008, and 2009.

2. Do the barrel cacti vary in how frequently they are tended by *C. opuntiae*, based on where they occur in relation to territory boundaries or nest entrances?

In order to determine the frequency with which *C. opuntiae* visited each plant, we used census data collected in 2006-2009. Approximately once each month, the ant species present at the nectaries of each of 351 permanently tagged *F. wislizeni* at sites 1, 2 and 3 were recorded. We categorized the data for each plant as *a*) *C. opuntiae* present, *b*) a different ant species present, or *c*) no ants present. Using the territory boundaries delineated during 2007-2009, we classified each barrel cactus as growing in one of three areas: habitat that for all three years was within the boundary of one *C. opuntiae* territory (interior), habitat that sometimes was within one territory and at other times within no territory (edge), and habitat that was included within the boundary of different territories in different years (contested).

3. Is ant behavior on different barrel cacti independent?
To determine whether increased *C. opuntiae* recruitment to one *F. wislizeni* individual would influence recruitment to neighboring conspecifics, we supplemented food on a focal cactus, then observed subsequent ant activity on that individual as well as on conspecifics 1 m, 5 m, and >10 m distant from it. We first recorded ant numbers on each of the four cacti every 10 min during a 60 min control period, in order to determine whether ant numbers were stable over time. If factors such as changes in weather and temperature, or the presence of other ant species appeared to cause the number of workers to increase or decrease dramatically during the control period, we aborted the trial and repeated it on a subsequent day. Once stable numbers were observed during the control period, we then added a cotton ball soaked with 25ml 1:10 diluted honey solution at the crown of the focal cactus. We continued to record the number of ants present on all four cacti at 10-min intervals for a further 60-min period. We repeated this experiment five times during 2009-2010. In one experimental trial, the *C. opuntiae* workers on the 5-m cactus were displaced by a different ant species partway through the experiment. We include this trial without data from that individual cactus.

**Results**

1. How are *C. opuntiae* colonies organized spatially in relation to the landscape of EFN-bearing plants?

   **Ant colony territories**

   Using the behavioral assay method, we were able to determine clear ant territory boundaries at each of the three sites (Figure 1). These boundaries were fairly consistent over the course of this study (Figure 2), with the majority of cacti and area remaining
within one territory over all three years. At all three sites we identified areas enclosed by
the same boundary all three years (60% of area, 71% of cacti), areas that were sometimes
within the boundary of a single territory (36% of area, 18% of cacti), and areas that
shifted ownership between years (4% of area, 11% of cacti).

At site 1, we found two territories during all three surveys (Figure 1a). During the
October 2007 and January 2008 surveys, Colony B occupied two cacti in the center of
this site and appeared to be surrounded by the donut-shaped Colony A. We were unable
to determine the outer boundaries or exact size of the territory of Colony A, because it
exceeded the boundary of our study site. In November 2008 the edge of the territory of
Colony B shifted 45 m southeast to include more cacti, but continued to be surrounded by
the donut-shaped Colony A (Figure 1a). On average, the territory of Colony A included at
least 5000 m$^2$ within the plot (indeterminate outer boundary), while the territory of
Colony B averaged 92 m$^3$ over the three surveys. During the behavioral assay censuses,
Colony A used an average of 26 out of 114 plants within its territory (23%), while
Colony B used an average of 3 out of 8 plants within its territory (37%). Most plants that
were not visited by *C. opuntiae* were visited by other ant species during the surveys on all
sites.

Site 2 contained the territories of three colonies (Figure 1b, Colonies C, D, and E)
during the October 2007 and January 2008 surveys, and an additional fourth colony
(Colony F) that first appeared during the November 2008 survey. We were unable to
determine the total size of the territories of Colonies D and E, as they extended beyond
the edge of the study plot. Because Colony C was bordered by other territories within the
plot and a cliff edge lacking cacti, we believe that we identified the true extent of this colony territory. The territory of Colony C averaged 475 m², and workers visited an average of 17 of 28 plants (61%) during the behavioral assay censuses. Colony F, which first appeared during the November 2008 census, occupied a small area with three cacti in an area formerly used by Colony C.

Site 3 contained five colonies (Figure 1c, Colonies G, H, I, J, and K). Two colony territories, G and H, were entirely contained within the site and bordered a road on their southern edge. A significant portion of the territory of Colony I was contained within the site, but we were unable to determine the northern and western boundaries that extended beyond the plot edge. The territories of Colonies J and K extended beyond the boundaries of the plot. The territories of Colony G and Colony H averaged 97 m² and 35 m², respectively. Colony I’s territory was at least 70 m² (beyond plot edge). Colony G visited an average of 13 of 31 cacti (42%) during the behavioral assay surveys, while Colony H visited an average of 13 of 21 plants (62%) and Colony I visited an average of 9 of 25 plants (36%).

The size of the colony territories was related to the density of C. opuntiae-tended cacti at the sites, with territory size increasing as density decreased (Linear Regression, \( F_{1,6} = 7.067, P = 0.0045 \), Figure 3). A similar pattern persisted even when we included cacti occupied by other ant species in the analysis (Linear Regression, \( F_{1,6} = 4.428, P = 0.0089 \)).

\textit{Nest and trail locations}
We mapped the location of nest entrances and trails within the territory of Colony G on Site 3 in August of 2007, 2008, and 2009 (Figure 4). Colony G had 22 nest entrances in 2007, 20 in 2008, and 24 in 2009. The nest entrances typically occurred in soil as small cryptic holes 2mm in diameter near rocks. Seventeen of the nests occurred in the same location all three years. Due to the difficult terrain and dense vegetation, we were only able to locate portions of the trails used by Colony G. However, the portion of the trails that we did identify were persistent over time. All trails we observed appeared with the onset of the monsoon season in mid-July, and remained in the same location until worker activity on the ground declined in October or November. Only nests that we could confirm as containing brood are shown in Figure 3. Because multiple, spatially segregated nest entrances were interconnected by trails, we can conclude that the colony occupying this area was polydomous. However, due to the difficult terrain we were unable to determine whether all nests within the territory of Colony G were interconnected by trails. Nests were more likely to be located near plants with more nectaries; as the number of nectaries per plant increased, the distance to the nearest nest decreased (Linear Regression, F\(_{1,22} = 9.5945, P = .0028\)).

Over the course of this study, we also noted that C. opuntiae created outstations (small, nest-like chambers that house only workers, not brood) and nectary shelters (small coverings over the cactus nectaries created from soil and debris) within the territories of Colonies A, B, C, G, H, and I. The outstations were distributed throughout the territories and were frequently located at the base of cacti, in vegetation next to the cacti, or in areas
of the cactus tissue that had previously been damaged by cactus beetles (*Moneilima gigas*).

2. **Do barrel cacti vary in how frequently they are tended by *C. opuntiae*, based on where they are growing in relation to territory boundaries or nest entrances?**

   Using the survey data on ant visitation to the cacti from 2006-2009, we compared the extent of *C. opuntiae* visitation received by plants growing in the edge and interior areas of territories (Figure 1, yellow and orange areas respectively). At sites 2 and 3, plants located in the interior of territories were visited by *C. opuntiae* in a larger proportion of the surveys than were plants growing on territory edges (Figure 5, Wilcoxon Rank-Sum Test, $\chi^2_1= 19.3235$, $P=0.0001$, Wilcoxon Rank-Sum Test, $\chi^2_1= 5.557$, $P=0.0184$). However, there was no difference in *C. opuntiae* visitation between the edge and interior areas on Site 1 (Figure 5, Wilcoxon Rank-Sum Test, $\chi^2_1= 1.348$, $P=0.246$).

   Distance to the nearest nest had a significant effect on how frequently *C. opuntiae* workers visited barrel cacti within the boundaries of Colony G. The proportion of surveys in which each plant within Colony G was occupied by *C. opuntiae* decreased significantly as the distance to the nearest nest entrance increased (Logistic Regression, $\chi^2=4.513$, $P = 0.034$).

3. **Is ant behavior on different barrel cacti independent?**

   Ant behavior on neighboring cacti was not independent. When we induced ant recruitment to a focal cactus through addition of nectar, we observed an increase in ant recruitment to nearby cacti. The change in the average number of ants was positive on the
1m and 5m plants, but recruitment decreased on the 10m plant (Fig. 6). There was a negative relationship between distance from the focal plant and the change in worker number we observed (Linear Regression, $F_{1,17} = 15.242$, $P=0.0011$).

**Discussion**

*Ant colonies as superorganisms*

Viewing the interaction between the ant *Crematogaster opuntiae* and the extrafloral nectar-producing barrel cactus *Ferocactus wislizeni* from an ant colony perspective, it is clear that the patchy, temporally persistent nature of this resource has important effects on colony structure. Nests in this ant species were numerous and distributed, temporally persistent over three years, and placed closest to cacti that had the greatest number of extrafloral nectaries. Colonies of this ant species were sessile and defended large territories and the cacti growing within them against neighboring conspecifics. Each colony used an average of 12 EFN-secreting cacti within their territory, many of which were visited by workers consistently over the three-year period. The size of the territories increased with decreasing density of cacti, suggesting that foraging range of individual colonies is influenced by the spatial distribution of EFN resources.

Based on the large size of *C. opuntiae* territories and the persistence of both nests and boundaries that we observed, we find it likely that these large colonies may be relatively long-lived superorganisms. Indeed, queens of many ant species are known to have life spans of up to 20 years, including other species of *Crematogaster* (Keller 1998). Although we did not excavate colonies in this study and thus do not yet know whether *C.*
opuntiae colonies have multiple or single queens, the adoption of new queens (secondary polygyny) is a potential mechanism used by some ant species that could extend colony lifespan even further. The most striking example of this can be found in the devil’s garden ant, Myrmelachista schumanni, colonies of which have thousands of queens and have been estimated to persist for up to 800 years (Frederickson et al. 2005). It is therefore not unreasonable to hypothesize that the ant colonies participating in this facultative mutualism could have life spans comparable to the F. wislizeni cacti they visit, which live up to 50 years (Bowers 2000).

However, despite the large, long-lived, and sessile nature of C. opuntiae colonies, plants in this system still experience turnover amongst the species of mutualistic ants that tend them. All of the C. opuntiae colony territories that we identified in this study also included a number of cacti that were not tended by C. opuntiae during some surveys, but that were frequently tended by other common ant species. Thus, although intraspecific aggression appears to produce the discrete territory boundaries we observed in C. opuntiae, other species of ants in this habitat are unaffected by these boundaries. A recent study of the competitive dominance hierarchy structuring the ant community at this site indicates that C. opuntiae is a weaker competitor at baits than two of the three other common ant species that collect EFN from F. wislizeni ((Lanan 2010) but see also Ness, Lanan, Bronstein, Morris, in prep). C. opuntiae also is less aggressive against insect herbivores and provides poorer protection for the plant than its more dominant competitors (Ness et al. 2006). However, this study shows that despite the presence of competitors in the environment, C. opuntiae colonies are able to occupy many EFN-
secreting plants within their territories for long periods of time. Polydomous colony structure, with numerous nests and outstations placed closest to the most rewarding plants (i.e. those with the most nectaries) may be a strategy enabling *C. opuntiae* to exploit this resource in the presence of competitors. Future studies of foraging and nest placement in other ant species in this community may yield more insight into how colony spatial structure influences interspecific competition among ants for EFN.

**Non-independence of worker behavior within colonies**

Our most striking result, that worker behavior was not independent among neighboring plants, highlights the importance of adopting a colony-level viewpoint when studying facultative ant-plant mutualisms. Ant colonies have the ability to share information among workers and coordinate collective actions at the colony level, and in many species foraging is a collective, rather than individual, activity (Hölldobler and Wilson 1990). In *C. opuntiae*, we found that increasing recruitment to one plant also caused an increase in recruitment to nearby plants. This result suggests that we should treat the colony, rather than the individual worker ants, as the unit with which each cactus interacts.

Because ant species differ in their recruitment behavior and nest structure, the distance over which recruitment is non-independent could vary considerably among ant species and between resource types. Without testing independence, our default assumption must be that all recruitment behavior within a single colony is non-independent. However, by testing independence in *C. opuntiae*, we determined that the change in recruitment did not differ from 0 at distances 5m and greater within the same
colony territory. The highly polydomous nest structure of *C. opuntiae* may account for this pattern. If workers from a single polydomous nest foraged on several nearby cacti, workers that returned after discovering the bait might induce other workers to leave the nest and forage without specifying which trail to take. More distant cacti visited by workers from a different polydomous nest would therefore not experience increased recruitment. We would expect to find a different pattern of distance effects on recruitment in ant species that are not polydomous.

Although we only observed non-independence of recruitment at short distances within colonies, other factors that influence entire colonies could have wider-ranging effects on worker behavior among plants. Ant species with polydomous nesting habits often transfer both food and workers between nests (Pfeiffer and Linsenmair 1998; Debout et al. 2007), and the diet of all members of the colony may therefore be fairly homogeneous, with food collected throughout the colony territory shared among workers. Colony-level nutritional needs can change the distribution of foragers among resources (Portha et al. 2002), and worker behavior (Ness et al. 2009). A longer-term food addition experiment on our *C. opuntiae* colonies might therefore have wider-ranging effects on worker behavior throughout the colony. For manipulative studies of facultative ant-plant mutualisms, we recommend that the only certain way to avoid these potential problems is to either test for independence or limit the unit of replication to the colony level.

**Acknowledgements**

We would like to thank Carolyn Camp, Rebecca Ruppel, and Andrew Waser for their assistance in the field, and all the participants in the Buffelgrass Eradication Effort
for their hard work toward protecting the Desert Laboratory and our study sites there against invasive species. We would also like to thank the International Arid Lands Consortium (No. 03R-25) for their grant to Judith Bronstein, Ido Izhaki, and Ran Nathan that funded part of this research.

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Figure 1: Maps showing the 2007, 2008, and 2009 territory boundaries that we deduced using the behavioral assay method for *C. opuntiae* colonies on a) Site 1, b) Site 2, and c) Site 3. Areas used by a single colony during all three years are shown in yellow, while areas that were sometimes used by one colony are highlighted in orange. Areas that changed colony ownership over the course of the study are shown in red.
Figure 2: Maps showing how *C. opuntiae* territory boundaries changed over the three year period for 2007, 2008, and 2009 on a) Site 1, b) Site 2, and c) Site 3.
Figure 3: Log territory size decreased as cactus density increased among the six colonies for which territory sizes could be determined.
Figure 4: Maps showing the trails and nests found for Colony G at Site 3 in August 2007, August 2008, and August 2009. Cacti are indicated as open circles, and nest entrances are represented by black stars. Trails with heavy traffic (10 – 30 ants per minute) are shown as solid lines, while trails with lighter traffic (<10 ants per minute) are shown as dotted lines. Some trails appear to dead-end on this map where they entered areas of vegetation that were impassible to researchers.
Figure 5: The proportion of the total surveys in which each cactus was occupied by *C. opuntiae* in edge and interior areas at sites 1, 2, and 3. Error bars indicate the upper 75% quartile of the mean, and stars indicate significance to $P=0.05$. 


Figure 6: The change in the number of *C. opuntiae* workers visiting each cactus before and after nectar addition. The average number of ants increased on the focal (0m), 1m, and 5m plants, and decreased on the 10m plants. The dotted line represents the linear regression.
APPENDIX C- CONTRASTING MODELS OF ANT COLONY FORAGING

BEHAVIOR: INDIVIDUAL DECISION-MAKING AND SYMMETRY BREAKING
Contrasting models of ant colony foraging behavior: individual decision-making and symmetry breaking

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Key Words: ants, symmetry breaking, decision making, social insects, collective behavior, foraging
Abstract

A social insect colony is an example of a complex system, in which the interactions of many individuals lead to colony-level collective behavior. Here, we examine symmetry breaking, an emergent pattern exhibited by some social insects in which colonies presented with two or more identical food sources allocate foragers among them asymmetrically, often foraging exclusively at one while ignoring others. Symmetry breaking may be an unavoidable epiphenomenon that occurs in all ant species that use pheromone trail recruitment, or it may be an adaptive behavior related to the ecology of a species. In this paper, we used a simulation model to determine how symmetry breaking would depend on the behavior of individual ants. Specifically, we varied the decision rule used by individuals to choose between trails, and the ability of individuals to discriminate between trails with similar amounts of pheromone. We found that although asymmetry was greater than expectation without recruitment in all simulations, some rules produced patterns of foraging that were much more asymmetrical than others. Furthermore, the relationship between forager group size and asymmetry also varied among simulations using different behavioral rules. It is therefore likely that asymmetrical foraging is not an unavoidable epiphenomenon of pheromone trail recruitment, and that symmetry breaking may vary among ant species based on their foraging ecology. Variation in the rule used by individual ants to choose trails is a plausible mechanism that could cause variation among species, and is a behavior upon which selection could act. The wide variation in model behavior that we observed in this
study due to small changes in individual behavior also highlights the importance of examining details of model construction in studies of collective behavior.
Introduction

Social insects are one of the most often cited examples of self-organized complex systems. The interaction of individual insects following various behavioral rules can lead to a variety of complex colony-level behaviors, including group foraging raids (Franks et al. 1991), traffic lane formation (Couzin and Franks 2003), and construction of nests (Bonabeau et al. 1998). As modern technology and networks become increasingly complex, our need to understand how collective behaviors lead to emergent properties of systems increases. The behaviors exhibited by social insect societies are therefore of great interest not only to biologists, but to computer scientists and mathematicians as well (Bonabeau et al. 1999; Franks et al. 2009).

In social insects the foraging behaviors manifested by a colony are not centrally directed, but are rather the result of collective behavior of numerous interacting individuals. One emergent phenomenon that can sometimes arise from the collective behavior of social insects is symmetry breaking. Symmetry breaking is a general term that refers to the transition of any system from an unstable symmetrical state to one of several possible asymmetrical, stable outcomes. In social insects, symmetry breaking occurs when the number of foragers visiting each of two or more identical food sources diverges over time, so that one food source becomes more visited than the other (de Vries and Biesmeijer 2002). The mechanism of this shift in colony foraging behavior is the positive feedback of recruitment. Stochastic variation in the numbers of workers that initially encounter each food source results in small differences in recruitment to each source. These differences are subsequently amplified as more insects visit the source with
stronger recruitment, and the foraging behavior of the colony becomes asymmetrical. An example of this phenomenon from a simulation of ant foraging (described below) is shown in Figure 1.

The benefits of the feedback in recruitment that leads to symmetry breaking become more obvious when we consider the natural environment in which social insects live. Here, we consider ant colonies, many species of which use pheromone trails to recruit to food sources. In a natural environment, ant colonies are unlikely to encounter food sources that are truly identical in quality, distance, and retrievability. When food sources differ, recruitment to the superior source will usually occur at a faster rate, enabling colonies to make a collective decision about where to forage (de Biseau et al. 1991; Collignon and Detrain 2010). A similar feedback mechanism also enables colonies to choose the shortest route to a food source (Goss et al. 1990). In addition, the ability to recruit numerous workers to one food source quickly may enable ants to repel competitors, as competitive interactions between colonies are often resolved through numerical dominance (Hölldobler and Wilson 1990; Holway 1999). Workers are also less likely to become lost while walking on a single strong trail that arises through symmetry breaking, rather than several weak trails (Pasteels et al. 1987; Nicolis and Deneubourg 1999).

However, in certain situations symmetry breaking could potentially be costly for ants. If two food sources do not differ strongly, initial stochastic differences in recruitment could lead a colony to choose the inferior food source. If too great an asymmetry arises, a colony could also lose the trail to the second source entirely, missing
out on an opportunity to exploit that source later. Honey bees are known to avoid this problem by sustaining low-level recruitment to sites that are currently less profitable, in order to keep them in reserve (Beckers et al. 1990). However, mechanisms that pheromone-recruiting ants might use to avoid symmetry breaking have yet to be explored. Some evidence that symmetry breaking may not occur universally among ants that use pheromone trails is provided by ant species that maintain many long-lasting trails to different food sources over time (Pfeiffer and Linsenmair 2000), and from the ant Lasius niger, a species that has been shown to forage symmetrically or asymmetrically depending on colony nutritional needs (Portha et al. 2002).

Many previous modeling studies have investigated symmetry breaking in ants and other social insects (e.g. (Deneubourg et al. 1989; Goss et al. 1989; Deneubourg et al. 1990; Goss et al. 1990; Franks et al. 1991; Beckers et al. 1993; Bonabeau 1997; Nicolis and Deneubourg 1999; Sumpter and Pratt 2003; Collignon and Detrain 2010)), yet there is currently a lack of data linking this collective behavior to the ecology of species (but see (Lanan 2010)). Indeed, although symmetry breaking has been reported for seven ant species under controlled laboratory conditions (Deneubourg et al. 1986; Pasteels et al. 1987; Beckers et al. 1990; Goss et al. 1990; de Biseau et al. 1991; Robson and Traniello 1995; Portha et al. 2002; Sumpter and Beekman 2003) this phenomenon has only been demonstrated in a natural setting for honey bees (Apis mellifara) (Seeley 1986; Seeley et al. 1991). We currently know nothing about the frequency or variability of symmetry breaking among social insect species.
Because symmetry breaking has the potential to be either beneficial or costly to pheromone-recruiting ant colonies depending on their foraging needs, we hypothesized that occurrence of this phenomenon is likely to vary among species. Our alternate hypothesis is that symmetry breaking is an unavoidable epiphenomenon that results from pheromone trail foraging, occurring in all trail-foraging species and unrelated to their ecology. In this study, we used a simulation model to determine whether variation in symmetry breaking can occur among trail-foraging ant species that vary slightly in individual behavior. Specifically, we investigated the effect of particular choice rules used by individual foragers to decide between trails. Despite the diversity of potential decision rules available, previous models have all relied on a single choice rule without consideration of the possible effects of alternative mechanisms. We propose that the individual choice rule is a likely mechanism that could produce variation in symmetry breaking, as it directly affects the number of ants visiting each food source. Individual-level behavior is a trait upon which selection could act, leading to changes in emergent collective behaviors at the colony level.

**Model**

We created an individual-based model of ant foraging using the Visual Basic .NET (VB.NET, Microsoft Corp. 2008) programming language. In the model, a colony of ants forages at four identical food patches over a period of 1000 time steps. The basic structure of the model is illustrated in Figure 2a. At any given time step, each ant in the nest has a 0.01 probability of leaving the nest. This parameter value causes 25% to 30% of the potential foragers to be outside of the nest at any given time in all simulations.
Once out of the nest, a forager encounters the paths to all four food sources and chooses one based on the particular decision rule used (each described below). After walking on the trail and encountering the food source, each ant can either stay and feed or return directly to the nest, based on whether the patch is already filled to its capacity of ants. We tested two food patch sizes, limited patches that accommodated only 15 ants per time step, and unlimited patches. In the model, only ants that successfully feed and return to the nest add a constant pheromone value to the trail they used, and the overall strength of each trail is multiplied by a decay rate of 0.9 at each time step to simulate evaporation of the pheromone over time. This is similar to the pheromone laying behavior of many ant species (Dornhaus and Powell 2010). The ants in our model take 18 time steps to reach food patches after choosing a trail, and feed for 6 time steps before returning to the colony. These numbers are based on field observations of *Forelius pruinosis* foraging at baits (Lanan, unpublished data), where each time step is equivalent to 5 seconds. We performed sensitivity analyses on the time step values and decay rate, which are summarized in Appendix 1.

For all sets of parameters tested, we ran 100 simulations for each of 25 colony sizes ranging from 5 to 2000 ants, yielding average active forager numbers ranging from 1 to 600. In addition, for our analyses of the effect of varying $k$ and discrimination (described below) on the behavior of the model at small forager numbers, we ran 500 simulations for 20 colony sizes ranging from 5 to 200.

*Choice rules*
We simulated three separate choice rules that individual ants could use to choose between trails upon leaving the nest. In addition, we simulated a null expectation of random choice between trails, to which we compared the simulations (Table 1, Figure 3).

Using the simplest rule, called the rank choice rule, individual ants chose the strongest trail with a 25% error rate. The procedure for breaking ties between trails with similar pheromone values is described in Figure 2b. A similar rank choice rule has been used previously in two Monte-Carlo type simulation studies that describe ants making a trail through 2D space (Robson and Traniello 1995; Jackson et al. 2004).

The second rule we tested was called the linear choice rule. With this rule, we assumed that the preference of an individual ant for a trail was directly proportional to the amount of pheromone it contained relative to all trails.

The third, and most complex rule we tested was called the sigmoidal choice rule. A sigmoidal curve was first proposed by Deneubourg et al. (1990) and adapted by Nicolis and Deneubourg (1999) to describe the preference of an individual Argentine ant worker for a trail based on the amount of pheromone on it. If $c_i$ is the amount of pheromone on trail $i$, then the preference for trail $i$ (i.e. the probability the ant will take trail $i$) is described as

$$prob_i = \frac{(k+c_i)^x}{\sum_{j=1}^{s} (k+c_j)^x}$$  \hspace{1cm} (1)$$

where $s$ is the total number of trails. This formula produces a sigmoidal curve, the specific shape of which is determined by the constants $x$ and $k$. Increasing $k$ causes a decrease in the asymptote of the curve, while increasing $x$ causes an increase in the
steepness of the curve (Appendix 1). In the Argentine ant *Linepithema humile*, $k = 20$ and $x = 2$ (Deneubourg et al. 1990), while in *Lasius niger* $k = 6$ and $x = 2$ (Beckers et al. 1993). Figure 3 shows four examples of how the probability that an individual chooses a particular trail will vary with pheromone strength depending on these constants. This model of choice is plausible, since preference might initially increase steeply, then plateau as pheromone becomes more saturated on the most preferred trail (Nicolis and Deneubourg 1999), and it has been frequently used in many modeling studies of ant foraging that used differential equation (Goss et al. 1989; Deneubourg et al. 1990; Bonabeau 1997; Nicolis and Deneubourg 1999; Sumpter and Pratt 2003) or simulation models (Deneubourg et al. 1989; Goss et al. 1990; Franks et al. 1991; Beckers et al. 1993; Collignon and Detrain 2010).

Because previous models have not explored the effects of varying $k$ and $x$ on the asymmetry produced by ants using the sigmoidal choice rule, we chose to compare the behavior produced by this rule with several combinations of parameters. Specifically, we varied $k$ from 0 to 6 and $x$ from 2 to 5. We did not use the value 1 for $x$, as this is equivalent to using the linear choice rule.

None of the simulations included any individual memory for the location of food sources, despite the importance of memory to ant foraging that has been reported for many species (e.g. (Sundstrom 1993; Schatz et al. 1999; Schilman and Roces 2003; Narendra et al. 2007)). Due to the variety of ways in which memory could affect the system, we included no memory in this study in order to focus solely on the effects of varying the individual choice rules. However, we intend to add memory to the model for
future work in order to investigate its effect on asymmetry depending upon the choice rule used.

**Discrimination between trails**

The basic version of the model assumes that ants can discriminate between trails that vary in infinitely minute amounts of pheromone. However, in nature it is likely that ants can only discriminate between trails that vary by more than some threshold amount. To explore the effect of discrimination ability on the behavior of the model, we added a discrimination value that varied from 0 to 5, representing the minimum difference between quantities of pheromone that an ant can detect. A high value therefore represents coarse discrimination ability, while a low value represents fine discrimination ability. Hereafter in the text, simulations with a discrimination value of 5 are referred to as having ‘limited’ or ‘coarse’ discrimination ability, while simulations using a value of 0 are referred to as ‘fine’ or ‘infinite’ discrimination ability. In the model, the discrimination value was applied before the decision rule, so that trails that varied by an amount below the discrimination value were treated as equal.

**Analysis**

To describe how asymmetrically each group of ants foraged over time in each simulation, we calculated the average proportion of ants that visited the most preferred bait, \( a \). We then standardized this value so that it ranged from 0 to 1, using this formula.

\[
\text{Asymmetry} = \frac{(an-1)}{(n-1)}
\]  

(2)

The value \( n \) is the number of food sources a colony could visit, in this case 4. Because asymmetry calculated using this formula is not dependent on the number of baits, it can
potentially be used in future work to compare foraging asymmetry between experiments with varying numbers of food sources.

The behavior of the model typically stabilized within the first 50-70 time steps, so only the last 900 of the 1000 time steps simulated were used to calculate asymmetry. Because the asymmetry value is bounded by 0 and 1 and the distribution of the model output was frequently non-normal, we used non-parametric statistics for comparing output of the different models and the null. However, in order to describe the direction of the relationship between asymmetry and forager number we used linear regression for all models.

**Results**

**Effect of choice rules**

We show that asymmetry is strongly affected by the choice rule used (Figure 4). Although all choice rules produced asymmetry that was significantly greater than the null, the median asymmetry varied greatly among the rules (Table 2). Some rules produced mean asymmetry values that were much higher (>0.5) than the null (i.e. ranked choice rule, sigmoidal choice rule with \(k = 0\) and \(x = 2\) or 3). Other rules produced mean asymmetry values that were fairly low (~0.05), although still significantly higher than the null (i.e. linear choice rule, sigmoidal choice rule with \(k=6\) and \(x = 2\)). The rules also varied greatly in how much variation occurred in asymmetry between simulations (Table 2).
**Effect of forager number**

The relationship between average forager number and asymmetry was also strongly affected by the choice rule used (Table 2). Asymmetry decreased with increasing forager number using the linear choice rule and the sigmoidal rule when \( k = 0 \) and \( x = 2 \) and 3. Conversely, asymmetry increased with increasing foraging number using the sigmoidal rule when \( k=6 \) or 2 and \( x = 2 \). In simulations using the ranked rule, we observed no relationship between forager number and asymmetry, and the mean asymmetry across all forager numbers was approximately 0.66. This value is equal to the set preference for the highest ranked trail (0.75), transformed using Equation 1. Sensitivity analyses for this rule indicated that average asymmetry is always approximately equal to transformed preference, regardless of the value used (Appendix 1).

**Parameters of the sigmoidal choice rule**

Varying the asymptote and steepness of the sigmoidal choice rule had a strong effect on the behavior of the simulations, influencing both the mean asymmetry and the relationship between forager number and asymmetry. To better demonstrate the effect of reducing the asymptote by increasing \( k \), we plotted linear regression lines from sets of simulations using \( k = 0, 1, 2, 3, 4, 5, \) and 6 (Figure 5). As \( k \) increased (and the asymptote decreased), the slope of the lines decreased (Linear Regression, \( F_{1,5}=363.556, P<0.0001 \)), indicating that forager number had less of an effect on asymmetry as the asymptote decreased. The value 0 produced unique behavior of \( k \), with a negative slope and much larger intercept (Figure 5). Although all values of \( k \) we tested generated asymmetry
values that were significantly greater than the null at large forager numbers, reducing the asymptote by increasing $k$ caused a reduction in the mean asymmetry of the simulations.

Reducing the asymptote of the curve had a second effect, causing a steep reduction in asymmetry relative to the null at very low forager numbers (Figure 6). However, even when the simulations produced asymmetry values only slightly higher than those of the null, the difference was always significant (Wilcoxon Rank-Sum Tests, all $P<0.05$).

Mean asymmetry increased as we increased the steepness of the curve (by increasing $x$ from 2 to 5 with $k$ constant at 0, Linear Regression, $F_{1,9999}=7992.074$, $P<0.0001$). We did not include the value $x=1$, as this produces a preference curve that is identical to the linear model.

**Effect of Discrimination ability**

Limiting the ability of ants to discriminate between trails with similar amounts of pheromone created a threshold number of foragers, below which simulations were not significantly different from the null, but above which colonies foraged asymmetrically (Figures 7 and 8, Wilcoxon Rank-Sum Tests, $P<0.05$ for all choice rules). This threshold forager number increased linearly with the size of the minimum difference in pheromone that workers could detect (Linear Regression, $F_{1,5}=1011.434$, $P<0.0001$).

Reducing the ability of ants to discriminate between similar trails also caused a change in the median asymmetry produced, but the direction of the effect varied between choice rules. Reducing discrimination ability caused an increase in median asymmetry for the linear choice rule and the sigmoidal choice rule with $k=2$ or $k=6$ and $x=2$, but a
decrease in median asymmetry for the ranked choice rule (Wilcoxon Rank-Sum tests, all $P<0.001$). There was no effect of reducing discrimination ability on median asymmetry for simulations using the sigmoidal choice rule with $k=0$ (Wilcoxon Rank-Sum, $\chi^2_1=3.576, P=0.0586$).

**Effect of patch capacity**

Regardless of the choice model used, limiting the capacity for ants at the patches produced a damping effect on asymmetry as average forager number increased (Figure 9). With all choice models, asymmetry declined above a threshold number of ants, and all simulations except those produced using the ranked choice rule became indistinguishable from the null model at high forager numbers (for detailed statistical analyses, see Appendix 1).

**Discussion**

Varying the decision rule used by individual foragers to choose between trails strongly affected how asymmetrically the ants foraged in our simulation. Although all choice rules produced asymmetry values that were statistically higher than without recruitment (our null model), the very small difference from the null produced by some rules may not be a relevant difference ecologically. In contrast, other rules produced intermediate or very high asymmetry values, indicating that the feedback leading to symmetry breaking occurred very strongly in these simulations. These results confirm that symmetry breaking can potentially vary greatly among species depending upon small differences in individual behavior. It is therefore unlikely that symmetry breaking is
simply an unavoidable epiphenomenon of pheromone trail foraging that does not vary between species.

The variety of relationships between asymmetry and average forager number that we observed highlight the unpredictability and non-intuitive nature of emergent collective behavior. Some choice rules produced asymmetry that decreased with increasing forager number, while others produced an opposite pattern or no relationship at all. However, without the simulations, the direction of the relationship would have been difficult to predict simply based on specific individual behaviors used by ants. Our results suggest that the relationship between forager number and asymmetry may vary greatly among trail-foraging ants. Many of the rules caused the asymmetry of foraging to either increase or decrease as forager number increased. Whether variation in this relationship serves an adaptive function, for instance enabling colonies to shift foraging strategies as they grow in size, is an interesting question that could be addressed in future studies.

The threshold and threshold-like effects produced by some versions of the model are similar to the relationship between forager number and asymmetry previously reported for several ant species. Tetramorium casepitum only exhibits symmetry breaking over a threshold colony size, below which they forage symmetrically (Deneubourg et al. 1986). Similarly, the foraging behavior of Monomorium pharaonis undergoes a ‘phase transition’ from individual foraging to mass trail recruitment as colony size increases (Beekman et al. 2001), and this transition is also predicted by a model of ant behavior (Nicolis and Deneubourg 1999). Interestingly, in these studies, the abrupt change in colony-level foraging behavior occurred simply due to changing group size, despite the
unchanged behavior of individual ants (Beekman et al. 2001). In our study the only true thresholds occurred when we limited the discrimination ability of the ants. Although small forager numbers produced asymmetry values that were very low relative to the null using the sigmoidal curve rule, this was not a true threshold in that asymmetry was still significantly higher than the null. It would be difficult to discriminate between these two similar patterns, however, using empirical studies of ant foraging such as those described by Beekman et al. (2991).

The results of this study support our hypothesis that symmetry breaking could vary among ant species, by demonstrating that this variation is possible via a simple mechanism upon which selection can act. If variation occurs, it is likely that this reflects the adaptive function of symmetry breaking and is related to the foraging strategy and ecology of species. Because competitive interactions among ant species are often resolved through numerical dominance (Hölldobler and Wilson 1990; Holway 1999), strong symmetry breaking may be a mechanism that enables dominant ant species to exclude other species from resources. In contrast, for species that are lower on the dominance hierarchy and rely on rapid discovery of new food sources, symmetry breaking may be maladaptive. In fact, the tendency to create one strong trail would prevent workers of these species from exploring new areas. A recent study (Lanan 2010) provides the first empirical evidence that asymmetry is related to dominance and foraging strategy in an ant community.

Our study highlights the importance of examining not only the sensitivity of models to parameters and initial conditions, but also the details of how the model is
constructed (Wood and Thomas 1999). The type of choice rule that we used drastically changed the behavior of the model, yet each rule we tested could be a plausible mechanism for the way that workers decide between trails. Previous modeling studies of symmetry breaking in social insects have all relied on a single choice rule, yet the justification for the rule chosen is stronger in some studies than in others. The individual-based models of honeybee foraging created by de Vries and Biesmeijer (1998, 2002) are based on strong empirical evidence of the way bees are recruited to food sources, and thus are using an appropriate choice rule. The sigmoidal choice rule we tested here was originally deduced by Deneubourg et al. (1990) based on observations of the preference of an individual Argentine ant worker for a trail based on the number of workers that had previously walked on it. Although the curve was a good match for the Argentine ant data, this data has only been replicated for one other species (Lasius niger, Beckers et al., 1993), and no attempt was made in either case to determine whether this curve was significantly more appropriate to describe the behavior than other possible models. Nevertheless, the sigmoidal choice rule has been used widely in models of ant foraging behavior, with most studies simply assuming that it is a good fit for their system based on the Lasius niger and Linepithema humile data. Neither study that used the ranked choice rule (Robson and Traniello 1995; Jackson et al. 2004) provided justification for this choice, but it is likely that it was chosen based on its simplicity. Both of these Monte-Carlo type simulations model the formation of trails in space, as workers choose the next point to occupy based on pheromone strength. However, our results show that this choice rule produces patterns that are dissimilar from all other choice rules, and that in the
absence of limits on patch size or worker discrimination ability the asymmetry produced by this rule is higher than for many other rules. It may be, therefore, that these simulations would have produced different patterns of trail formation if different choice rules were tested.

Although symmetry breaking is an emergent property of collective behavior in pheromone-recruiting ants, we have provided evidence that this phenomenon has the potential to vary greatly among species based on small differences in individual behavior. Variation in symmetry breaking may reflect the ecology and foraging behavior of species, and we expect that future studies will reveal a wealth of variation in the collective behaviors of social insects, not only in symmetry breaking but in other emergent phenomena as well. However, because emergent phenomena in groups of organisms are frequently non-intuitive, we wish to emphasize the importance of examining details of model construction in studies of these systems.

Acknowledgements

We thank Benjamin Blonder, Jenny Jandt, Annie Leonard, Raine Kaczorowski, Matina Donaldson, Scott Powell, and Aimee Dunlap for comments on this manuscript. We would also like to thank the Center for Insect Science of the University of Arizona and National Science Foundation grant no. IOS 0841756 for funding this research.

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Sundstrom L (1993) Foraging responses of Formica truncorum (Hymenoptera, Formicidae) - exploiting stable vs spatially and temporally variable resources. Insectes Sociaux 40:147-161
Figure 1: Typical output of two simulations of ants foraging at 4 identical food sources A-D (model described in text). In simulation a), symmetry breaking does not occur, and asymmetry (calculated using Equation 2) is 0.065. In simulation b) symmetry breaking occurs, and asymmetry is 0.570. Simulation a) had 500 potential foragers and used the linear choice rule, while simulation b) had 500 potential foragers and used the sigmoidal curve choice rule with $k=0$ and $x=2$. 
Figure 2: Flow charts describing a) the basic individual-based model, and b) the ranked decision rule.
Figure 3: Several examples of models describing the relationship between an ants’ preference for a trail and the proportion of pheromone on that trail. The example of the ranked choice rule has a 25% error rate in this figure.

\[ \text{Sigmoidal curve (Equation 1)} \]

\[ \text{prob}_i = \frac{(k + c_j)^i}{\sum_{j=1}^{n} (k + c_j)^i} \]
### Table 1: Choice rules used in the simulations.

<table>
<thead>
<tr>
<th>Rule</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random choice rule</td>
<td>Ants choose trails randomly, never add pheromone, and have a 1/4 chance of choosing each trail</td>
</tr>
<tr>
<td>(null model)</td>
<td></td>
</tr>
<tr>
<td>Linear choice rule</td>
<td>Preference for each trail is directly proportional to the amount of pheromone it contains</td>
</tr>
<tr>
<td>Sigmoidal curve choice</td>
<td>Preference for each trail is calculated using Equation 1</td>
</tr>
<tr>
<td>rule</td>
<td></td>
</tr>
<tr>
<td>Ranked choice rule</td>
<td>Ants rank trails by strength, and choose the strongest with a 25% error rate (75% preference). If the strongest trail is not chosen the worker then uses the same decision-making process to choose between the remaining trails. (Fig. 3b)</td>
</tr>
<tr>
<td>Choice rule</td>
<td>Median asymmetry</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Ranked</td>
<td>0.662</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.0341</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmoidal $k=0$, $x=2$</td>
<td>0.604</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmoidal $k=2$, $x=2$</td>
<td>0.336</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmoidal $k=6$, $x=2$</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmoidal</td>
<td>0.844</td>
</tr>
</tbody>
</table>
\[ k = 0, x = 3 \]

\[
\text{Linear Regression, } F_{1,2499} = 473.530 \quad P < .0001
\]

\[
\text{Wilcoxon Rank-Sum, } \chi^2_1 = 152.423, \quad P < .0001
\]

Table 2: The different choice rules produced differing median asymmetry values, and also differed in the relationship between forager number and asymmetry and the spread of asymmetry values produced.
Figure 4: The asymmetry calculated for the simulations varied greatly depending on the decision rule used. In this figure we show the relationship between average forager number and asymmetry for a) the rank choice rule, b) the linear choice rule, c) the sigmoidal choice rule with \(k=0\) and \(x=2\), and d) the sigmoidal choice rule with \(k=2\) and \(x=2\), e) the sigmoidal choice rule with \(k=6\) and \(x=2\), and f) the sigmoidal choice rule with \(k=0\) and \(x=3\). The upper 95% quantile of the null is shown as a solid line on all graphs.
Figure 5: The linear regression lines for simulations using $k = 0, 1, 2, 3, 4, 5,$ and 6, when patch capacity is unlimited and discrimination ability is infinite. The upper 95% quantile of the null is included, and for all linear regressions shown, $P < 0.0001.$
Figure 6: The 95% quantiles of the asymmetry produced using simulations with different values of $k$. At every average forager number shown, the sigmoidal choice simulations produced asymmetry significantly higher than the null (Wilcoxon Rank-Sum Tests, all $P<0.05$).
Figure 7: Limiting the ability of individual ants to distinguish between trails with similar amounts of pheromone caused asymmetry to become indistinguishable from the null at low average forager numbers for a) the rank choice rule, b) the linear choice rule, c) the sigmoidal choice rule with $k=0$ and $x=2$, and d) the sigmoidal choice rule with $k=0$ and $x=3$. The upper 95% quantile of the null is shown as a solid line on all graphs.
Figure 8: The 95% quantiles of the asymmetry produced using simulations with the linear choice rule and varying discrimination values.
Figure 9: Asymmetry decreased at large average forager numbers when patch size was limited for a) the rank choice rule, b) the linear choice rule, c) the sigmoidal choice rule with $k=0$ and $x=2$, and d) the sigmoidal choice rule with $k=0$ and $x=3$. The upper 95% quantile of the null is shown as a solid line on all graphs.
Appendix 1

Sensitivity analysis: time step values

In order to examine the sensitivity of the simulations to the time step values, we tested several combinations of trail and feeding times that varied in the total time outside of the nest and the ratio of time spent on the two activities (Figure 1).

We found that the relationship between the average number of foragers and asymmetry remained similar for each decision model tested (Linear regressions, all $P<0.05$). However, there was a positive relationship between the proportion of foragers at any given time and the total forager group size as the total time outside the nest increased (linear choice rule, Linear regression: $F_{1,7499}=222995.2$, $P<0.0001$, sigmoidal choice rule, Linear regression: $F_{1,7499}=234656.3$, $P<0.0001$). This indicates that if we had used larger time step values, our results would have been similar, but with data across a larger span of forager numbers.
Figure 1: asymmetry across average forager numbers for a) the linear choice model and b) the sigmoidal choice model with $k=0$ and $x=2$ for different combinations of time step values.

*Sensitivity analysis: pheromone decay rate*

Because all trails are added to by the same amount and decay at the same rate, the specific decay value used does not influence the proportion of pheromone between trails.
However, decay rate could potentially influence the outcome of simulations that used limited discrimination ability (discrimination value of 5), because the ability of ants to detect differences in these simulations is based on the amount rather than proportion of pheromone.

We found that a wide range of pheromone decay rates produced asymmetry values that did not differ significantly (Figure 2), even when we included a discrimination value of 5. We compared the asymmetry produced by the linear rule and discrimination 5, using decay rates of 0.95, 0.5, 0.1, and 0.01 (Figure 2a, Wilcoxon Rank-Sum, $\chi^2_3=0.676$, $P=0.879$). Similarly, there was no difference for simulations using the sigmoid curve rule with $k=0$ and $x=2$ (Figure 2b, Wilcoxon Rank-Sum, $\chi^2_3=3.010$, $P=0.390$). These results indicate that the behavior of the model is consistent across a wide range of decay values, and that 0.9 was a reasonable parameter choice for our study.
Figure 2: The asymmetry values produced across a range of decay values for a) simulations using the linear choice rule and a discrimination value of 5, and b) simulations using the sigmoid curve rule with $k=0$ and $x=2$ and a discrimination value of 5. Boxes indicate quantiles.

**Sensitivity analysis: error in the ranked rule**

Sensitivity analyses for the effect of the percentage error used with this rule (i.e. 1- preference) indicated that average asymmetry is always approximately equal to the preference for the highest ranked trail transformed using Equation 1 (Figure 3, Linear regression, $F_{1,7499}=620714.3$, $P<0.0001$). The slope of the regression line is slightly less than 1 because we are using the most preferred trail to calculate asymmetry, and thus even equal preference for all trails (0), the values will all be distributed above 0.

![Figure 3: the relationship between asymmetry and preference (1-error) transformed by Equation 1, using the ranked rule.](image)

**Sensitivity analysis: effects of varying x and k on the sigmoidal choice rule**
The parameters $x$ and $k$ determine the shape of the sigmoidal curve. Increasing the value $k$ causes a decrease in the asymptote of the curve (Figure 4a), while increasing $x$ causes an increase in the steepness of the curve (Figure 4b).

Figure 4: a) Increasing the constant $k$ from 0 to 8 with $x$ held constant at 2 causes a decrease in the asymptote of the curve. b) Increasing $x$ from 1 to 7 with $k$ held constant at 0 causes the steepness of the curve to increase.
**Effect of patch capacity**

In Table 1, we provide detailed information on the point at which limiting patch capacity causes a decrease in asymmetry, as well as the point at which asymmetry with limited patch size becomes indistinguishable from the null.

<table>
<thead>
<tr>
<th>Choice rule</th>
<th>Divergence point with simulations of unlimited patch capacity</th>
<th>Convergence point with null simulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear choice rule</td>
<td>203 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 10.2924, P=0.0013$)</td>
<td>398 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 3.467, P=0.0626$).</td>
</tr>
<tr>
<td>Ranked choice rule</td>
<td>90 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 4.8466, P=0.0277$).</td>
<td>Always significantly greater than null</td>
</tr>
<tr>
<td>Sigmoidal curve choice rule, k=0</td>
<td>146 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 10.324, P=0.0013$).</td>
<td>449 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 0.752, P=0.3857$).</td>
</tr>
<tr>
<td>Sigmoidal curve choice rule, k=2</td>
<td>174 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 6.482, P=0.011$).</td>
<td>427 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 3.667, P=0.0665$).</td>
</tr>
<tr>
<td>Sigmoidal curve choice rule, k=6</td>
<td>174 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 4.143, P=0.0418$).</td>
<td>398 foragers (Wilcoxon Rank-Sum, $\chi^2_1 = 0.646, P=0.421$).</td>
</tr>
</tbody>
</table>

Table 1: The average number of foragers at which simulations with patch capacity 15 diverged from simulations with unlimited capacity (i.e., the point at which asymmetry values were significantly lower), and the number of foragers at which simulations with patch capacity 15 converged with the null (i.e. no significant difference detected).
APPENDIX D- FORAGING STRATEGY AND SYMMETRY BREAKING IN DESERT ANTS
Foraging strategy and symmetry breaking in desert ants

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Key Words: ants, symmetry breaking, social insects, collective behavior, foraging, dominance, competition, colony size
Abstract

We explore the ecological and evolutionary significance of symmetry breaking, an emergent property of collective behavior in ant colonies. Symmetry breaking occurs when colonies presented with identical food sources allocate foragers among them asymmetrically, due to initial stochastic variation and positive feedback in pheromone recruitment. We compared the occurrence of symmetry breaking across species in an ant community with a clear dominance hierarchy and evidence of a trade-off between numerical dominance and discovery ability. Competitive dominance was strongly correlated with how asymmetrically colonies foraged, with the highest-ranking species exhibiting the most asymmetry. In a community structured by a trade-off between numerical dominance and discovery ability, symmetry breaking may enable dominant species to recruit large numbers of workers to resources. Species lower in the hierarchy may need to avoid symmetry breaking in order to rapidly explore their environment and discover new food sources. Thus, symmetry breaking is not simply an epiphenomenon of recruitment. The degree to which it occurs can be related to the ecology of a species.
Introduction

Ants are of great interest to ecologists due to the wide variety of ecological roles they play, their ubiquity in habitats around the world, and the variety of significant behaviors they exhibit (Hölldobler & Wilson 1990). Ant colonies are also a common and accessible example of a complex system, in which a number of sophisticated colony-level behaviors arise from the collective interactions of many workers following simple behavioral rules. For this reason, ants are also of interest to biologists, computer scientists, and mathematicians studying the emergent properties of self-organized complex systems (Bonabeau et al. 1999; Franks et al. 2009). However, the collective nature of ant colonies also poses a problem, due to the difficulty of distinguishing emergent behaviors that are simply unavoidable epiphenomena of collective behavior from emergent behaviors that evolved to serve adaptive functions.

One example of an emergent behavior occurs in ant colonies that use pheromone recruitment during foraging, when the colony is presented with two identical food sources at equal distances from the nest. Although workers initially discover both sources, the number of foragers visiting each often diverges over time until one source becomes preferred (Deneubourg et al. 1986). This pattern of asymmetrical foraging, symmetry breaking (Pasteels et al. 1987), can occur because initial stochastic variation in the number of workers that encounter each source becomes amplified via pheromone recruitment. Symmetry breaking has been demonstrated under laboratory conditions for seven ant species that use pheromone recruitment (e.g. (Deneubourg et al. 1986; Pasteels et al. 1987; Beckers et al. 1990; Goss et al. 1990; de Biseau et al. 1991; Robson &
Traniello 1995; Portha et al. 2002; Sumpter & Beekman 2003). Although it is presumed to occur in nature as well, this has not yet been demonstrated. Herein we provide the first evidence for its existence in the wild.

Symmetry breaking may simply be a consequence of pheromone trail formation in ants, perhaps an unavoidable result of the feedback that occurs during recruitment. Alternately, it may be an evolved adaptive trait, and mechanisms could exist that enable species to enhance or avoid symmetry breaking depending on their foraging needs. It is currently unclear whether all ant species that use pheromone recruitment will exhibit symmetry breaking, or whether the phenomenon varies among species. Many authors have speculated about possible adaptive benefits of symmetry breaking in ants. For instance, by concentrating the workforce in one location, colonies may be better able to defend a food source against competitors (Deneubourg et al. 1986); alternatively, workers might be less likely to become lost while walking on one strong trail rather than several weak trails (Pasteels et al. 1987; Nicolis & Deneubourg 1999). In the vast majority of situations that ant colonies encounter in the wild, resources are not perfectly symmetrical. When food sources differ in distance or quality, the same feedback mechanism that causes symmetry breaking can enable colonies to make the correct decision about which food source to visit (de Biseau et al. 1991; Collignon & Detrain 2010).

Despite the existence of ecological hypotheses for its function, previous studies have generally explored symmetry breaking from a purely mechanistic perspective under laboratory conditions. The occurrence of symmetry breaking has not as yet been linked to
the ecology of individual ant species, and no interspecific comparisons of this phenomenon have yet been conducted.

Here we examine whether symmetry breaking is simply an emergent property that arises through the interaction of many individuals in an ant colony, or whether this phenomenon might serve an evolved adaptive function. If symmetry breaking were simply an epiphenomenon associated with pheromone recruitment, we would expect it to be ubiquitous among pheromone-recruiting ant species, with any variation being uncorrelated with ecological factors. We sought to relate the occurrence of symmetry breaking to the competitive dominance and foraging strategy of species in an ant community. We hypothesized that in an ant community structured by a trade-off between dominance and discovery ability (Fellers 1987; Davidson 1998), highly dominant species would exhibit the most asymmetrical foraging, due to their reliance on numerical dominance to displace competitors (Hölldobler & Wilson 1990; Holway 1999).

Methods

Study system

Experiments were conducted in the field at the Desert Laboratory in Tucson, Arizona, USA (32°13’11”N; 111°00’14”W), a research station that has been continuously protected from grazing since 1907 and that consists of Sonoran Desert scrub habitat. Although previous studies have investigated ants that interact with nectar-secreting cacti at this site (Morris et al. 2005; Ness 2006; Ness et al. 2006, 2009), little is known about its ant community as a whole. We used two locations approximately 1km apart for the baiting experiments. One location, at an elevation of 739m, contained
relatively flat alluvial soil. The second location was at an elevation of 850m, on rocky, sloped terrain. The diurnal ant fauna at these two locations is similar except that one species, *Solenopsis xyloni*, is less common at the second location (W.F. Morris, J. Ness, and J.L. Bronstein, *unpubl. data*). The experiments for assessing symmetry breaking were conducted at the same locations, as well as at two other sites 2-3 km away. Choice of sites for the symmetry breaking experiments was limited by the availability of ant nest entrances on flat soil with space for the experimental apparatus, described below.

**Measuring dominance and discovery ability**

In order to measure the dominance and discovery ability of different ant species, we observed their foraging behavior and interactions on protein baits. This technique has been successfully used to assess competitive hierarchies in a variety of ant communities (Fellers 1987; Holway 1999; LeBrun 2005; Lebrun & Feener 2007; Feener *et al.* 2008), and has become a standard method for assessing ant dominance hierarchies (Parr & Gibb 2010). During July and August 2009 and August 2010, we placed baits every 5 m along transects and observed them every 10 min for one hour, then every 20 min for 40 min, for a total of 8 observations over 100 min. We observed the baits between 0600h and 0800h or between 1700h and 1900h, when all members of the diurnal ground-foraging ant community were active (Lanan, *personal observation*). Each bait consisted of 14-15 ml of tuna canned in water, placed directly onto the ground. Because many of the ant species in this community prefer to forage on wet food (Lanan, *personal observation*), we periodically refreshed the liquid on each bait with more liquid from the tuna can during the experiment. After each baiting session we removed the remaining tuna from the field.
site. A new transect was used for every trial. In total, we observed 292 baits. During our observations, we recorded the number and identity of the ant workers that were in physical contact with each bait.

We used the Colley matrix method for assessing dominance (Colley 2002). This method has been used to describe ant dominance hierarchies in several recent studies (Lebrun & Feener 2007; Feener et al. 2008), and is superior to ranking methods that use only the total number of confrontations won (e.g., Santini et al. 2007; Parr & Gibb 2010) because it also accounts for the relative strengths of the competitors in each interaction. Thus, a species that won one confrontation against a strong competitor (i.e., a competitor that won a large proportion of the total interactions it participated in) received a higher Colley ranking than a species that won one confrontation against a weak competitor (i.e., a competitor that lost a large proportion of the total interactions it participated in). Based simply on the total proportion of interactions won, these two species would be ranked equally.

To construct the dominance interaction matrices needed to calculate the Colley rankings, we used the methods for assessing interaction outcomes described by Feener et al. (2008). An ant species was recorded as winning a confrontation if (a) it discovered the bait first, then successfully prevented a second species that arrived later from foraging on the bait, or (b) it arrived second at the bait, displacing the first arrival. Two other possible interactions described by Feener et al. (2008), that (c) a species was expelled temporarily from a bait but returned to displace the new arrival, and (d) a species co-occurred with another on a bait but removed most of the resource, did not occur during our
observations. Interactions in which we could not assess the outcome (for instance, cases in which two species continued to interact on the bait until the end of the 100 minute observation period) were not included in the analysis.

We used these data to construct the Colley matrix. Diagonals in the matrix contain the total number of interactions that each species participated in plus two. The off-diagonals contain the number of interactions between each pair of species, multiplied by -1. To calculate the Colley dominance values, the matrix is multiplied by a column containing win and loss data for each species, calculated as 1+(wins-losses)/2. For more information on the Colley matrix ranking method, see Colley (2002).

Because some ant communities are structured by a trade-off between dominance and discovery ability (Fellers 1987; Davidson 1998), we examined our baiting data for evidence of this trade-off in our community. For every occurrence of each species that we observed on the baits, we measured discovery time as the time it took for at least one worker to discover the bait, and recruitment as the maximum number of workers we observed on the bait at one time over the 100 min observation period. All baits were considered independent and data from all transects were pooled.

**Measuring symmetry breaking in the field**

In order to investigate the relationship between foraging asymmetry and dominance, we compared the foraging behavior of three ant species. We chose these species before conducting the dominance tests, because they were abundant enough at the sites for sufficient replication of the experiment. The species that we chose a priori were *Solenopsis xyloni*, *Forelius pruinosus*, and *Dorymyrmex insanus*. 
To test whether these species would exhibit symmetry breaking in the field, we allowed colonies to forage on a symmetrical maze with four identical food sources at equal distances from the nest entrance (Figure 1). Four baits, which consisted of 1 cm slices of Ball Park brand hot dogs, were placed on the endpoints of the four arms of the maze. A new cardboard maze was used for every experimental trial to ensure that no pheromones were present at the start of the experiment.

We observed each colony as it foraged on the maze for a total of five hours, and recorded the number of ants walking on each of the four arms of the maze, as well as the number of workers in physical contact with the baits. During the first hour observations were made every 10 min, after which observations were made every 20 min for the remainder of the experiment, for a total of 18 observations. We replicated this experiment 10 times for each of the three ant species. Data for 3 trials with S. xyloni and 2 trials with D. insanus were discarded, because these colonies ceased foraging partway through the trial due to changing weather conditions.

To describe how asymmetrically each colony foraged on the maze (i.e., how “strongly” symmetry breaking occurred), we calculated the average proportion of ants that visited the most preferred bait, \( a \). We then standardized this value so that it ranged from 0 to 1, using the formula:

\[
Asymmetry = \frac{(an-1)}{(n-1)}
\]  
(2)

The value \( n \) is the number of baits a colony could visit, in this case 4. The lowest asymmetry value, 0, indicates that the colony foraged completely symmetrically (25% of workers visiting each bait), while the highest value, 1, indicates that the colony foraged
completely asymmetrically (100% of workers visiting one bait). Asymmetry for each trial was calculated using the 12 observations made during the last four hours of the experiment. The first six data points were not used, ensuring that all four baits had already been discovered in every trial.

Experimental asymmetry values were compared to a null expectation for the range of asymmetry values we would expect to find if colonies were not using pheromone recruitment and ants chose trails randomly. To generate this null expectation, we used an individual-based model of ant foraging (Lanan, Dornhaus, Jones, Waser, Bronstein, in prep) in which ants had a 25% chance of choosing each of the four trails. We used this model to generate asymmetry values for 500 simulations at each of 20 total forager pool numbers ranging from 5 to 2000 workers. Here we present both the experimental data and the null simulations in terms of the average number of foragers on the trails and baits over the course of the experiment.

**Results**

**Dominance and discovery ability**

The baiting experiment revealed a clear dominance hierarchy among the 11 most common ant species we observed at the baits (Table 1). Ants visited 91.1% of the baits, and encounters that could be classified as clear wins for one species occurred on 41.4% of the baits. 26.7% of the baits were discovered by only one ant species. On the remaining 23.0% of the baits, we could not determine the outcome of the interaction because neither species was displaced before the end of the observation period, or the ants did not overlap in the times that they visited the baits. The Colley dominance
rankings (Table 1) were highly correlated with the proportion of confrontations won (Spearman Rank Correlation, $\rho=0.9358$, $P<0.0001$).

There was clear evidence of a trade-off between dominance and discovery ability among the species in this ant community (Figure 2). The maximum number of workers of each species recruited to each bait over the course of the observations increased with increasing Colley dominance rank (Figure 2a, Spearman Rank Correlation, $\rho=0.3628$, $P<0.0001$). Similarly, time to initial discovery of the baits increased as the Colley dominance ranking increased (Figure 2b, Spearman Rank Correlation, $\rho=0.1071$, $P=0.0226$).

**Symmetry breaking in the field**

Because the goal of this study was to compare symmetry breaking among species across a community dominance hierarchy, we chose three species to test with high, intermediate, and low Colley rankings. These species were *Solenopsis xyloni* (Colley ranking 0.788), *Forelius pruinosus* (Colley ranking 0.597), and the introduced species *Dorymyrmex insanus* (Colley ranking 0.393). The average maximum number of ants recruited to the baits varied significantly among species (ANOVA $F_{2,214}=26.409$, $P<0.0001$, Tukey-Kramer HSD test), and increased with increasing Colley rank. However, these three species did not differ significantly in the time it took them to discover baits (ANOVA $F_{2,214}=0.230$, $P=0.795$).

Foraging asymmetry was positively correlated across species with Colley ranking (Figure 3a, Linear Regression, $F_{1,24}=5.614$, $P=0.026$), with symmetry breaking occurring most strongly in the most dominant species, *S. xyloni*. To examine how the asymmetry
values varied in comparison to the null expectation of random foraging, we subtracted the upper 95% quartile of the null model from each value. This difference is plotted in Figure 3b for each of the three species. The difference between asymmetry and the null expectation also increased significantly with Colley ranking (Linear Regression, $F_{1,24}=22.442$, $P<0.0001$).

Across species, the average number of foragers observed during the symmetry breaking experiments had a significant, yet contrasting effect on asymmetry (Figure 4). The asymmetry exhibited by colonies of the most dominant species, *S. xyloni*, did not change significantly as forager number increased (Linear Regression, $F_{1,6}=2.820$, $P=0.154$). In contrast, the asymmetry exhibited by *F. pruinosus* colonies decreased significantly as forager number increased (Linear Regression, $F_{1,9}=8.361$, $P=0.020$). The *D. insanus* colonies we tested had a much smaller, more limited range of average forager numbers. For three of the seven colonies of this species that we tested, the asymmetry values fell below the 95% quartile of the null model, and we could not reject the null hypothesis of random trail choice by foragers in those trials. In contrast, all asymmetry values calculated for *S. xyloni* and *F. pruinosus* were significantly above the 95% quartile of the null model, suggesting that we can reject the hypothesis of random trail choice for these species.

Two examples of data from the symmetry breaking experiment are presented in Figure 5. In Figure 5a, a colony of *F. pruinosus* foraged relatively symmetrically among the four baits, and had an asymmetry value of 0.094. In contrast, a similarly-sized colony of *S. xyloni* (Figure 5b) exhibited strong symmetry breaking with an asymmetry value of
0.701. Asymmetry values for both examples were higher than the 95% quartile of the null simulations (0.02) at the same forager group size.

**Discussion**

This study provides the first evidence that symmetry breaking occurs in the field among ant species that use pheromone trail foraging. Furthermore, we show that competitive dominance in the three species tested was correlated with how asymmetrically colonies foraged, demonstrating that symmetry breaking may be correlated with ecological variables. The species that ranked at the top of the dominance hierarchy in this community, *S. xyloni*, also exhibited the most strongly asymmetrical foraging, while the asymmetry exhibited by the comparatively less dominant *D. insanus* was low and could not be distinguished from the null in all cases. We also present the first clear evidence that the degree to which symmetry breaking occurs varies among species that use pheromone trail recruitment, and that the relationship between forager number and foraging asymmetry varies among species. Because we have evidence that foraging asymmetry in this community is associated with competitive ability, we can therefore reject the hypothesis that symmetry breaking is simply a nonadaptive epiphenomenon of pheromone recruitment. Instead, symmetry breaking could potentially be an evolved adaptation to the particular foraging ecology of a species. Although we present data from just three species here, future studies of a wider range of species with enable us to explore hypotheses about the evolution of this collective behavior.

The differing degrees to which *S. xyloni*, *F. pruinosus*, and *D. insanus* foraged asymmetrically may reflect differences in their foraging strategies, and should be
considered in the context of the ant community to which they belong. Like several previously described ant communities, the Sonoran desert ant community we described has a clear dominance hierarchy and appears to be structured by a trade-off between numerical dominance and discovery ability (Fellers 1987; Davidson 1998). Symmetry breaking is likely to have an effect on both of these factors. Dominant species such as S. xyloni that tend to rely on numerical dominance to displace competitors would benefit from strongly asymmetrical foraging patterns, because the majority of the workforce could be recruited to one source. However, this numerical dominance may come at a cost, because as recruitment to one source increases, trails to other sources become rarely travelled. The resulting loss of trails may limit the number of food sources a colony can exploit at one time. In contrast, symmetry breaking may be maladaptive for species such as D. insanus that rely more on rapid discovery of new food sources. Workers of these species may frequently explore their environment searching for new, unexploited sources, with a lower tendency to follow pheromone trails to previously discovered sources. Thus, in ant species that use pheromone recruitment, symmetry breaking is a single colony-level trait that could account for the trade-off between numerical dominance and discovery ability. Variation in the behavioral responses of individual workers to pheromone trails is a likely mechanism that can cause differences in symmetry breaking among species (Lanan, Dornhaus, Jones, Waser, Bronstein, in prep), and is a trait upon which selection could act.

Not only did the three species we described vary in the degree to which they exhibited symmetry breaking, they also differed in the relationship between the number
of active foragers in the colony and asymmetry. For *S. xyloni*, asymmetry increased as forager number increased, but we observed the opposite pattern in *F. pruinosus*. This result suggests that the relationship between colony size and symmetry breaking is not consistent among ants, as neither of these species followed the patterns described for *Monomorium pharaonis*, *Tetramorium caespitum*, and *Lasius niger* (Deneubourg et al. 1986; Beekman et al. 2001; Portha et al. 2002). Modeling studies show that the relationship between forager number and asymmetry can change depending on the specific behavioral responses of individual workers to trails (Lanan, Dornhaus, Jones, Waser, Bronstein, *in prep*). Specifically, the rule used by individual workers to choose between trails can strongly affect the relationship between forager number and asymmetry. Furthermore, in a previous study of *Lasius niger* the degree to which colonies forage asymmetrically depended upon the amount of brood and colony nutritional needs (Portha et al. 2002), suggesting that symmetry breaking can be a flexible trait within a single species.

In this study, we demonstrated that although symmetry breaking is an emergent property that arises through the interaction of many individuals in an ant colony, the degree to which it occurs and its relationship to forager number varies among species. It is not simply an epiphenomenon of pheromone trail foraging, and foraging asymmetry can be related to the competitive dominance and foraging strategy of a species. Further, we have proposed a mechanism through which selection could act on symmetry breaking, through variation in the behavioral responses of individual workers to trails. This study provides an example of how investigating emergent group behaviors in an ecological
context can help us understand the adaptive function of these phenomena in groups of social organisms.

Acknowledgements

We would like to thank Blake Pellman for assistance in the field. We would also like to thank the Center for Insect Science of the University of Arizona and National Science Foundation grant no. IOS 0841756 for funding this research.

References


Figure 1: The experimental apparatus used to measure symmetry breaking in the field. The apparatus consisted of a laser-cut cardboard maze, supported 5 cm above the ground with five cardboard support posts. A central post was placed directly on the entrance of the ant nest, allowing ant workers to climb through holes to the center of the maze. Four identical arms of the maze radiated outward from the center, and were supported by cardboard posts at their endpoints. The total length of each arm of the maze was 75 cm, and ants were prevented from accessing the arms from the ground via the support posts by petri dishes coated in Fluon on the outside and inside.
<table>
<thead>
<tr>
<th>Species</th>
<th>Number of observations</th>
<th>Number of confrontations</th>
<th>Colley dominance ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solenopsis xyloni</em></td>
<td>12</td>
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<tr>
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<td><em>Pheidole sp. 4</em></td>
<td>1</td>
<td>0</td>
<td>-</td>
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</table>

Table 1: The number of observations on the baits, number of confrontations observed, and Colley dominance ranking for the 14 species that visited the baits. Colley rankings cannot be calculated for species for which we did not observe confrontations.
Figure 2: a) The maximum number of workers of each species recruited to each bait over the course of the baiting experiment increased with increasing Colley dominance ranking. 

b) The time to initial discovery of the baits for each species increased with increasing Colley dominance ranking. Error bars indicate the standard deviations of the mean, and data for the three species used for the symmetry breaking experiment are labeled. Dotted linear regression lines are shown to indicate the direction of the relationship between the variables (all $P<0.05$), although these correlations are described using non-parametric Spearman Rank Correlation tests in the text.
Figure 3: a) The amount of asymmetry exhibited by each colony increases with the Colley ranking for each species. b) The difference between the asymmetry values and the upper 95% quartile of the null simulations increases with the Colley rankings for each species. Linear regression lines (both $P<0.05$) are shown as dotted lines.
Figure 4: The asymmetry exhibited by *S. xyloni*, *F. pruinosa*, and *D. insanus* over a range of forager group sizes. The top 95% quartile of the asymmetry for the null simulations of random trail choice is plotted across forager numbers as a black line. Linear regression lines are shown for *S. xyloni* (dashed line) and *F. pruinosa* (dotted line).
Figure 5. Two examples of experimental symmetry breaking data for a) a colony of *F. pruinosus* and b) a colony of *S. xyloni*. The lines represent the number of workers visiting each of the four baits over time. The experimental trial for the colony shown in graph a) had an asymmetry value of 0.094, and the number of foragers on the maze at any given time, averaged over the whole experiment, was 216. The colony shown in graph b) had an asymmetry value of 0.701, and the average number of foragers was 225.