CAUSES AND CONSEQUENCES OF INTRASPECIFIC VARIATION IN BEHAVIOR OF THE RED IMPORTED FIRE ANT

A Dissertation

by

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ABSTRACT

Organisms vary at the individual and population level in many ecologically relevant traits. This study documents and quantifies colony-level variation in ecologically important behaviors of a widespread invasive social insect, demonstrates multitrophic ecological effects of this colony-level variation, and explores genetic factors that may affect and predict behavior at the colony-level.

I quantified significant, persistent regional and colony-level variation in the red imported fire ant (*Solenopsis invicta*) in behaviors such as extra-nest activity, exploration, and resource discovery speed and recruitment effort. Colony behavior correlated with both colony productivity and colony growth. Using single-lineage colonies, I estimated broad-sense heritability of between 0.45 and 0.5 for the observed colony behaviors.

I created experimental microcosms comprised of fire ant colonies, plants, and insect herbivores. Differences in fire ant colony behavior linked to carbohydrate attraction directly impacted herbivore mortality and indirectly impacted plant damage.

I quantified colony differences colony differences in the expression of the fire ant *foraging* gene (*sifor*) as well as colony-level differences in behavior for fire ant colonies collected from across a large area of Texas. Expression of *sifor* was more than three-fold higher in fire ant foragers than in fire ant workers in the interior of the nest, and colony-level differences in *sifor* expression of foragers and interior workers correlated with

colony behavior. Higher *sifor* expression in foragers correlated with higher foraging activity, exploratory activity, and recruitment to nectar in fire ant colonies.

Finally, I explored the hypothesis that fire ant foundress groups could maximize inclusive fitness benefits and alter cooperative and competitive behaviors in response to cues indicating higher relatedness of foundresses. I found that group and queen performance was significantly affected by group composition. Groups composed of foundresses that were less likely to be related produced no more workers than queens founding alone, while groups composed of foundresses from the same site produced the most workers of all group types.

The conclusions of this study have widespread implications for many social insects and their ecological interactions. By further exploring these effects at the mechanistic, organismal, and ecological level we will improve our understanding of collective behavior, social evolution, and intraspecific variation.

DEDICATION

I’d like to dedicate this to work to my polydomous, highly cooperative family, who have supported me every step of the way. To my brothers Eric, Scott, and Ryan: thanks for being the best worker-sibs a sister could have. Eric, you know how many times you saved the day. To Mom, our queen ant: thanks for always believing in my dreams and giving me the tools and encouragement I needed to succeed. I told you when I was five that I was going to become a tap-dancing doctor. I’m sure I’ll work in some tap-dancing lessons soon.

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CHAPTER I

INTRODUCTION

1.1 Intraspecific variation

Animal behavior is a fundamental topic of ecology. Behavior affects every aspect of an organism's life from the way it seeks out the resources it needs to survive, to the way it interacts with members of its own or other species. Many studies have looked at how these behaviors are regulated as well as how they vary between species (Alcock and Farley 2001). A surge of recent studies, however, have increasingly highlighted the widespread biological significance of behavioral variation within a species (Smith et al. 2008, Bolnick et al. 2011, Jandt et al. 2014). Organisms vary at the individual and population level in many ecologically relevant traits (Bolnick et al. 2011). As has been long understood, heritable variation in a trait allows natural selection to act on behaviors, affecting everything from adaptation to speciation (Fisher 1930). Additionally, however, increasing evidence has demonstrated the importance of not just the mean, but the variance of a trait in predicting ecological effects (Benedetti-Cecchi 2003, Bolnick et al. 2003, Hughes et al. 2008a). Intraspecific variation in behavior has important ecological consequences because it affects both the adaptive pathways available to an organism and also the way in which the organism interacts with its environment and other members of the ecosystem (Hughes et al. 2008a).

1.2 Foraging behavior and social insects

Foraging behavior provides a particularly appropriate trait for the exploration of intraspecific variation. The ability to successfully acquire resources is an essential component of fitness. For many animals, foraging is an activity that occupies a large portion of their lifespan and carries some of the greatest risks they will face (Barbosa and Castellanos 2005, Stephens et al. 2007). Foraging may require entering dangerous or unknown environments, may attract or expose organisms to predators, and may place organisms in direct or indirect competition with others. Models of the "ecology of fear" suggest that animals must balance the potential costs of risky behaviors against their other fitness needs (Brown et al. 1999). For this reason, foraging strategies can be expected to be under intense selection. Indeed, optimal foraging theory suggests that animals must weigh costs and benefits of different foraging strategies to maximize fitness (Stephens and Krebs 1986).

Social insects employ very complex foraging behaviors. The individual must make foraging decisions based not on individual state but the nutritional state of the group (Dussutour and Simpson 2009). The selective unit is the colony, but regulation of behavior, whether genetic or environmental, must happen at the level of the individual (Wilson 1968). Moreover, for many social insects such as ants and bees, foraging excursions, in which workers must leave the relative safety of the nest and the group, represent a substantial risk to the individual (Oster and Wilson 1979, Porter and Jorgensen 1981). Social insects have evolved complex gene regulatory pathways in order to modulate division of labor (Ben-Shahar 2005, Page et al. 2006). Caste systems allow worker specialization which may extend from the behavioral phenotype to worker morphology, and may be fixed, or vary with environmental or developmental effects (Wilson 1968, Wheeler 1991, Smith et al. 2008). In multilineage colonies, genetic variation in behavior between lineages can increase the behavioral range of the colony, allowing better and more rapid response to environmental changes, and improving the colony's ability to maintain homeostasis (Oldroyd and Fewell 2007, Hughes et al. 2008b). For example, Wiernasz et al. (2008) found that harvester ant colonies with more patrilines of workers foraged for a longer period of time throughout the day, and certain patrilines were more likely to initiate foraging. Thus, genetically diverse colonies spent more time foraging, collected more food and grew faster (Cole et al. 2008, Wiernasz et al. 2008). Likewise, honeybee colonies with experimentally increased genetic diversity had higher foraging activity, increased food storage, and more rapid growth than honeybee colonies with a singly mated queen (Mattila and Seeley 2007). Such heritable variation in foraging related behaviors provides both a tool for enriching colony fitness and a mechanism by which different colonies or populations may impact ecosystems in consistently different ways.

1.3 The *foraging* gene

When searching the literature for candidate genes that may influence foraging behavior one gene stands out immediately—the aptly named *foraging* gene (*for)*. This gene appears to be highly conserved across *Animalia*, and differences in expression of *for* orthologs have been shown to influence foraging behavior in a wide variety of organisms from nematodes to the social Hymenoptera (de Belle et al. 1989, Fitzpatrick and Sokolowski 2004, Ben-Shahar 2005, Ingram et al. 2005, Tobback et al. 2008, Lucas and Sokolowski 2009, Lucas et al. 2010b, Tobback et al. 2011, Tarès et al. 2013).

The *for* gene encodes a cGMP-dependent protein kinase (PKG) involved in the cGMP/PKG signaling pathway (Fig. 1.1) (Ben-Shahar 2005). The gene is expressed as an inactive PKG. Outside stimulus causes cyclic guanosine monophosphate (cGMP) signaling in the body, and cGMP binds to PKG, activating the enzyme by exposing the catalytic core. The active PKG enzyme can then use ATP to phosphorylate target molecules, leading to downstream effects in the organism. cGMP-dependent protein kinases are found in a broad range of eukaryotic organisms, and appear to play a crucial role in functions related to energy acquisition and homeostasis (Hofmann 2005, Kaun and Sokolowski 2009).

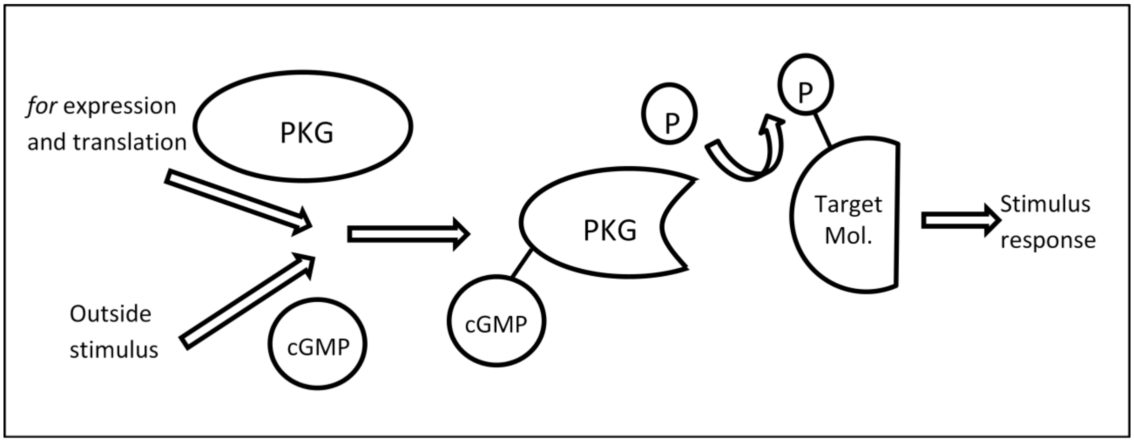

Figure 1.1: The foraging gene is expressed as mRNA and translated into inactive cGMP-dependent protein kinase (PKG). Outside stimulus causes cGMP signaling. cGMP binds to PKG, exposing the catalytic core. The activated PKG phosphorylates target molecules leading to downstream responses to the original stimulus.

The *foraging* gene was first discovered and investigated in *Drosophila melanogaster*, where wild populations maintain a stable polymorphism through frequency and density-dependent selection (de Belle et al. 1989, Sokolowski et al. 1997, Fitzpatrick et al. 2007). Variation in the *for* gene affects the foraging behavior of both adult and larval flies, with flies with the "rover" phenotype generally exhibiting more active foraging than flies with the "sitter" phenotype (Sokolowski 1980, de Belle and Sokolowski 1987, de Belle et al. 1989, Pereira and Sokolowski 1993, Kent et al. 2009). In fact, differences in *for* expression between the two phenotypes correlate with a suite of behavioral and physiological differences, including metabolic activity, fat storage, learning and memory, motor activity and gustatory response (Scheiner et al. 2004, Kaun et al. 2007, Kent et al. 2009). The increased foraging activity associated with the "rover" phenotype is associated with increased *for* expression as well as increased PKG activity (the enzyme product of *for*).

A behavioral syndrome is a suite of correlated behaviors (Sih et al. 2004). Because *for* regulates a major pathway, the *for* gene can coordinate a suite of downstream effects, providing a mechanism for this single gene to comprehensively alter an entire behavioral syndrome. In fruit flies, *for* creates the rover and sitter phenotypes by altering the expression of genes involved in metabolism, the insulin pathway, and other pathways, as well as affecting learning, memory, and habituation (Kaun et al. 2007, Kent et al. 2009). This latter is particularly interesting, as in mammals (and therefore humans) PKG is involved in long-term potentiation and long-term depression, which are thought to underlie learning and memory (Whitlock et al.

2006, Kaun et al. 2007). Among the social Hymenoptera, *for* orthologs have been demonstrated to be associated with the division of labor in a broad range of species, including bees, wasps, and ants (Ben-Shahar et al. 2002, Ingram et al. 2005, Tobback et al. 2008, Kodaira et al. 2009, Lucas et al. 2010a, Tobback et al. 2011, Lucas et al. 2015). Significant work on honeybees has demonstrated the role of *amfor* upregulation in creating the age-dependent transition from nurse to forager caste (Ben-Shahar et al. 2002, Heylen et al. 2008). *amfor* appears to act primarily through the visual processors to alter phototaxic responses (the transition from nurse to forager is accompanied by a switch from negative to positive phototaxis) (Ben-Shahar et al. 2003).

The novel regulatory role of the *foraging* gene in social insects supports the idea of changes in gene regulation allowing the evolution of behavior (Robinson and Ben-Shahar 2002). Because sociality has arisen independently in multiple hymenopteran lineages this is a striking indication of multiple novel adaptations of the *for* gene for a convergent purpose (Andersson 1984). Indeed, while honeybees follow a similar pattern to fruit flies with increased *for* expression leading to increased foraging activity, a variety of patterns appear to exist in wasps and ants (Ingram et al. 2005, Tobback et al. 2008, Lucas and Sokolowski 2009, Lucas et al. 2015). In the harvester ants, it was first thought that *Pogonomyrmex barbatus* foraging workers had significantly lower *pbfor* expression levels than callow (newly pupated) workers and workers for from other castes (Ingram et al. 2005). Later, it was determined in a related species that expression of the protein kinase gene varied cyclically, in sync with the circadian foraging patterns of the harvester ants (Ingram et al. 2011). Similarly, in the big-headed ants, *Pheidole* *pallidula,* where foraging is largely divided on the basis of genetically-determined polymorphic castes, the minor caste (foragers) exhibited lower levels of PKG activity than the major caste (guards) (Lucas and Sokolowski 2009). The observations of these and other species suggest that the gene serves a conserved or convergent purpose in fashions that can be expected to vary based on the ecology of the organism. Notably, several papers documented variation in *foraging* gene expression amongst colonies, but no previous studies have sought to determine if this variation has biological significance for behavior at the colony level.

1.4 Intraspecific variation and cooperative and competitive behavior during founding

For social insects, the founding period is among the most vulnerable periods in the "lifespan" of a colony, and therefore likely to be subject to strong selection (Whitcomb et al. 1973, Hölldobler and Wilson 1990). Many social insects practice either haplometrosis (single-queen founding) or pleometrosis (cooperative founding) (Tschinkel and Howard 1983, Keller and Wilson 1993, Roisin 1993). In the latter case, groups of foundress queens cooperate in the establishment of a new colony. The foundress associations may persist throughout the lifespan of the colony, or last only through the founding period, after which supernumerary queens may be killed or evicted from the nest (Balas 2005). Even when associations persist, queens may sacrifice some of their reproductive potential by sharing resources with a group, leading to a conflict of interests between the interests of individual queens and those of the colony as a whole

(Vargo 1992). These conflicts of interest in foundress association are likely to lead to trade-offs between the cooperative and competitive behaviors (Choe and Perlman 1997). Among ants, these foundress groups are very often formed of unrelated individuals (Bernasconi and Strassmann 1999). Although there is evidence that foundress relatedness affects sex ratio and inclusive fitness returns of subordinate foundresses in bees and wasps, it is less clear if relatedness or other traits of foundresses in ants can affect conflict and performance of these groups (Metcalf and Whitt 1977, Frank 1985, Schwarz 1987). Understanding how the composition of foundress groups affects the behavior and fitness of these groups has widespread implications for understanding the evolution of social and cooperative behavior in general.

1.5 The red imported fire ant

The red imported fire ant, *Solenopsis invicta*, is an invasive pest species, with negative ecological, economic, and health consequences for much of the southern United States (Vinson 1997, Tschinkel 2006). Our preliminary work on this insect has found strong evidence for both regional and colony-level variation in foraging behavior, which may have important consequences for management and ecology. For instance, the vast majority of variation in bait efficacy may be explained by variation in ant attraction (Tobler et al. in press). Moreover, the success of the fire ant in the United States has been linked to changes in its foraging access to plant-based carbohydrates (Wilder et al. 2011b) as well as the evolution of polygyny in North America(Ross et al. 1996). The potential for both genetic and environmentally regulated variation as well as the relevance of such variation to studies of ecology, evolution, and the design of fire ant management practices makes the red imported fire ant an ideal candidate to address the topic of intraspecific variation in foraging behavior.

1.6 Ecological impacts

Ants play a vital role in many ecosystems, dispersing seeds, turning over soil, and altering paths of energy and nutrient flow (Folgarait 1998, Allen et al. 2004). As opportunistic omnivores and disturbance specialists, red imported fire ants disrupt and alter natural communities. They have been associated with substantial population declines and displacement of a broad range of native wildlife from insects to vertebrates (Lofgren and Adams 1982, Morrison 2002, Allen et al. 2004). In their invasive range, fire ants have been linked to declines in terrestrial invertebrate, bird, and mammal populations (Holway et al. 2002).

In addition, the introduction of fire ants alters interactions between ants and honeydew-producing hemipterans (Kaplan and Eubanks 2002, Lach 2003). Ant-hemipteran mutualisms are common in both natural and agricultural ecosystems and can significantly impact their plant hosts and ecosystems (Way 1963, Hölldobler and Wilson 1990, Styrsky and Eubanks 2007). Such ant-hemipteran relationships have been suggested as ‘keystone interactions’ in ecosystems, as they can alter the abundance and spatial distribution of both natural enemies and non-honeydew-producing herbivores on plants (Styrsky and Eubanks 2007, Lach et al. 2010, Powell and Silverman 2010, Rudgers et al. 2010, Helms et al. 2011). For example, Kaplan and Eubanks (2005) found that the mutualistic relationship between fire ants and cotton aphids (*Aphis gossypii*) affected the abundance of 27–33% of herbivore taxa and 40–47% of predator taxa. Fire ant tending of aphids can significantly increase aphid populations, indirectly reduce leaf damage to plants by suppressing chewing herbivores, and potentially have positive or negative indirect effects on plant fitness depending on their interactions with other arthropod communities (Kaplan and Eubanks 2002, Diaz et al. 2004, Coppler et al. 2007, Styrsky and Eubanks 2007, 2010).

1.7 Importance for fire ant management

Intraspecific variation in fire ant behavior has significant consequences for agriculture as well. In a one year period the estimated cost of fire ant damages and control to Texas agriculture exceeded $90 million, with the largest damages being to crop yield and equipment (Lard et al. 2002). As omnivores, fire ants can have both beneficial and detrimental effects on crop systems, for example by forming mutualisms with crop pests such as aphids while simultaneously eliminating other potentially more damaging insect herbivores (Styrsky and Eubanks 2007). The effects of fire ants and fire ant control methods may be altered by crop system and season (Tobler et al. in press).

Moreover, while pest control methods for other insects typically focus on a quick kill, toxic ant baits rely on foraging workers to collect the bait and feed it to queens and larvae in order to effectively eliminate a colony. A recent review of the literature and my own preliminary work suggest that variation in foraging behavior and food preferences among fire ant colonies may significantly affect attraction to baits and subsequent bait efficacy (Tobler et al. in press). A comprehensive understanding of foraging behavior is, therefore, important for managing fire ants as well as predicting their impacts on agriculture and agricultural pests

In this study, I characterize intraspecific variation in fire ant behavior and begin to untangle the relative contributions of environmental and genetic effects in creating this variation. Differences in the success and fitness of fire ant founding groups can help to predict and explain their spread. Documenting the existence of regional and colony-level variation in fire ant foraging behavior has important implications for understanding their impacts on biodiversity and natural and agricultural ecosystems. Additionally, understanding variation in foraging behavior is vital for the development of more efficient baits and better control practices. Quantifying the ability of fire ant colonies to have consistently different ecological effects of fire ants on the surrounding communities has exciting implications for studies of both ecology and evolution, as well as for fire ant control and wildlife management practices.

1.8 Importance for invasion ecology

Interactions between species and communities are an important factor in understanding and predicting invasions (Lodge 1993). Studying the impacts of variation in foraging behavior on fire ants and their surrounding communities may enhance our understanding of the success and spread of fire ants in the southern United States since their introduction from South America more than seventy years ago.

Much research has gone into determining the characteristics of successful invaders and predicting their patterns of invasion and ecological impacts (Williamson and Fitter 1996, Kolar and Lodge 2001, Holway et al. 2002). Recent studies suggest that many worldwide introductions of alien organisms stem not from native populations but from particularly successful invasive populations, a phenomenon termed the invasive bridgehead effect (Lombaert et al. 2010). Indeed, this pattern of repeated subsequent invasions can be seen in the red imported fire ant, where the invasive US population is believed to be responsible for at least nine separate worldwide invasions (Ascunce et al. 2011). Notably, the bridgehead effect suggests that the genotypes of successfully invasive populations may preadapt them for further invasions. This provides strong support for the pivotal role of genetics in invasion ecology in general, and in invasions of the red imported fire ant in particular.

Moreover, fire ants provide an avenue to explore questions on how invasions can both drive evolutionary changes and be driven by genetic variation. Introduction to a novel habitat may free a species from selective pressures such as co-evolved natural enemies and competitors and open up new ecological niches (Lockwood et al. 2007). This introduces the potential for increased genetic drift as well as evolution due to novel environments and genetic bottlenecks. Changes in genetic structure following an invasion may alter species physiology or behavior (Lee 2002). At the same time, a growing body of examples have emphasized the importance of genetic variation in the propagation of successful invasions. (Carroll et al. 2001, Lee 2002, Dlugosch and Parker 2008, Vellend et al. 2009). In some circumstances, introduction events may even increase genetic variation (Wilson et al. 2009). For example, in both canary reedgrass and the brown anole, the successful invasions and subsequent range extensions have been linked to increased genetic diversity created by multiple introductions (Kolbe et al. 2004, Lavergne and Molofsky 2007). Exploring the potential genetic causes of variation in fire ant foraging patterns and founding behavior may help to explain the success of fire ants as invaders, as well providing insight into the role of variation in invasion ecology. For this reason, the results of this study have potential applications for other invasive species as well as invasion theory as a whole.

1.9 Objectives

In this study I seek to better understand the causes and consequences of intraspecific variation in fire ant behavior by addressing four questions:

1. Does genetic or environmentally-derived variation in foraging behavior exist in the red imported fire ant?
2. What are the potential ecological consequences of colony-level variation in foraging behavior of the red imported fire ant?
3. Is the foraging gene associated with variation in foraging behavior in the red imported fire ant?
4. How does group composition affect the cooperation and competition of group-founding queens?

CHAPTER II

INTRASPECIFIC VARIATION AMONG SOCIAL INSECT COLONIES: PERSISTENT REGIONAL AND COLONY-LEVEL DIFFERENCES IN FIRE ANT FORAGING BEHAVIOR

2.1 Overview

Individuals vary within a species in many ecologically important ways, but the causes and consequences of such variation are often poorly understood. Foraging behavior is among the most profitable and risky activities in which organisms engage and is expected to be under strong selection. Among social insects there is evidence that within-colony variation in traits such as foraging behavior can increase colony fitness, but variation between colonies and the potential consequences of such variation are poorly documented. In this study, we tested natural populations of the red imported fire ant, *Solenopsis invicta*, for the existence of colony and regional variation in foraging behavior and tested the persistence of this variation over time and across foraging habitats. We also reared single-lineage colonies in standardized environments to explore the contribution of colony lineage. Fire ants from natural populations exhibited significant and persistent colony and regional-level variation in foraging behaviors such as extra-nest activity, exploration, and discovery of and recruitment to resources. Moreover, colony-level variation in extra-nest activity was significantly correlated with colony growth, suggesting that this variation has fitness consequences. Lineage of the colony had a significant effect on extra-nest activity and exploratory activity and explained approximately half of the variation observed in foraging behaviors, suggesting a heritable component to colony-level variation in behavior. Colony-level variation among red imported fire ants has important implications for improving predictive ecological models of the impacts of this widespread invasive species and for furthering our understanding of the evolution of social behavior.

2.2 Introduction

Individuals vary within populations in many ecologically important ways (Post et al. 2008, Duffy 2010, Bolnick et al. 2011, Dall et al. 2012) and there is mounting evidence that this variation can have large effects on populations and communities (Benedetti-Cecchi 2003, Bolnick et al. 2003, Hughes et al. 2008a). Consistent individual variation in behavior (i.e., personality and behavioral syndromes) may be particularly important in determining the outcomes of inter and intraspecific interactions (Sih et al. 2004, Jandt et al. 2014). In terrestrial ecosystems, social insects are often abundant and provide critically important ecosystem functions (Price et al. 2011). Social insects (such as ants and many species of bees and wasps) are the most important pollinators of flowering plants, act as major seed predators and dispersers, prey on agricultural pests and other arthropods, and are major ecosystem engineers that alter soil aeration and nutrient content (Folgarait 1998, Price et al. 2011, Sanders and van Veen 2011). Despite the pervasive ecological importance of social insects, very little is known about colony-level variation in their behaviors. The goal of this study is to document colony-level variation in the foraging behavior of an ecologically dominant invasive social insect: the red imported fire ant (*Solenopsis invicta;* Hymenoptera: Formicidae).

Among social insects there is substantial evidence that within-colony variation in traits such as foraging behavior can increase colony fitness (Mattila and Seeley 2007, Oldroyd and Fewell 2007, Cole et al. 2008, Hughes et al. 2008b). Such variation may extend the behavioral range of the colony and allow better and more rapid response to environmental changes. For example, in harvester ants, workers from different patrilines vary in the time of day they begin foraging, resulting in increased seed collection in colonies with more patrilines (Cole et al. 2008). Surprisingly, behavioral variation at the colony level (among colonies) has been much less well studied. Consequently, the extent, persistence, and potential consequences of variation among colonies of social animals are poorly understood. Among ants, research-to-date on colony-level behavioral variation has been limited to only a scattering of species from out of the hundreds of ant genera (e.g. *Myrmica*: (Chapman et al. 2011); *Pogonomyrmex*: (Cole et al. 2010); *Linepithema*: (Hui and Pinter-Wollman 2014); *Temnothorax*: (Bengston and Dornhaus 2014)). For example, recent work on the harvester ant *Pogonomyrmex barbatus* found that colonies differ in the baseline rates at which foragers leave the nest, and also differ in their behavioral plasticity—specifically the degree to which they adjust their foraging activity based on outside stressors (Gordon et al. 2011). Daughter colonies may exhibit similar behavior to their mother colonies, indicating a potential heritable component (Gordon 2013). If colonies consistently vary in foraging behavior and other important traits, then quantifying and understanding colony-level variation will be critical to accurately predict the effects of social insects on interacting species.

We predict that foraging behavior of fire ants will vary significantly. For social insects, as with most animals, foraging occupies a large portion of their lifespan, is vital for growth, reproduction, and survival; yet, it carries some of the greatest risks they will face (Barbosa and Castellanos 2005, Stephens et al. 2007). Foraging may require entering dangerous or unknown environments, may attract or expose organisms to predators, and may place organisms in direct or indirect competition with others (Lima and Dill 1990). Animals must balance the potential costs of risky behaviors against other fitness needs (Brown et al. 1999). If foraging is energetically costly or risky, then we would predict that fire ant colonies will show evidence of trade-offs between foraging activity and colony growth.

We tested for the existence and extent of variation in foraging behavior in natural populations of the red imported fire ant by quantifying colony and regional-level variation. We quantified variation in ground and arboreal foraging, the persistence of variation over five weeks and across two microhabitats, and quantified trade-offs in fire ant foraging and colony growth by regressing foraging activity, food collection, and colony biomass. We also estimated the broad sense heritabilities of foraging behavior using single-lineage colonies.

2.3 Methods

2.3.1 Study system

Fire ants are an invasive pest species across much of the southern United States and many other areas around the world (Ascunce et al. 2011). As such, they have significant ecological, economic, and health consequences (Tschinkel 2006). Like most ants, fire ants forage by sending worker scouts into their territory to locate resources. These scouts return to the nest or to nearby foraging tunnels and recruit other workers to the resource using pheromone trails (Tschinkel 2006). We selected collection sites in Texas and Mississippi because in previous field work we observed differences in the arboreal and ground-level foraging behavior of fire ants from the two regions (Wilder et al. 2011b). This is of particular interest because differences in the use of arboreal resources have been linked to the invasive success of fire ants in the United States and the ecological dominance of multiple ant species (Blancafort and Gómez 2005, Grover et al. 2007, Styrsky and Eubanks 2007, 2010, Wilder et al. 2011a).

2.3.2 Experiment 1: Colony and regional-level variation

2.3.2.1 Field colony collection and maintenance

We collected red imported fire ant colonies from Texas (Texas A&M Field Laboratory, Burleson Co., TX; permission granted by Texas A&M Agrilife Research) and Mississippi (Homochitto National Forest, Amite Co., MS; permission granted by

Mississippi Dept. of Wildlife, Fisheries & Parks) in order to quantify colony and regional-level variation in foraging behavior. We extracted colonies from soil using drip floatation (Banks et al. 1981) and used each field colony (colony of origin) to create two standardized experimental colonies of 2 queens, 50 brood, and 1 gram of workers (~2000 ants). Only field colonies found to contain multiple queens were included in this experiment (TX n=17, MS n=16). Each experimental colony was placed in a fluon-lined (Insect-a-slip Insect Barrier, BioQuip Products, 2321 Gladwick St., Rancho Dominguez, CA) foraging arena (38x55x6cm) containing a nest dish (15cm diameter black-lidded petri dish with dampened plaster) and water tube.

Throughout the experiment, experimental colonies were maintained in standardized laboratory conditions (temperature 24-32ºC, 40-70% humidity, 12:12 light/dark cycle) and fed three times per week, alternating between two 3 mL tubes of artificial nectar (Wilder and Eubanks 2010a) and one male and one female adult cricket, *Acheta domesticus*. By observing the foraging behavior of standardized colonies in the lab, we controlled for variation due to environment and colony size and ratio of brood to workers. All food was removed from the foraging arenas 24hrs prior to behavioral assays and all assays were conducted at a standardized time (10AM).

2.3.2.2 Experiment 1a) Survey of colony and regional-level variation in foraging behavior of natural populations

To quantify variation in ground-level foraging among experimental colonies, we placed a freshly killed cricket 30 cm from the nest entrance and recorded the number of ants present at the cricket after 10 minutes, and then every 30 minutes for 150 minutes. We also observed colonies every minute for 10 minutes and then at 30 minutes intervals to determine time to discovery of resource and time to formation of a visible trail of recruiting ants. Colonies which had not discovered or formed a trail to the resource within the observation period were scored with the final time value. The following day we assayed variation in climbing behavior by recording discovery, trail formation, and recruitment to an elevated cricket placed at the top of a 30 cm dowel placed 30 cm from the nest entrance. Observations were made as above, with an additional final observation at 330 minutes. At the end of the week, we measured extra-nest activity by counting the number of ants active outside the nest in the foraging arena three times and taking the average. We measured exploratory activity by introducing a novel climbing structure comprised of two halves of a 7.6 x 12.7 cm index card skewered vertically at the top of a 30 cm bamboo skewer. We then counted the number of ants exploring the structure at 20 minute intervals for 2 hours and took the average of these counts.

These data were used to compare variation at both the level of region (Texas vs. Mississippi populations) and colony of origin (nested in region). We used multiple regression with region and colony (nested in region) as covariates in the model to determine the effects of these variables on variation in the activity, exploration, and ground-level and elevated foraging recruitment traits. Count data were square-root transformed. All results in this study were analyzed using SAS software, Version 9.3 of the SAS System for Windows. Copyright 2011 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA. Data available from the Dryad Digital Repository (doi:10.5061/dryad.94r7j).

2.3.2.3 Experiment 1b) Persistence of variation (before and after exposure to different foraging habitats)

After one week of equilibration to laboratory conditions and one week of foraging assays as described above, we divided the experimental colonies into treatments of two different foraging habitats in order to determine if behavioral variation would persist across exposure to different environmental complexities and foraging contexts. Each colony of origin was represented in each treatment group by one standardized experimental colony. In the first treatment, we provided all colonies with six 30-cm upright wooden dowels and all food items were placed at the top of two randomly-selected dowels throughout the experiment (“elevated” foraging habitat), requiring ants to climb and forage in a more complex environment. In the second treatment, we placed all food items at ground level, next to horizontal wooden dowels (“ground-level” foraging habitat). Ants were maintained in these conditions for five weeks. In the following week we temporarily removed all elevated foraging structures and then assayed the behavior of all colonies as described previously, first in the ground-level foraging habitat and then in the elevated foraging habitat.

We used repeated measures analysis of variance to compare the behavioral variables (discovery, trail formation, and recruitment to a ground-level or elevated cricket; extra-nest activity; exploration) before and after exposure to different habitats. Foraging habitat treatment, region, and colony (nested in region) were included as covariates in the model, and count data were square-root transformed. We analyzed within-subjects effects using the more conservative multivariate analysis of variance which does not assume sphericity of variance. Interactions that were not significant were sequentially excluded from the model.

2.3.2.4 Experiment 1c) Food collection and colony growth

We measured the dry weight of cricket collected by each colony during foraging assays at the beginning, middle, and end of the experiment. Crickets were weighed before and after 24 hours of fire ant foraging, and the total dry weight removed by fire ants was estimated using previously established methods (Wilder and Eubanks 2010a). Upon completion of the experiment, we measured the final dry weight of workers and brood in each colony in order to compare colony growth.

We used repeated measures analysis of variance as previously described to test for the effects of foraging habitat, region, and colony (nested in region) on dry weight of cricket collected over time. We used multiple regression to analyze the effects of foraging habitat, region, and colony (nested in region) on final colony size. To determine correlations between weight of food collected, final colony size, and colony behaviors (as first measured) we calculated Pearson's product-moment coefficients.

2.3.3 Experiment 2: Single-lineage colony experiments colony experiments

2.3.3.1 Single-lineage colony collection and maintenance

In order to establish colonies with a minimum of environmental and within-colony genetic variance, we collected newly-mated foundress queens and reared single-lineage colonies in a standardized laboratory environment. Invasive fire ant colonies may be monogyne or polygyne (having a single queen or multiple, unrelated queens in a nest), creating the potential for many genetic lineages in a single field colony. Fire ant queens mate monandrously (or primarily monandrously) so that a single queen produces only workers from a single patriline, or genetic lineage (Tschinkel 2006, Lawson et al. 2012). Fire ants mate in nuptial flights hundreds or thousands of feet in the air and attempts to artificially cross them in the lab have proved challenging and largely ineffective (Cupp et al. 1973, Ball et al. 1983). Studies of heritability must be approached via indirect methods. Foundresses were collected after two mating flights in College Station, TX and Conroe, TX and cloistered individually in darkened nest tubes (permission for collection was obtained from owners of private land). Seven days after the first worker eclosed, we moved colonies into standard nest dishes and arenas as described above for field colony maintenance. To promote colony growth, we added up to four additional nest dishes over time. All colonies were maintained in standardized environmental conditions, as previously described for field-collected colony maintenance, on a standard diet: water *ad libitum*, 14mL artificial nectar replaced weekly, and up to two crickets provided three times weekly (increased as colonies grew). All colonies were at least six months old prior to the experiment.

2.3.3.2 Experiment 2) Variation among single-lineage colonies

In order to estimate the contribution of lineage to intraspecific variation in fire ant foraging behavior, we created three standardized experimental colonies each composed of .65g workers (~1300 ants) and about 100 brood for each of 15 single-lineage colonies and assayed their foraging behavior. Workers were collected randomly from disturbed ants both inside and outside the nest dish in order to ensure a representative selection of all task-groups. The experimental colonies were maintained in individual trays under standardized conditions. Colonies were assayed as previously described for extra-nest activity, exploratory activity, and time to discovery and number of ants recruiting to a single cricket placed at ground-level 30cm outside the nest-entrance. The number of ants at the cricket was recorded after five minutes and then every ten minutes for 60 minutes.

The behavioral data of the single-lineage colonies were analyzed as described for field colonies, using multiple regression to test for effects of region and colony (nested in region). Queen mating flight of origin had no effect and was excluded from the model.

The R^2 value of the model was used to estimate the percentage of variation explained by colony of origin, a rough estimate of broad-sense heritability (Kover and Schaal 2002, Fjerdingstad 2005).

2.4 Results

2.4.1 Experiment 1a) Colony and regional-level variation in foraging behavior of natural populations

We observed significant variation among fire ant colonies in extra-nest activity ($F_{31,33}$=3.93, p=<0.0001), exploratory activity ($F_{31,33}$=1.87, p=0.0405), and recruitment to ground-level ($F_{31,33}$=4.63, p<0.0001) and elevated food ($F_{31,33}$=5.08, p<0.0001). Behavioral variation among colonies was often large. For example the most active colonies recruited more than 40 times more workers to crickets on average than the least active colonies (Fig. 2.1). When foraging at ground-level, the number of ants recruited to crickets varied significantly by colony of origin (Fig. 2.1A) as did recruiting trail formation ($F_{31,33}$=2.43, p=0.0069), but not discovery time ($F_{31,33}$=0.92, p=0.5907). Colony-level variation in foraging behavior at elevated foods was even more pronounced, with a highly significant effect of colony of origin (nested in region) for all measured variables (Fig. 2.1B: discovery: $F_{31,33}$=3.16, p=0.0008; trail: $F_{31,33}$=7.51, p=<0.0001).

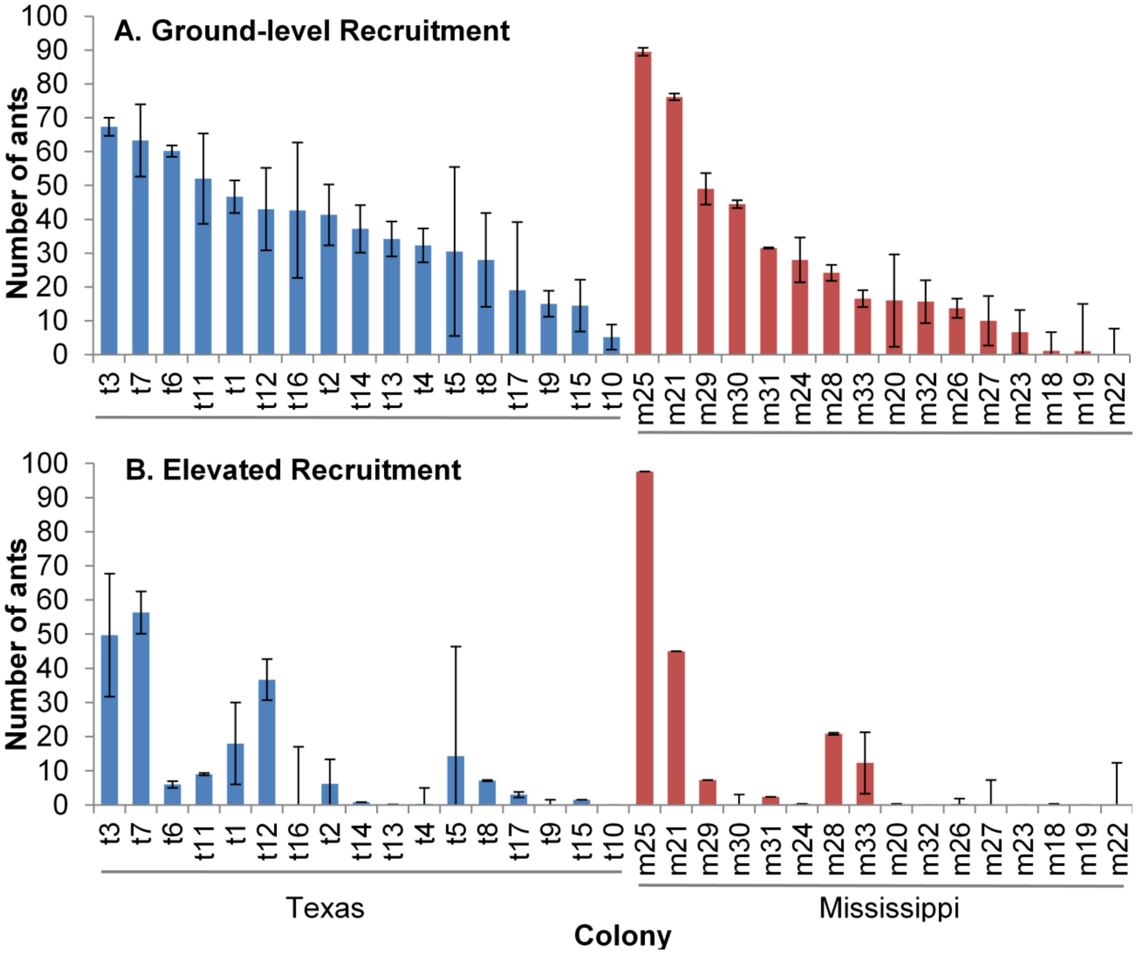

Figure 2.1: Average number of fire ants recruiting to ground-level or elevated crickets. Crickets were placed at (A) ground-level or (B) 30cm up a wooden dowel, prior to experimental treatment. Each bar represents a single colony of origin (n=2); error bars show standard error.

We also observed significant regional differences in ant behavior, which fit our expectations for behavioral patterns in relationship to site invasion history. Fire ants from Texas colonies (closer to the invasion front) recruited to ground-level crickets in significantly higher numbers than ants from Mississippi colonies (closer to the invasion origin)(Fig. 2.2A: $F_{1,31}$ =17.08, p=0.0002) with on average 40% more ants foraging at crickets. Ants from Texas colonies also discovered and formed recruiting trails to ground-level crickets significantly faster than those from Mississippi colonies (Fig. 2.2B: discovery: $F_{1,33}$=7.17, p=0.0115; trail: $F_{1,33}$=13.53, p=0.0008). When ants were required to climb 30cm to reach crickets, the regional differences in discovery and trail formation times were similar to those at ground-level, with Texas colonies locating and developing foraging trails to elevated crickets significantly faster than colonies from Mississippi (Fig. 2.2D: discover: $F_{1,33}$=4.61, p=0.0391; trail: $F_{1,33}$=13.16, p=0.0010). Fire ant colonies collected in Texas also had, on average, more workers active outside the nest ($F_{1,33}$=3.51, p=0.0700) , more workers exploring a novel structure ($F_{1,33}$=1.79, p=0.1901), and more workers recruiting to an elevated cricket (Fig. 2.2C: $F_{1,33}$=1.55, p=0.2226) than fire ant colonies collected in Mississippi, although these differences were not significant for α=0.05.

2.4.2 Experiment 1b) Persistence of variation (before and after exposure to different foraging habitats)

Colony and regional-level variation in behavior generally persisted over time and across exposure to different foraging habitats. After five weeks, colony of origin remained a significant factor in the variation observed for all measured foraging variables (summarized Table 2.1; Table A.1; Fig. 2.3 A-D). Additionally, extra-nest activity, trail formation to elevated resources, and average recruitment to both ground-level and

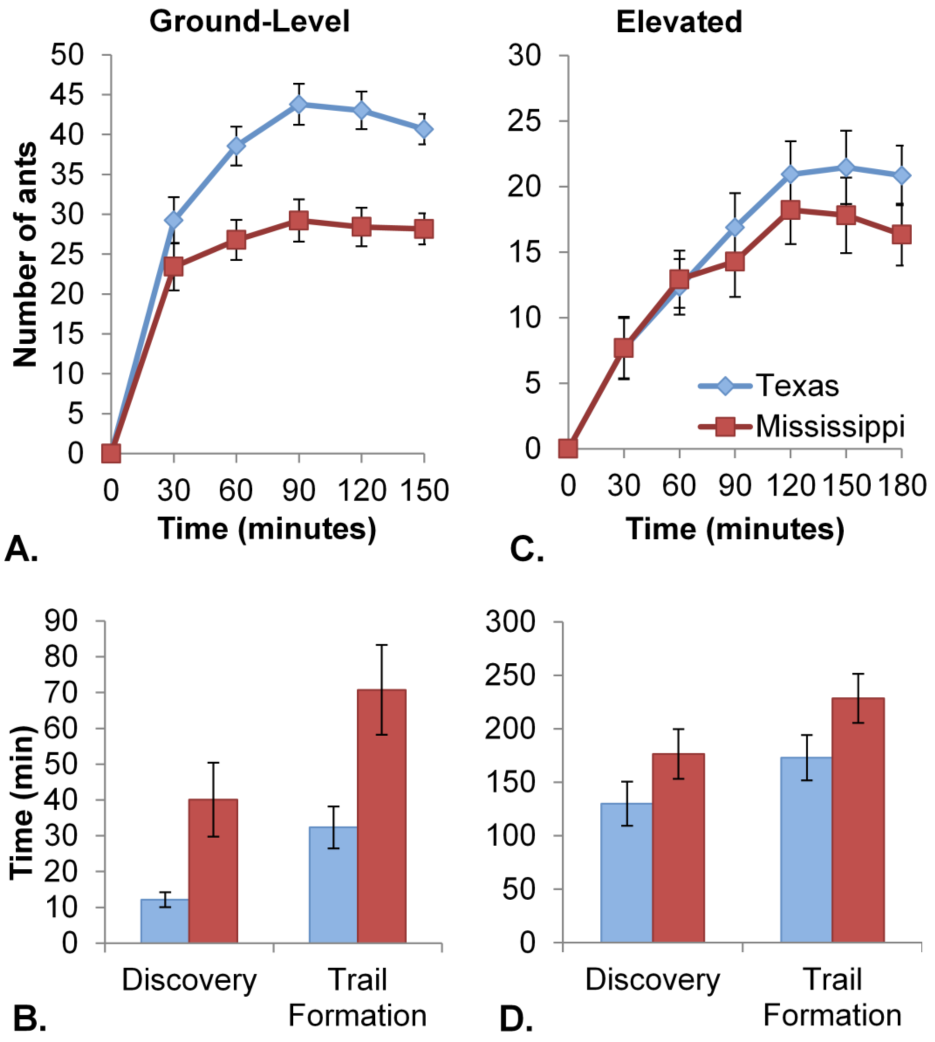

Figure 2.2: Regional differences in recruitment of fire ants to ground-level or elevated crickets. Colonies were collected in Texas (light diamond, n=17) or Mississippi (dark square, n=16) and crickets were placed at ground-level (A and B) or 30cm up a wooden dowel (C and D). (A) and (C) show least square mean number of ants observed at the cricket over time while (B) and (D) show the average time to resource discovery and time to formation of a recruiting trail. Error bars show standard error.

Table 2.1: Analysis of behavior before and after exposure to different foraging habitats. Table summarizes repeated measures analysis of variance for standardized experimental colonies in standardized foraging habitats before and after being exposed to different foraging habitats for five weeks. Within subjects effects use multivariate analysis of variance; lambda is converted to the appropriate F value. Aterisks denote significance at α=0.05.

Trait	Effect	p	F	df 1	df 2
Average recruitment to ground-level cricket (30-90min)	Foraging Habitat	0.3554	0.88	1	32
	Region	0.0007*	14.11	1	32
	Colony(Region)	<.0001*	4.98	31	32
	Time	<.0001*	202.61	1	32
	Time*F.Habitat	0.6727	0.18	1	32
	Time*Region	0.0015*	12.05	1	32
	Time*Colony(Region)	0.0001*	3.88	31	32
Average recruitment to elevated cricket (30-90min)	Foraging Habitat	0.9678	<0.01	1	32
	Region	0.1328	2.38	1	32
	Colony(Region)	<.0001*	5.22	31	32
	Time	0.2443	1.41	1	32
	Time*F.Habitat	0.1431	2.25	1	32
	Time*Region	0.6077	0.27	1	32
	Time*Colony(Region)	0.0002*	3.68	31	32
Extra-nest Activity	Foraging Habitat	0.0791	3.29	1	32
	Region	0.9922	<0.01	1	32
	Colony(Region)	0.0002*	3.76	31	32
	Time	0.0835	3.19	1	32
	Time*F.Habitat	0.3572	0.87	1	32
	Time*Region	0.0039*	9.66	1	32
	Time*Colony(Region)	0.0092*	2.35	31	32
Exploration	Foraging Habitat	0.0443*	4.38	1	32
	Region	0.0980	2.91	1	32
	Colony(Region)	0.0224*	2.07	31	32
	Time	0.7344	0.12	1	32
	Time*F.Habitat	0.1255	2.48	1	32
	Time*Region	0.9130	0.01	1	32
	Time*Colony(Region)	0.1680	1.41	31	32

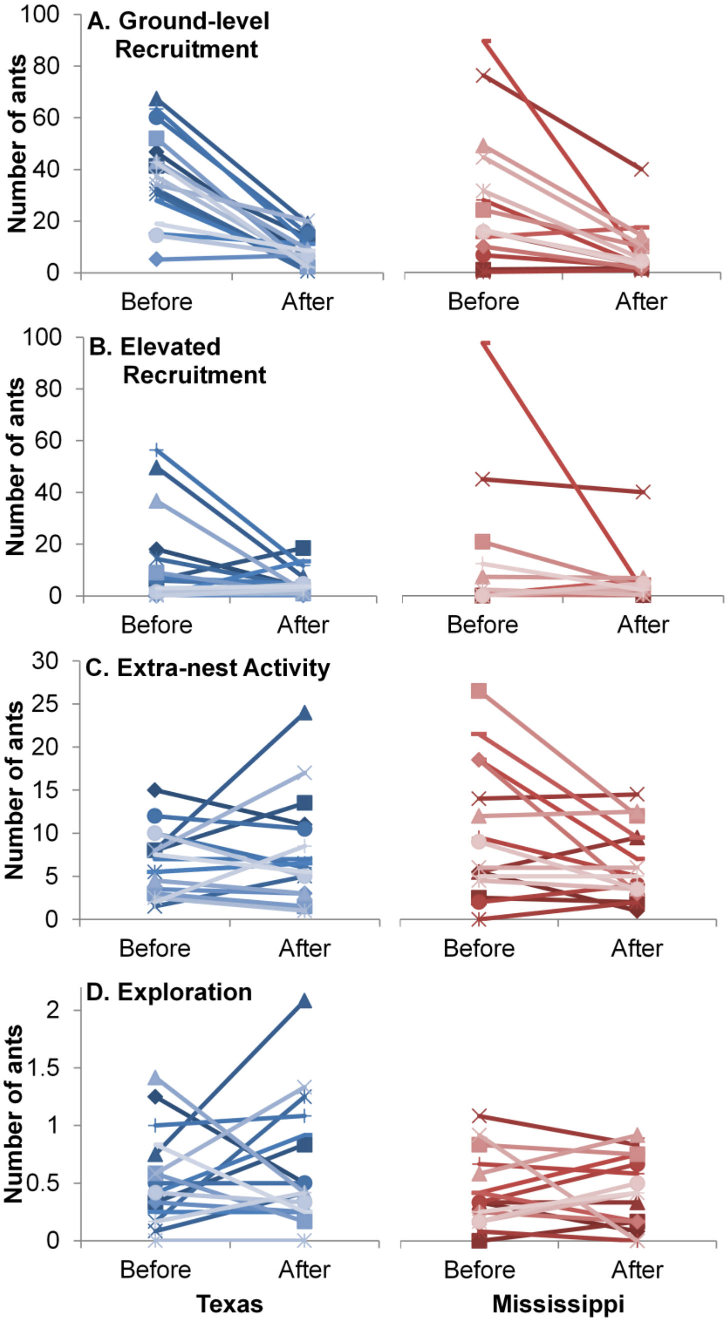

Figure 2.3: Colony differences in fire ant foraging behavior before and after exposure to different foraging habitats. Graphs show average number of ants (A) at a ground-level cricket, (B) at a cricket elevated 30cm up a wooden dowel, (C) active outside the nest and (D) exploring a novel structure. Each line represents a colony-of-origin (n=2), for fire ant colonies from Texas (left) and Mississippi (right) before and after five weeks exposure to different foraging habitat treatments.

formation to elevated resources, and average recruitment to both ground-level and elevated resources of experimental colonies from the same colony of origin tended to increase or decrease over time in a colony-specific manner (Table 2.1; Fig. 2.3A-C). Only ground-level recruitment showed a significant effect of time independent of colony effects, with significantly less ants on average recruiting to resources at the end of the experiment. Neither foraging habitat (treatment) nor time by treatment effects were significant for any measured traits (Table 2.1; Table A.1).

Significant regional differences in many foraging behaviors also persisted before and after exposure to different foraging habitats (Table 2.1; Table A.1: ground recruitment, discovery, and trail; elevated discovery and trail). Fire ants from Mississippi maintained significantly lower average ground-level recruitment than ants from Texas. The ground-level recruitment of Texas ants changed more over time than that of ants from Mississippi, decreasing significantly (Table 2.1, Fig. 2.4A). Colonies from Mississippi also took longer to discover resources and form recruitment trails than colonies from Texas (Table A.1; Fig. A.1). Average recruitment to elevated crickets continued to exhibit no significant regional differences (Table 2.1, Fig. 2.4B). Over the course of the experiment, Texas ants significantly increased extra-nest activity while Mississippi ants decreased their activity (Table 2.1, Fig. 2.4C). Ants from different regions did not significantly alter their exploratory activity over time (Table 2.1, Fig. 2.4D).

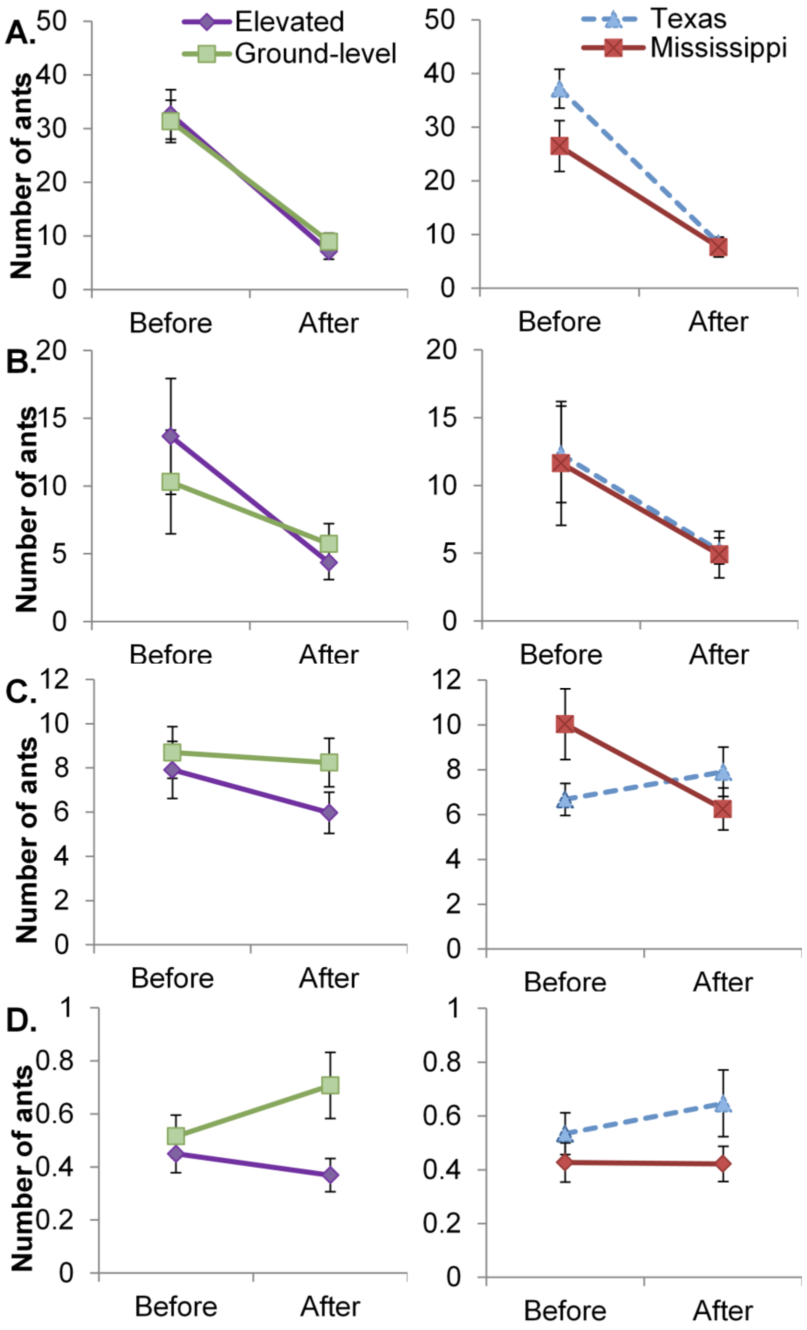

Figure 2.4: Foraging habitat and regional effects on fire ant foraging behavior. Average number of ants (A) at a ground-level cricket, (B) at a cricket placed 30cm up a wooden dowel, (C) active outside the nest and (D) exploring a novel structure, before and after five weeks exposure to different foraging habitats. Graphs show colonies grouped by foraging habitat treatment (left: elevated, dark diamond (n=33) vs. ground-level, light square (n=33)) and by region of origin (right: Texas, light triangle (n=17) vs. Mississippi, dark square (n=16)). Error bars show standard error.

2.4.3 Experiment 1c) Food collection and colony growth

Although all measured foraging variables were significantly correlated with each other (Pearson's test $p<0.05$), we observed differences in their relationships with the amount of food collected by each colony and colony growth. Average dry weight of cricket collected per experimental colony correlated with increased recruitment to crickets (Fig. 2.5A; ground-level: $r= 0.36859$, $n=66$, $p=0.0023$; elevated: $r= 0.25270$, $n=66$, $p=0.0014$) and faster discovery and trail formation to ground-level resources (discovery: $r= -0.32257$, $n=66$, $p=0.0083$; trail: $r= -0.38477$, $n=66$, $p=0.0014$), but not extra-nest activity or exploration. Ants from Texas collected significantly greater dry weight of crickets than ants from Mississippi, collecting upwards of 50% more cricket by weight by the end of the experiment ($F_{1,32}=6.19$, $p=0.0182$). Weight of cricket collected was not significantly affected by either foraging habitat or colony of origin, although weight collected increased over time ($F_{2,31}=46.33$; $p=<0.0001$).

Colony of origin had a highly significant effect on final colony size ($F_{31,32}=3.32$, $p=0.0006$). Colony size correlated negatively with extra-nest activity (Fig. 2.5B; $r=-0.30761$, $n=66$, $p=0.0120$). Colonies with higher activity at the beginning of the experiment had lower final weights, and were as much as three times smaller than less active colonies by the end of the experiment. Final colony weight did not correlate significantly with recruitment or weight of cricket collected and neither exposure to different foraging habitats nor region of origin had a significant effect on final colony size.

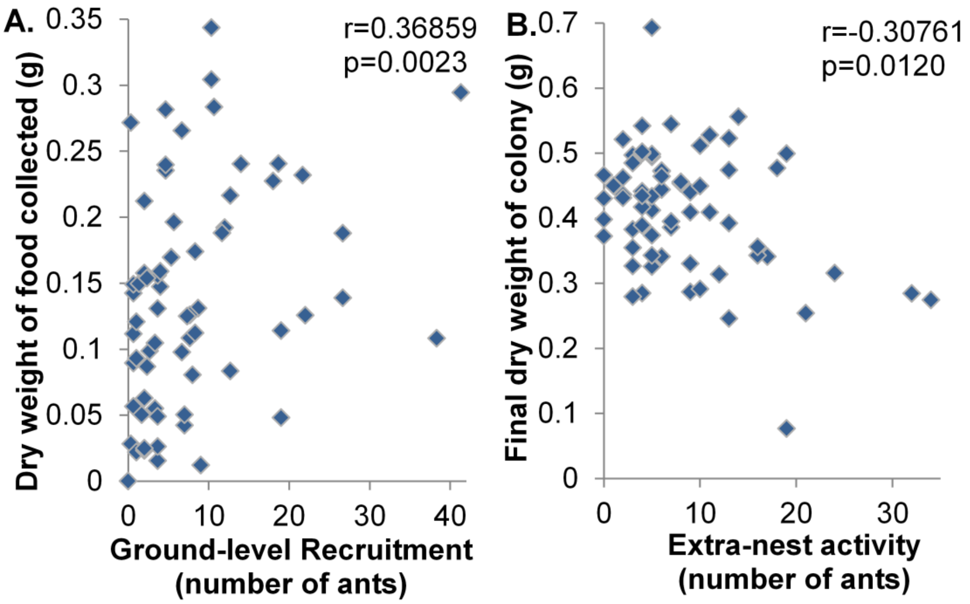

Figure 2.5: Effects of behavior on fire ant colony fitness measures. Graphs show (A) Ground-level recruitment versus colony food collection and (B) extra-nest activity versus colony size (final dry weight of workers and brood).

2.4.4 Experiment 2) Variation among single-lineage colonies

When reared from single queens in standardized environments, worker lineage (single-lineage colony of origin) explained nearly half of the total observed behavioral variation among colonies for all measured traits (R^2: exploratory activity=49.65% extra-nest activity=45.50%, recruitment=45.48%). Groups of workers varied significantly in extra-nest activity and exploratory activity, and these differences were significantly affected by workers colony of origin (Fig. 2.6; extra-nest: $F_{13,30}$=2.15, p=0.0414; exploratory: $F_{13,30}$=3.25, p= 0.0038; recruitment $F_{13,30}$=1.96; p= 0.0632). The R^2 values provide an estimate of the broad-sense heritability of the traits.

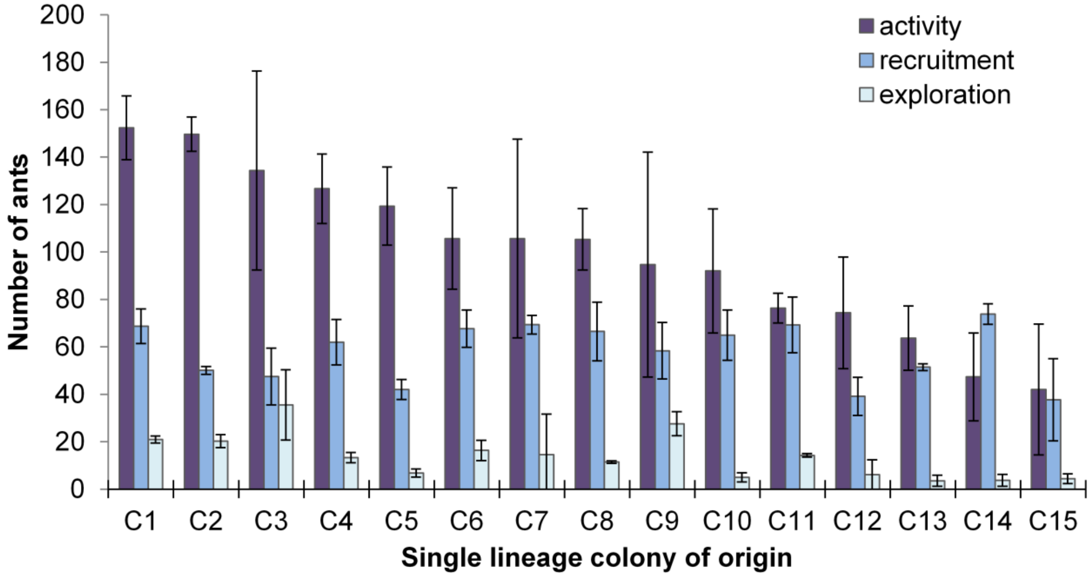

Figure 2.6: Foraging behavior of fire ants from different colony lineages. Graphs show average extra-nest activity (dark), recruitment to cricket between ten and forty minutes (medium), and exploratory activity (light) of each colony lineage (C1-C15, n=3). Error bars show standard error.

2.5 Discussion

The results of this study suggest that natural populations of fire ants exhibit substantial colony-level variation in foraging behavior both among and within populations, that this variation persists over time and across environments, and that this variation has significant consequences for colony fitness. Previous research has focused largely on behavioral variation within colonies (Robinson 1992, Hunt et al. 2007, Oldroyd and Fewell 2007) and studies specifically demonstrating variation in behavior among colonies are limited and are often snapshots in time (Crosland 1990, Raine et al. 2006, Cole et al. 2010, Gordon et al. 2011). We know of only a few that demonstrate persistence of variation (Wray and Seeley 2011, Gordon et al. 2013). In one of the best studies to date, Bengston and Dornhaus (2014) found colony-level variation in activity, aggression, and foraging effort in a cavity-dwelling ant *(Temnothorax rugatulus)*. They concluded that *T. rugatulus* colonies could be roughly classified as “risk-averse” and “risk-prone,” with risk-averse colonies combining high foraging effort with low aggression and shorter foraging distances. *T. rugatulus* colony activity levels did not correlate with other behaviors. We found that fire ant colonies also varied in their foraging effort (*e.g.* discovery time, and trail formation, and recruitment), and higher foraging correlated with higher exploratory and extra-nest activity. Intriguingly, in our study increased foraging activity increased the amount of food collected by colonies, while increased extra-nest activity decreased colony growth, and these fitness measures varied independently. More studies are needed to discover the extent and effects of colony-level behavioral variation in social insects.

We found evidence for a trade-off between colony activity level and growth. Studies have shown trade-offs associated with foraging behavior within colonies and among social insect species (Nonacs and Dill 1991, Bestelmeyer 2000, LeBrun and Feener 2006), but there is very little work to demonstrate trade-offs among colonies (Gordon 2013). The most active colonies in our study were as much as three times smaller at the end of our experiment than the least active colonies. Colony size is strongly correlated with the fitness of social insect colonies (Houston et al. 1988, Tschinkel 1998b), suggesting that high activity colonies pay a substantial fitness cost. In our experiment, colony growth may have been negatively affected by worker mortality associated with desiccation while foraging outside the nest, increased senescence associated with overworking, and/or worker allocation away from critical interior nest maintenance tasks (e.g., nursing and colony hygiene)(Calabi and Porter 1989). Under field conditions, foraging workers would additionally be exposed to predators, competitors, and other hazards. All of these factors could provide selection to reduce foraging activity. We predict, however, that more active colonies may ameliorate these costs in some field conditions where they may also be more likely to discover and dominate patchily distributed food resources and/or more effective at patrolling and controlling larger territories (Hölldobler and Lumsden 1980, Tschinkel et al. 1995). For example, in harvester ants, increased foraging activity resulted in higher reproductive success only when environmental conditions were poor (Gordon et al. 2013). Future studies are needed that not only document trade-offs but also further explore the selective forces that produce them.

Our results demonstrate that colonies may express substantial behavioral variation independent of significant environmental variation during establishment and growth. We estimated a broad-sense heritability of between 0.45 and 0.5 for the foraging-related behaviors observed. Estimates of heritability of behavioral traits are rare among ants, but our results are comparable to the narrow-sense heritability estimates for worker and gyne mass in acorn ants, *Temnothorax* (h^2=0.37, 0.74) as well as for colony-level behavioral variation in pollen-hoarding behavior in honey bees (h^2=0.5) (Hellmich et al. 1985, Linksvayer 2006). Although maternal and other environmental effects may be widespread in social insects, we think that the colony-level variation we observed likely has a genetic basis. In harvester ants, for example, daughter colonies resemble their mother colonies in the choice of days in which they reduce foraging activity (Gordon 2013). If the level of broad-sense heritability we estimated even remotely reflects narrow-sense heritability (true genetic variation among colonies), then it is highly likely that fire ant foraging behavior is under selection and is evolving. Page and Fondrk (1995) demonstrated that selection could alter pollen-hoarding behavior of honey bee colonies (h^2=0.5) within a single generation. Future studies should seek to identify genes that vary between colony lineages, and compare the behavior and fitness of these colonies under different environmental conditions and selective pressures.

The colony-level variation that we observed is likely to have broad ecological consequences. We expect field colonies of fire ants with high or low patterns of foraging to impact interacting species in consistently different ways. For example, ant foraging behavior can alter dominance hierarchies and diversity of competing ant communities, initiate both top-down and bottom-up trophic cascades, and change seed shadows of plants (Ness 2004, Kaplan and Eubanks 2005, Sanders and van Veen 2011). Our data suggests that these important ecological effects will vary depending on the behavior of neighboring fire ant colonies. Incorporating measures of heredity and intraspecific trait variation can significantly improve models of community assembly and alter predictions of extinction risk, population spread, and the outcomes of species interactions (Fox and Vasseur 2008, Bolnick et al. 2011, Laughlin et al. 2012). There is increasing interest in the ability of more heritable traits to shift the balance of evolution and ecological dynamics (Bolnick et al. 2011). Higher heritability and increased intraspecific variation of ecologically important traits allows more rapid adaptation, increasing the chance of persistence in novel environments and decreasing the window of time in which a species may be displaced by better adapted competitors (Urban et al. 2008). We expect that comparing the relative heritabilities of foraging behavior and other competitively important traits between ant species will increase accuracy of predictions of success and spread of invasive ants. Colony-level variation in the foraging behavior of other social insects is also likely to have important ecological effects. For example, Gordon et al. (2011) found that harvester ant colonies vary in their baseline foraging rate. This suggests that the impact of harvester ants as seed predators could vary significantly among ant colonies and there could be significant subsequent variation in the effect of harvester ants on neighboring plant communities (MacMahon et al. 2000). Documenting colony-level variation in behaviors associated with the ecosystem functions provided by social insects will be critical to more accurately predict and potentially manage the ecological effects of these pervasive and critically important animals.

The regional variation we observed suggests that macro-environmental factors may affect colony-level behavioral variation. Throughout the experiment, colonies from the Texas site exhibited on average higher activity and recruitment, faster resource discovery, and greater weight of food collected compared to colonies from the Mississippi site. Texas colonies also tended to increase their extra-nest activity over time, while Mississippi colonies tended to reduce activity. Imported fire ants (species complex *S. invicta* and *Solenopsis richteri)* were introduced into Alabama more than 70 years ago and expanded through the coastal US, reaching Mississippi around 1940 and spreading into Texas around 1975 (Callcott and Collins 1996). Intriguingly, our results align with the “spatial sorting” hypothesis, which predicts that faster or more active individuals will move further from the invasion origin, leading to assortative mating and the evolution of faster individuals at the spreading edge of an invasion (Shine et al. 2011). Bengston and Dornhaus (2014) found colony behavior of native *T. rugatulus* ants varied along a latitudinal gradient. It would be extremely interesting to test fire ant colony behavior patterns along an invasion gradient. Recent studies have hypothesized that behavioral syndromes, particularly those geared toward higher activity and aggression, may contribute to invasive success (Pintor et al. 2009, Fogarty et al. 2011, Chapple et al. 2012, Knop et al. 2013). Comparisons across native and invasive range may help to clarify the role of evolution and changing environment in the success of invasions and the evolution of collective behavior.

The results of this study lend new support to the idea that colony-level variation in social insect behavior is likely to be widespread in natural populations and can have significant consequences for colony fitness which selection may act upon. Most work on intraspecific variation has focused on the organismal level (Jandt et al. 2014). Our work provides evidence that “individual” variation and personality is likely to be relevant at the level of the superorganism, highlighting an important area for future research.

CHAPTER III

THE SEARCH FOR SUGAR EXPLAINS COLONY-LEVEL VARIATION IN THE DIRECT AND INDIRECT ECOLOGICAL IMPACTS OF AN INVASIVE ANT

3.1 Overview

Intraspecific variation in behavior appears to be widespread among social insects, but the ecological impacts of such colony-level differences are poorly documented. We provide the first empirical test of the multitrophic effects of colony-level behavioral variation of a widespread and invasive social insect pest. Red imported fire ant colonies (*Solenopsis invicta*) vary persistently in their foraging activity and effort at different resources. We quantified this variation and assayed the direct and indirect impacts of these colony-level differences on an insect herbivore and its host plant. Colonies with higher foraging activity at carbohydrates predated caterpillars significantly faster and reduced herbivory damage of cotton plants by as much as seven fold. Colony differences in foraging activity at proteins improved predictions of ant effects on caterpillar mortality only when differences in carbohydrate foraging were first accounted for. Our study demonstrates that colony-level behavioral variation can have dramatic, cascading consequences for other organisms, and suggests that differences in carbohydrate-attraction may drive variation in the ecological impacts of fire ants. Incorporating differences in nutritional ecology may improve our ability to predict variable effects of social insects on other organisms.

3.2 Introduction

A growing body of literature on social insects documents the existence of colony-level differences in behaviors such as aggression, cooperation, hygiene, foraging, exploration, and decision-making (Jandt et al. 2014)(Chapter II). Differences among individuals within a species can fundamentally alter ecological interactions and reshape community dynamics (Bolnick et al. 2011). Despite this, efforts to incorporate intraspecific variation into ecological models have lagged (Sih and Bell 2008, Bolnick et al. 2011). Social insects are major ecosystem engineers whose behavior can fundamentally alter plant and arthropod communities through roles in pollination, soil turbation, seed dispersal, and nutrient flow (Folgarait 1998, Price et al. 2011). Colonies function as "superorganisms" comprised of sometimes hundreds of thousands of individuals, magnifying their potential impacts (Hölldobler and Wilson 2009). Despite this, we know of no empirical studies that examine the relationship between colony-level behavioral variation and the impact of social insects on other species.

Foraging is one of the primary ways most organisms interact with their environment (Stephens et al. 2007), making nutritional ecology a promising area for further exploration. Protein-derived nitrogen is thought to be a limiting resource for many predators and omnivores, driving their behavior and ecology (Denno and Fagan 2003). On the other hand, recent research has increasingly emphasized the role of carbohydrates in fueling the workforces of social insects (Wilder and Eubanks 2010b). The aggressiveness and activity of Argentine ants, for example, is positively correlated with carbohydrate consumption (Grover et al. 2007), while differences in foraging at arboreal carbohydrate resources has been linked to the invasive success of fire ants (Wilder et al. 2011b).

In our previous work, we identified substantial, persistent colony-level variation in the foraging and resource use of the red imported fire ant, *Solenopsis invicta* (Chapter II). Fire ants are a widespread invasive pest species in the southern US (Tschinkel 2006, Ascunce et al. 2011), whose activity has been shown to re-shape native ant communities, form the foundation of “keystone” mutualisms with other insects, and ignite multi-level trophic cascades (Porter and Savignano 1990, Wojcik et al. 2001, Styrsky and Eubanks 2007). Here, we use experimental microcosms to test for the direct and indirect impacts of colony-level variation in fire ant foraging activity on an insect herbivore and its host plant. Specifically, we test the hypothesis that colony differences in foraging activity at carbohydrate versus protein/lipid resources will predict differences in caterpillar mortality and cotton plant defoliation.

3.3 Methods

3.3.1 Colony collection and maintenance

We collected 10 fire ant colonies (Texas A&M University, Brazos Co., TX). From each field-collected colony of origin we established two standardized experimental colonies with 0.1 gram of brood (~200 larvae and pupae) and 1 gram of workers (~2000 ants) and maintained them under standardized laboratory conditions using previously established protocols (Wilder et al. 2011a)(Chapter II). All food was removed from trays 24 hours before each assay.

3.3.2 Assays: Foraging activity

We allowed colonies to acclimate for at least two days and then assayed foraging activity at crickets (a protein/lipid resource) and artificial nectar (a carbohydrate resource)(Chapter II). For the cricket assay (day zero) we placed one freshly killed cricket 30 cm from the nest entrance and recorded the number of ants foraging at the cricket at five minute intervals for 70 minutes. We calculated the average number of foraging ants across the peak period of activity (15 to 40 minutes) to determine each experimental colony's average recruitment to crickets. The nectar assay was carried out the following day (day one) in the same manner, using a 3mL tube of artificial nectar.

3.3.3 Assay: Ecological effects of foraging variation

After nine days we introduced two cotton plants (3-weeks old, 3-6 leaves per plant) into each microcosm. We allowed ants and plants to acclimate for 24 hours and removed all food from trays. On day ten we transferred five third-instar beet armyworm caterpillars (*Spodoptera exigua*) to random leaves of the cotton plants in each colony. We quantified the direct effects of fire ants from different colonies on caterpillar mortality by observing colonies for 4.5 hours and recording the number of surviving caterpillars in each colony at 45 minutes intervals. Caterpillar predation rate was calculated as the maximum number of caterpillars killed divided by the number of minutes to reach this maximum. On day eleven (after total caterpillar mortality) we quantified indirect effects of fire ant colonies on herbivory by using a grid to estimate the percent damage per leaf for each plant. Defoliation was calculated by standardizing percent leaf damage relative to the ratio of caterpillars per leaf in order to control for plant differences.

3.3.4 Analysis

We used one-way ANOVA to test for the effect of colony of origin on each variable (recruitment to nectar, recruitment to cricket, predation rate, herbivory). We then calculated Pearson's product-moment coefficients to determine how variables were correlated. Finally, we used multiple regression to determine the effects of nectar and cricket recruitment levels on predation rate and herbivory. We calculated type II squared partial correlation coefficients (partial R^2 values) for each variable to determine the percentage of variation explained when the other factors were accounted for. Interaction effects were non-significant and were excluded from all models. We square root transformed count data and arcsine square root transformed percentage data for all tests. Results were analyzed using SAS 9.3.

3.4 Results

Fire ant colonies varied significantly in their foraging activity (Fig. 3.1A; Nectar: $F_{9,19}$=14.14, p=0.0001, R^2= .9271; Fig. 3.1B; Cricket: $F_{9,19}$=51.37, p= <0.0001, R^2=.9788). Ants from the most active colony of origin recruited nearly three times more workers to artificial nectar than average and recruited more than six times more workers to crickets. Colonies with high activity at nectar also tended to have high activity at crickets (Table 3.1; r=0.7547, p=<0.0001).

Colony of origin also explained a substantial amount of the variation in both caterpillar predation rate (Fig. 3.1C; $F_{9,19}$=2.98, p=0.0520, R^2=.7284) and herbivory by caterpillars (Fig. 3.1D; $F_{9,19}$=2.99, p=0.0513, R^2=0.7293). The rate at which fire ants predated caterpillars varied more than seven fold among colonies, and the amount of leaf damage that plants received from caterpillars varied by the same factor. Furthermore, plants paired with ant colonies that had higher rates of caterpillar predation received significantly lower percent defoliation (Table 3.1, r=-0.6220, p=0.0034).

Colony impacts on predation and subsequent herbivory correlated significantly with colony differences in nectar foraging, rather than protein/lipid foraging (Table 3.1). Colonies with high foraging recruitment to nectar predated caterpillars faster (Table 3.2, Fig. 3.2A, r=0.5438, p=0.0132) and were associated with lower herbivory of cotton plants (Fig. 3.2B, r=-0.6033; p=0.0049). Colony differences in cricket foraging were significant predictors of colony predation rates only when differences in nectar foraging were first accounted for (Table 3.2, nectar: $F_{1,19}$=17.21, p=0.007; cricket: $F_{1,19}$=7.37,

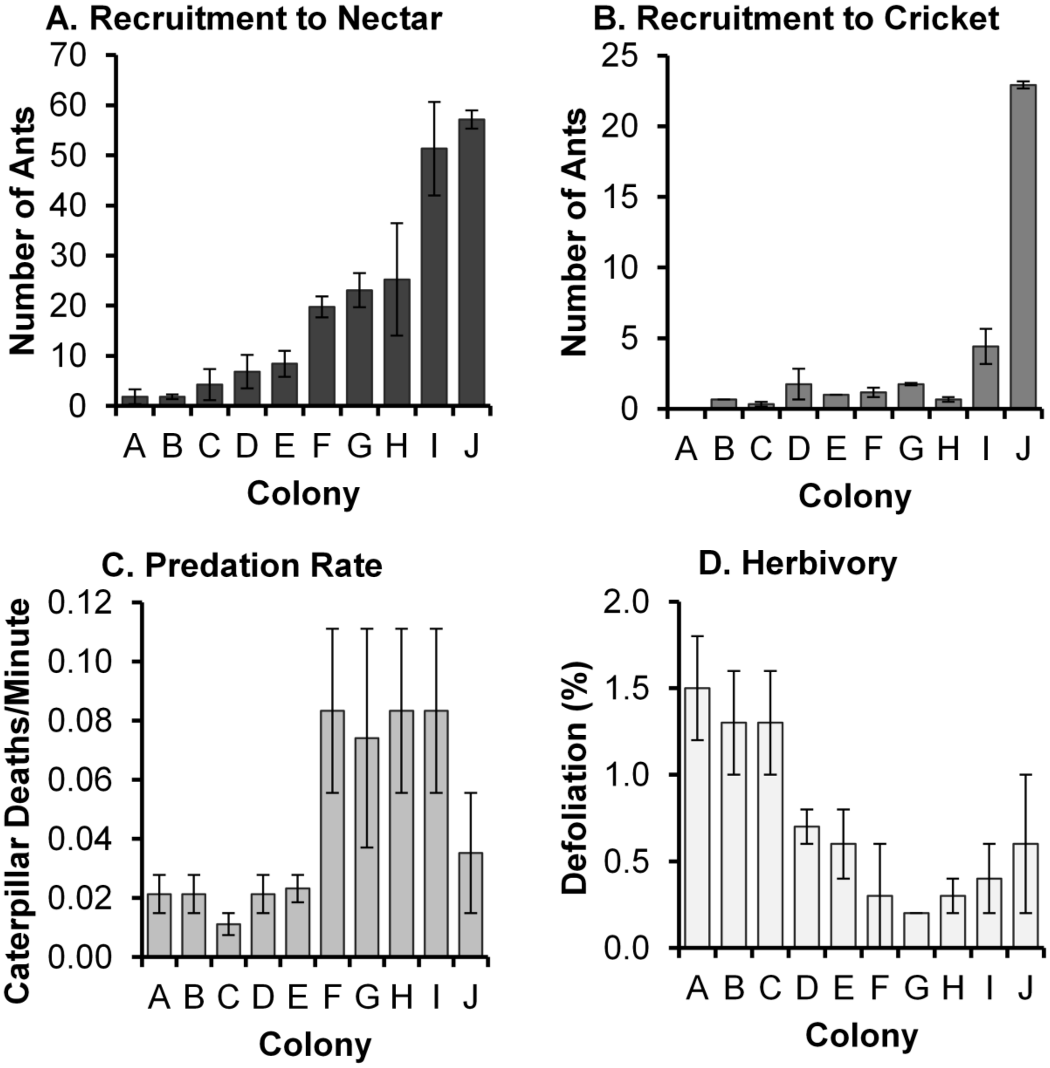

Figure 3.1: Fire ant foraging activity (A, B) and impacts (C, D) grouped by colony. Bars are calculated from number of ants recruiting to artificial nectar (A), number of ants recruiting to a cricket (B), predation rate (caterpillar deaths/minute) (C) and percent defoliation (D). Error bars show standard error.

Table 3.1: Pearson's product-moment coefficients (r, n=20) and p-values for foraging activity and impacts.

	Rec. Nect.	**Rec. Crick.**	**Predation Rate**	**Herbivory**
Recruitment to Nectar		0.75472 p=0.0001*	0.54380 p=0.0132*	-0.60333 p=0.0049*
Recruitment to Cricket	0.75472 p=0.0001*		0.10761 p=0. 6516	-0.25759 p=0.2729
Predation Rate	0.54380 p=0.0132*	0.10761 p=0. 6516		-0.62201 p=0.0034*
Herbivory	-0.60333 p=0.0049*	-0.25769 p=0.2729	-0.62201 p=0.0034*	

p=0.0147) and both recruitment types explained more variation together than either independently (Table 3.2, model R^2=0.5088; nectar partial R^2=0.5030, cricket partial R^2=0.3025). Intriguingly, only colony recruitment levels to nectar explained a significant portion of the variation observed in defoliation (Table 3.2, nectar: $F_{1,19}$=12.12, p=0.0029, partial R^2=0.4161; cricket: $F_{1,19}$=2.83, p=0.1106, partial R^2=0.1428).

Table 3.2: The effects of nectar and cricket recruitment levels of colonies of fire ants on (A) caterpillars killed per minute and (B) percent leaf damage due to herbivory as ascertained with two multiple regressions.

	Recruitment Level	**Partial *R*^{2}**	***F***	***p***
A) Predation Rate	Nectar	0.5030	17.21	0.0007*
	Cricket	0.3025	7.37	0.0147*
B) Herbivory	Nectar	0.4161	12.12	0.0029*
	Cricket	0.1428	2.83	0.1106

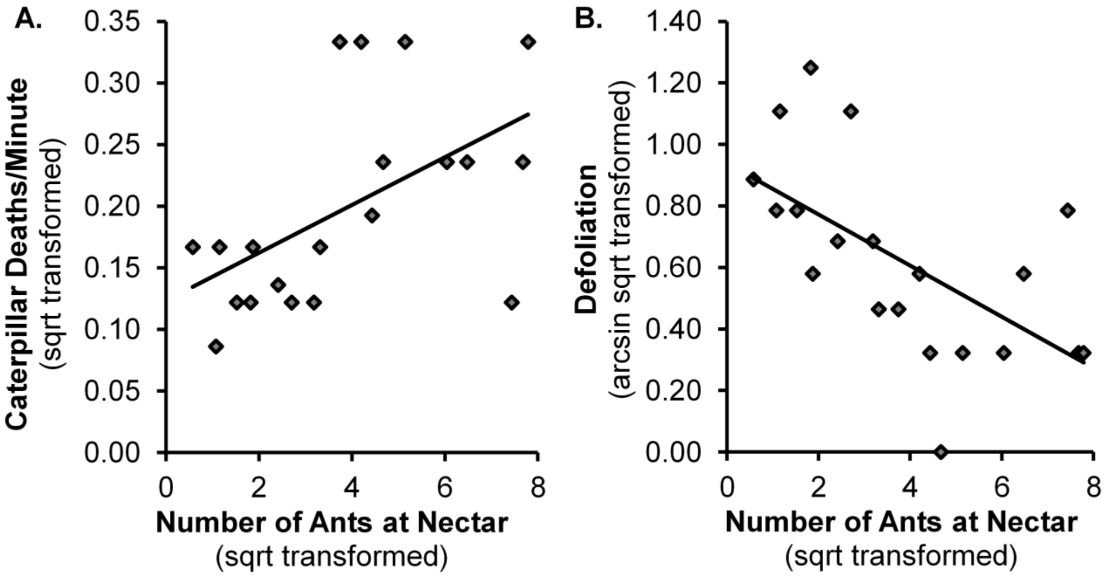

Figure 3.2: Colony recruitment to artificial nectar vs. predation rate (A) or caterpillar herbivory (B).

3.5 Discussion

Our study demonstrates that fire ant colonies vary in foraging behavior and this has significant direct and indirect effects on herbivores and plants. Moreover, our results suggest that carbohydrate attraction plays a central role in mediating the ecological impacts of colony-level variation. Colonies that were more strongly attracted to carbohydrates predated caterpillars faster and reduced herbivory by as much as seven fold. Studies of predators and omnivores often focus on protein-derived nitrogen as the primary driver of foraging behavior and the consequent ecological impacts (Fagan and Denno 2004, Wilder and Eubanks 2010b). Our results, however, found only a limited correlation between protein/lipid foraging activity and colony impacts on caterpillar mortality, and only after accounting for the substantial impact of carbohydrate foraging. Further studies are needed to untangle the relationship between carbohydrate attraction, activity, and predation.

Carbohydrates provide the primary fuel for social insect worker activity and have been linked to faster colony growth in fire ants (Tennant and Porter 1991, Cook et al. 2010, Wilder et al. 2011a). Access to carbohydrates has been linked to invasive success (or failure) of several social insects, including fire ants, and subsequent dominance of these resources by invaders can have substantial ecological impacts (Beggs 2001, O'Dowd et al. 2003, Hoffmann and Kay 2009, Wilder et al. 2011b). We suggest that the impacts of fire ant colony behavioral types will be driven by their interactions with carbohydrate resources in the ecosystem, such as plant extrafloral nectaries and honeydew-producing hemipterans. We expect colony-level differences in carbohydrate-motivated activity to substantially alter food web interactions, the frequency and impacts of ant-aphid mutualisms, the growth and spread of colonies, the effectiveness of ant baits, and many other ecologically important factors (Styrsky and Eubanks 2007, Pintor et al. 2009, Eubanks and Finke 2014)(Chapter II*)*.

This is the first documented example of ecological effects of colony-level variation in behavior. Moreover, the effects of variation were multitrophic, with direct effects on herbivores leading to indirect effects on plants. The presence of fire ant colonies with more active foraging behavior decreased herbivore density and increased plant biomass. This suggests that the ecological effects of colony-level differences could be wide-reaching. Other factors that affect level of fire ant foraging, such as aphid presence, have been shown to result in community-wide changes, such as outbreaks of specific herbivores, changes in plant quality, and effects on plant reproductive fitness and crop yields (Styrsky and Eubanks 2007). Top-down effects on herbivory, such as we observed, can themselves initiate bottom-up trophic cascades, through effects on plant nutritional quality, induced plant defenses, induced regrowth, and ecosystem engineering (Ohgushi et al. 2012). If colony-level differences correlate with other environmental variables we would also expect the ecological impacts of species to covary with these factors. For example, more active colonies could be more frequent at invasion fronts, where we would expect their effects on herbivores and subsequent effects on plants to be more pronounced (Fogarty et al. 2011). Our results are likely to have widespread significance for many social insects and their ecological interactions (Bolnick et al. 2011, Jandt et al. 2014). Exploring these effects, as well as the interactions between behavioral types and different environments will be an important avenue for future research.

CHAPTER IV

COLONY-LEVEL BEHAVIORAL VARIATION CORRELATES WITH DIFFERENCES IN EXPRESSION OF THE *FORAGING* GENE IN RED IMPORTED FIRE ANTS

4.1 Overview

Among social insects, colony-level variation is likely to be widespread and have significant ecological consequences. However, very few studies have documented how genetic mechanisms influence behavior at the colony level. Differences in expression of the *foraging* gene have been associated with differences in foraging and activity of a wide variety of organisms, and specifically with division of labor in social insects. We quantified expression of the red imported fire ant *foraging* gene (*sifor*) in foragers and interior workers from 21 colonies collected across a wide range of natural Texas populations of fire ants. Colonies varied significantly in their behavior, with the most active colonies having as much as ten times more foragers active outside the nest on average as the least active colonies. Expression of *sifor* was on average 3.2 fold higher in foragers than interior workers. Moreover, expression differences among colonies correlated with colony-level behavioral variation. Colonies with higher *sifor* expression in foragers had, on average, significantly higher foraging activity, exploratory activity, and recruitment to nectar compared to colonies with lower expression. These results provide insight into the genetics and physiology underlying collective differences in social behavior, and may provide an important tool for examining and predicting the ecological consequences of colony-level behavioral variation.

4.2 Introduction

Intraspecific behavioral variation is nearly ubiquitous, can have significant evolutionary and ecological consequences, and remains poorly documented and understood in many organisms (Bolnick et al. 2011). In social insects, the primary unit of selection is the colony, and individual behaviors and other traits manifest as collective colony phenotypes (Seeley 1997, Hölldobler and Wilson 2009). Much work has been done examining the genetic, environmental, and social mechanisms that influence individual variation, specialization, and/or division of labor within a colony (Oster and Wilson 1979, Robinson 1992, Mattila and Seeley 2007). There have been surprisingly few studies, however, examining how the same mechanisms influence variation among colonies of social insects (Page Jr et al. 1995, Gordon et al. 2011, Pruitt et al. 2013, Jandt et al. 2014). Few, if any, studies have directly linked differences in gene expression among individuals to differences among colonies in collective behavior. In this study, we use a promising candidate gene to compare how colony-level variation in gene expression relates to colony-level differences in behavior of the red imported fire ant, *Solenopsis invicta*.

The *foraging* gene (*for*) and its homologs have been shown to influence foraging and related behaviors in a wide variety of organisms (e.g., nematodes (Fujiwara et al.

2002), fruit flies (de Belle et al. 1989), grasshoppers (Lucas et al. 2010b), aphids (Tarès et al. 2013), honeybees (Ben-Shahar 2005), harvester ants (Ingram et al. 2011)). The gene encodes a cGMP-dependent protein kinase (PKG), which can respond to cGMP signaling to phosphorylate target molecules, triggering a suite of downstream effects. For example, in fruit flies higher expression correlates with increased sucrose responsiveness (Scheiner et al. 2004), increased metabolic plasticity in response to food presence/absence (Kent et al. 2009), increased movement between food patches (Nagle and Bell 1987), and decreased long-term memory retention (Engel et al. 2000). In the insect order Hymenoptera (ants, bees, and wasps), differences in expression of this gene have been linked to differences in worker foraging and division of labor (Tobback et al. 2008, Kodaira et al. 2009, Lucas and Sokolowski 2009, Ingram et al. 2011, Tobback et al. 2011). For example, in honeybees (*Apis mellifera*), upregulation of *amfor* expression is involved in the transition of nurses to foragers (Ben-Shahar et al. 2003). Although several of the previously mentioned studies examined individuals from multiple colonies and found expression differences among the colonies tested, no previous studies have documented how or if these differences in expression relate to colony-level behavior.

The red imported fire ant is an invasive pest species, with significant ecological, economic, and health consequences for the southern United State and many areas around the world (Tschinkel 2006, Ascunce et al. 2011). The foraging behavior of these ants negatively impacts native wildlife, alters seed dispersal, and can fundamentally shift multitrophic interactions (Allen et al. 2004, Ness 2004, Kaplan and Eubanks 2005). In previous work, we found persistent colony-level variation among fire ants in behaviors such as extra-nest activity, exploration, and foraging recruitment to resources (Chapter II). We also demonstrated that behavioral variation among fire ant colonies significantly altered their effects on herbivores and plants, with higher foraging colonies increasing caterpillar mortality and decreasing defoliation of cotton plants (Chapter III). Here, we survey natural populations of fire ants and quantify the relationship between colony-level variation in behavior and *S. invicta foraging* (*sifor*) expression of foragers and interior workers. We provide the first evidence that expression levels of a single gene can predict colony-level differences in ecologically relevant behaviors.

4.3 Methods

4.3.1 Colony collection and maintenance

We collected fire ant colonies across a 300km east-west range of Texas, spanning multiple ecoregions (Table A.2). This experiment was performed in two blocks, starting on June 30th 2012 and September 24th 2012. We collected up to four colonies per site from 11 sites. Only colonies with sufficient workers and brood for the experimental replicates were included in the analysis, for a total of 21 colonies of origin. All colonies in a block were dug up and established in the laboratory within a 72 hour period and allowed to acclimate to lab conditions for 1 week prior to behavioral assays. From each field colony of origin we created 3 standardized experimental colonies of 0.6g workers (~1200 ants) and 0.1g brood (~200 larvae and pupae). Experimental colonies were maintained in individual colony habitats in standardized conditions in the laboratory and maintained on diets of crickets and artificial nectar using previously established protocols (Chapter II).

4.3.2 Behavioral assays

We next assayed colony behavior. All behavioral assays were performed midafternoon and all food was removed from colony habitats at least 12 hours prior to the assay. To quantify colony recruitment to carbohydrate resources, we placed a tube of artificial nectar in each foraging arena, 30cm from the nest entrance. We recorded the number of ants foraging at the nectar after five minutes and then every ten minutes for one hour. We calculated average colony recruitment to nectar as the mean number of ants observed at the nectar between 10-40 minutes after resource placement. These assays were performed 7/10/12 and 10/2/12 for each block, respectively. To quantify colony recruitment to protein/lipid resources, we placed a freshly killed cricket in each foraging arena, 30cm from the nest entrance and recorded the number of foraging ants as previously described. These assays were performed 7/11/12 and 10/3/12. To quantify average colony extra-nest activity, we counted the number of ants active outside the nest in the foraging arena (with no food present for at least 12 hours prior) at the same time on three consecutive days. These assays were performed on 7/9-11/12 and 10/1-3/12 and averaged across the three days. To quantify average exploratory activity exploratory activity we placed a novel climbing structure comprised of two halves of a 7.6 x 12.7 cm index card skewered vertically at the top of a 30 cm bamboo skewer into each colony

40cm from the nest entrance on three consecutive days. We counted the number of ants exploring the structure after 5 minutes and then every ten minutes for one hour. We scored average colony exploratory activity by taking the sum of these counts and averaging them across the three days. These assays were performed on 7/13-15/12 and 10/5-7/12.

In order to quantify daily weight of food collected per colony and colony foraging activity at a stable resource, we removed all food from colonies and switched colony maintenance diets from crickets and artificial nectar to a composite ant diet. Two grams of a dry, pelleted ant diet at a p60:c20 ratio (Cook et al. 2010) were provided to each colony in a petri dish. Assays began after 24 hours acclimatization to the new diet. Every 24 hours for 3 days, the diet was replenished with a new dish, and the previous diet was removed, dried, and weighed. The average weight of food collected per colony per day was calculated by averaging the three measurements. To assay average colony foraging activity at a stable resource, we counted the number of ants actively foraging at the dry diet at the same time on three consecutive days. Both diet collection and foraging activity assays were performed on 7/14-16/12 and 10/6-8/12.

4.3.3 Analysis

We used one-way ANOVA to test for effect of colony of origin on all behavioral variables (recruitment to nectar and crickets, extra-nest activity, exploratory activity, foraging activity, weight of diet collected). We observed no effect of block, site of origin, or longitude or latitude of origin, so these factors were excluded from the analysis. Count data was square root transformed. All results in this study were analyzed using SAS software, Version 9.3 of the SAS System for Windows. Copyright 2011 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

4.3.4 Primer design

We used sequence data from the *S. invicta* draft genome (Munoz-Torres et al. 2011) to design flanking primers for exon-coding regions of the *S. invicta* ortholog of *foraging* (*sifor*). We used these to sequence DNA extractions from individual fire ants from six different colonies (QIAGEN DNeasy kit; Applied Biosystems BigDye Terminator v1.1 Cycle Sequencing kit). We aligned the transcribed regions of the gene with each other using Vector NTI AlignX and found the sequenced regions to be non-polymorphic among colonies. We used these sequences and OligoPerfect Designer (Life Technologies) to design specific primers for *sifor*. Primers were designed around an intron region to minimize the possibility of DNA contamination during RNA quantification. We used sequence data from GenBank and the *S. invicta* draft genome to design specific primers for *actin* 87E isoform 1 with Primer Express software v3.0 (Life Technologies). ß-actin has been successfully used as an endogenous control gene in previous work on fire ants (Lu and Pietrantonio 2011). (Primer sequences: *sifor*-F: 5'-

ACGATCCAGAAGGAGTGAGC; *sifor*-R: 5'-ACGCCAAGAGTCGCAATAGT; *siact*-F: 5'-AATCTTGCGGTATCCACGAA; *siact*-R: 5'-TCGACGTCGCACTTCATG.)

4.3.5 Quantification of sifor *expression*

At the end of behavioral assays we collected workers foraging at a cricket and workers from the interior of the nest (near the brood pile) from each experimental colony and flash froze them in liquid nitrogen. All samples were collected at the same time of day to control for circadian effects on expression patterns. For each worker type for each colony replicate, total RNA from pooled samples of 10-20 worker heads was isolated using phenol-chloroform extraction with TRIzol reagent (Life Technologies) and protocols. Total RNA at 300ng/uL was DNAse treated using RQ1 RNase-Free DNase kit (Promega) and reverse transcribed into cDNA with the Applied Biosystems High Capacity cDNA Reverse Transcription kit (Life Technologies) and anchored oligo dT (20) primers (Integrated DNA Technologies).

We assayed expression of *sifor* mRNA in fire ant heads relative to an endogenous control gene (*actin* 87E isoform 1) using real-time quantitative reverse transcription-polymerase chain reaction (qPCR) techniques on an Applied Biosystems 7300 RealTime PCR System with PerfeCTa SYBR Green SuperMix, ROX reagents and protocols. We used specific primers for *sifor* and *actin* to amplify 160ng and 10ng cDNA, respectively. Samples were pipetted from a standard concentration (20ng/uL) to minimize variation and loading error. We included negative and positive control reactions on each plate and used melting curve analysis on a subsample of plates to confirm primer specificity. Relative expression was quantified based on the difference in the number of PCR cycles (Ct) at which a fluorescent threshold crossed, using $\Delta\Delta$Ct analysis (ABI User Bulletin 2). Each cycle difference (ΔCt) between *actin* and *sifor* represents a two-fold relative difference in expression, and ΔCt scores were adjusted relative to starting concentrations. Each colony replicate (n=3) of each task group type (foragers or interior workers) was amplified and quantified three times, and the Ct values averaged. Technical replicates with Ct values significantly different from the others were excluded from the analysis.

We used a t-test of ΔCt scores to compare the average colony *sifor* expression of foragers versus interior workers. We used multiple regression to determine the effects of forager *sifor* expression and interior worker *sifor* expression on the assayed colony behaviors (recruitment to nectar, recruitment to cricket, foraging Activity, extra-nest Activity, exploratory Activity).

4.4 Results

4.4.1 Behavioral assays

Fire ant colonies varied significantly in all measured behaviors, and ants from the same colony of origin behaved most similarly to each other (nectar recruitment: $F_{19,33}=6.84$, $p<0.0001$; cricket recruitment: $F_{19,33}=2.84$, $p=0.0041$; foraging activity: $F_{19,33}=3.09$, $p=0.0022$; extra-nest activity: $F_{19,33}=13.13$, $p<0.0001$; exploratory activity:

$F_{19,33}$=2.57, p=0.0084; diet collected: $F_{19,33}$=2.38, p=0.0140). For example, more active colonies had as many as 10 times more foragers active on average compared to the least active colony, and more than 16 times as many workers outside the nest.

4.4.2 Quantification of sifor *expression*

We observed a significant effect of worker task group on *sifor* expression. Foragers had on average 3.2 fold higher *sifor* expression than workers collected from the interior of the nest (Fig. 4.1A; $F_{1,16}$=12.17, p=0.0013). This pattern was consistent across all colonies of origin except one, which had unusually high *sifor* expression in the interior workers sampled (Fig 4.1B).

Most notably, we observed a significant correlation between expression of *sifor* and colony behavior. Fire ant colonies with higher *sifor* expression in foragers had higher recruitment to nectar, foraging activity, and exploratory activity (Fig. 4.2; nectar recruitment: $F_{1,50}$=6.98, p=0.0110; foraging activity: $F_{1,50}$=14.98, p=0.0003; exploratory activity: $F_{1,50}$=11.04, p=0.0017). The *sifor* expression of interior workers did not significantly correlate with colony behavior (p>0.1, all behaviors), but including *sifor* expression of interior workers as a covariate in the model with *sifor* expression of foragers explained more variation than forager *sifor* expression alone (nectar recruitment: R^2=0.1393; foraging activity: R^2=0.2714; exploratory activity: R^2=0.1922;

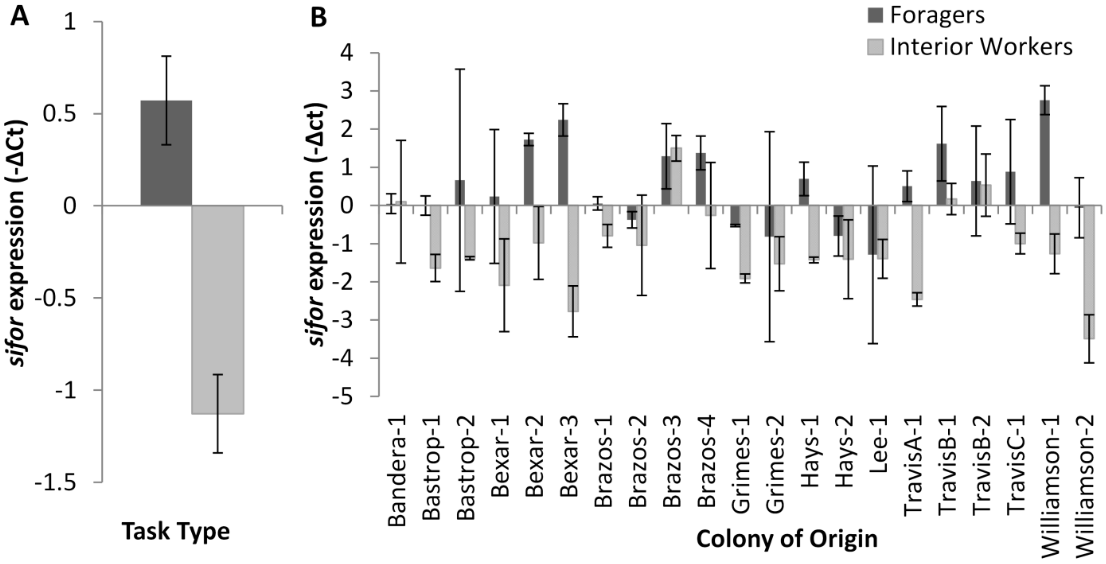

Figure 4.1: Average *sifor* expression in heads of workers collected foraging (dark grey) or in the interior of the nest (light grey), relative to *actin* expression. Bars show data averaged across (A) task groups and (B) colony of origin. Colonies of origin are listed by collection site and colony number. Expression scores are presented as –ΔCt because lower ΔCt scores reflect higher expression compared to the endogenous control. Error bars show standard error.

extra-nest activity: R^2=0.0717; cricket recruitment: R^2=0.0174;). The *sifor* expression levels of foragers and interior workers explained as much as 27% of the colony differences in average foraging activity, and about 19 and 13% of the colony differences in exploratory activity and recruitment to nectar, respectively. Extra-nest activity also had a positive, though non-significant, correlation to forager *sifor* expression (extra-nest activity: $F_{1,50}$=3.67, p=0.0612). Intriguingly, colony *sifor* expression of foragers did not correlate with differences in recruitment to crickets or with the weight of dry diet collected per day (cricket recruitment: $F_{1,50}$=6.98, p= 0.3789; diet collected: $F_{1,50}$=0.28, p=0.4399).

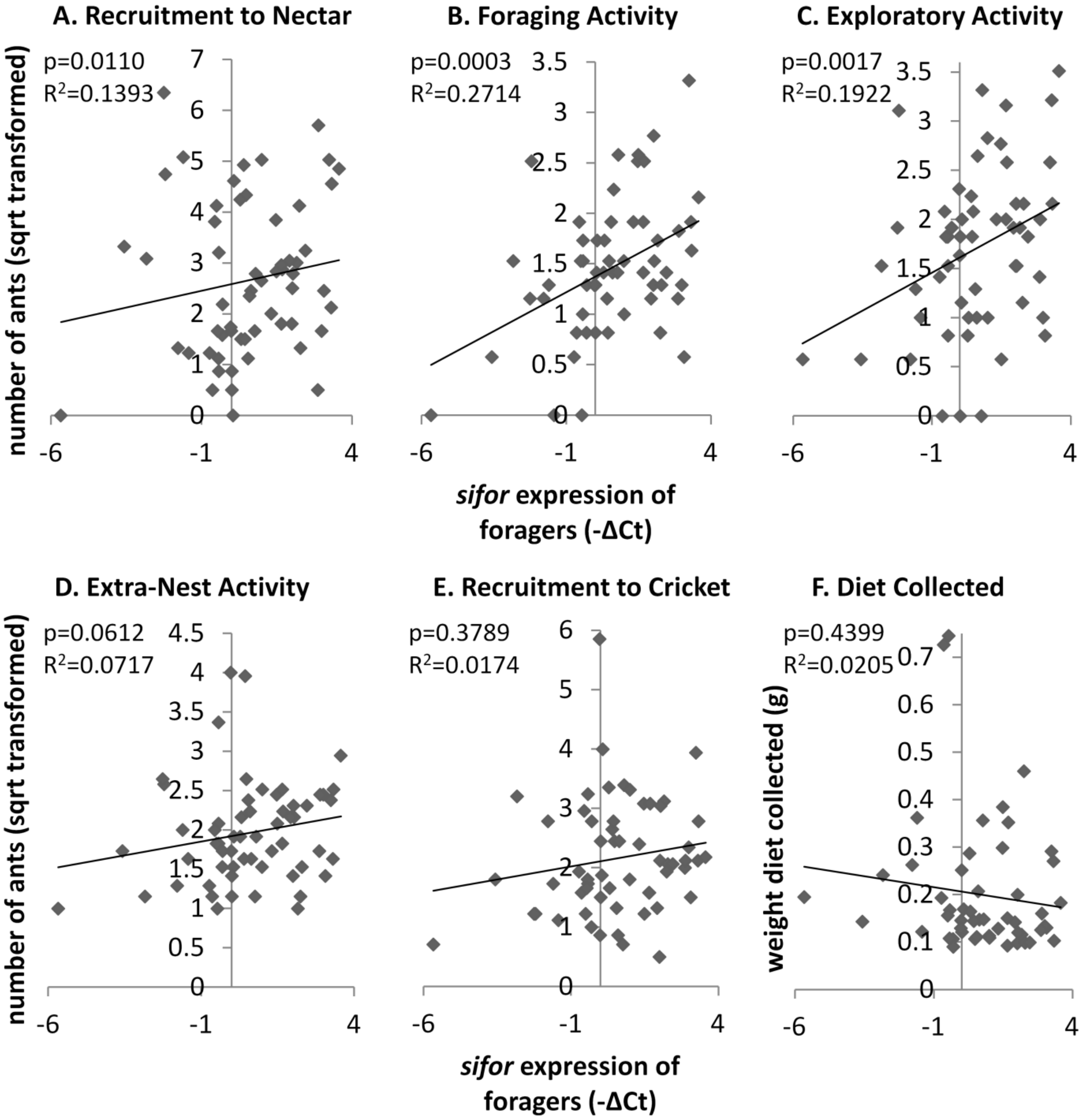

Figure 4.2: The relationship between average *sifor* expression in the heads of foragers versus the behavior of experimental colonies of fire ants. Graphs depict expression relative to (A) average number of ants recruiting to artificial nectar; (B) average number of ants present at a permanent dry diet resource each day; (C) average number of ants exploring a novel structure; (D) average number of ants recruiting to a freshly killed cricket; and (F) average weight per day collected from a permanent dry diet. Expression scores are presented as –ΔCt because lower ΔCt scores reflect higher expression compared to expression of the endogenous control.

4.5 Discussion

Understanding the genetic mechanisms behind variation in complex social behaviors is a fundamental goal of behavioral research. The results of this study demonstrate that *sifor* expression in fire ants correlates with worker task type, and provide the first evidence that the average *sifor* expression levels of foragers in a colony correlate with colony-level differences in foraging activity, exploratory activity, and recruitment to carbohydrate resources. Our study is the first study to directly link *foraging* expression levels of groups of workers to collective behavior and colony phenotype. These results indicate that the *sifor* expression of foragers and interior workers could be used to predict the behavior of fire ant colonies and their impacts on the surrounding ecosystem.

We observed more than three-fold higher *sifor* expression on average in foraging workers compared to workers collected in the interior of the nest. Our task-group results fit with those of a recent study, which found higher *sifor* expression in fire ant workers collected in the foraging area versus in the nest area, and indicate that *sifor* plays a role in the division of labor among fire ants (Lucas et al. 2015). The higher *sifor* expression we observed in foragers of colonies with increased foraging and exploratory activity suggest two possible mechanisms by which *sifor* expression could affect the collective behavior of fire ant colonies. The colony differences in average *sifor* expression we observed could reflect differences either in the proportion of high *sifor* expressing workers per colony, differences in the relative expression levels of individual workers, or a combination of both. The former would indicate differences in colony regulation of division of labor, while the latter could suggest variation among ants in foraging threshold. Ingram et al. (2011) found that harvester ants, which forage in specific circadian cycles, upregulate *foraging* gene expression during their period of peak foraging activity. Foragers from different harvester ant patrilines also vary in the time of day they begin foraging (Wiernasz et al. 2008). Fire ants forage actively throughout the day and night as temperatures and humidity permit (Porter and Tschinkel 1987, Tschinkel 2006). Our colonies were maintained under standardized, environmentally controlled conditions and sampled at the same time each day. It would be very interesting to test both daily patterns of *sifor* expression, which we would not expect to vary, and the *sifor* expression levels of workers from different lineages of fire ants. Foraging behavior in fire ants is likely to have a heritable component (Chapter II). We predict that fire ants from different lineages will vary in average *sifor* expression, and that multilineage colonies with a high diversity of *sifor* expression may be more flexible in their response to different environmental conditions and have higher fitness (Mattila and Seeley 2007, Pruitt and Riechert 2010). Quantification of *sifor* expression levels of individual workers in a colony would further assist in untangling the mechanisms behind colony differences in behavior, and may become more manageable as techniques for quantification of very low yield mRNA improve.

Our study suggests that *sifor* expression affects colony behavior in a quantitative or dose-dependent manner, with increased mRNA expression levels correlating with increased number of foragers active outside the colony, increased exploratory activity, and higher recruitment effort to artificial nectar. In fruit flies, the *foraging* gene has been shown to alter the threshold of feeding response to appetitive stimulus (Scheiner et al. 2004), and in several model organisms cGMP signaling has been demonstrated to modulate sensory neuron responsiveness and axon connectivity (Firestein and Bredt 1998, Schmidt et al. 2002). This suggests that *sifor* expression, through the cGMP-PKG signaling pathway, may modulate the responsiveness of individual workers to foraging cues. This aligns with observations in the big-headed ant, *Pheidole pallidula*, where differences in foraging activity among workers are associated with differences in PKG activity, the enzyme product of *ppfor*. Similarly, Gordon et al. (2011) found that harvester ant colonies vary in the baseline rate at which foragers leave the nest, a behavior regulated by forager response to the stimulus of incoming workers. They theorized that colony differences in foraging rate could be attributed to differences in the response threshold of foragers to stimulus (Gordon et al. 2011). Further work to localize the expression of *sifor* in different tissues and to determine the downstream biological effects could shed light on this issue.

Intriguingly, our results suggest that *sifor* expression may specifically relate to fire ant foraging for carbohydrates. Studies of ants and other predators have often assumed their foraging to be nitrogen-limited and protein-driven (Fagan and Denno 2004), but recent research has increasingly placed the acquisition of carbohydrates in a central role (Wilder and Eubanks 2010b, Cook et al. 2011)(Chapter III). Our results indicate that differences in fire ant *sifor* expression correlate with colony differences in recruitment to artificial nectar (a carbohydrate resource), but not recruitment to crickets (a protein and lipid resource). Previous work in fire ants suggests that foraging for protein, which is necessary for brood development, and foraging for carbohydrates, which fuel the large pool of workers, are regulated separately and vary in response to programmed seasonal cues (Cook et al. 2010, 2011). We propose that the *sifor* gene may be involved in regulating this macronutrient-specific behavior. In fruit flies, *for* expression affects the threshold of fly feeding response to sucrose, as well as the levels of gene products involved in carbohydrate metabolism and insulin signaling (Scheiner et al. 2004, Kent et al. 2009), suggesting that this gene can direct complex behavioral and metabolic responses underlying different strategies of foraging. Intriguingly, our previous work further demonstrates that differences in fire ant colony recruitment effort to carbohydrates (rather than proteins and lipids) best predict the differential effects of fire ant colonies of herbivore mortality rate and plant defoliation (Chapter III). The correlation we observed between *sifor* expression and colony behavior is therefore extremely likely to correlate with ecological effects of colonies. This suggests that colony expression of *sifor* may prove a useful assay tool, allowing researchers to categorize of colonies from expression levels of a small sample of workers and then make predictions about the behavior and ecological effects of these colonies. Moreover, if expression differences are heritable, the regulation of carbohydrate foraging among colonies is likely to be under selection and evolving. The *foraging* gene is conserved across *Animalia*, and is likely to play a similar role in the division of labor and the regulation of foraging in many social insects . Variation in carbohydrate foraging has been linked to invasion success in both fire ants and Argentine ants (Rowles and Silverman 2009, Wilder et al. 2011b). We would similarly expect colony-level variation in the carbohydrate foraging of honeybees, where *foraging* gene expression has already been tied to division of labor, to have dramatic impacts on pollination and crop yields (Ben-Shahar et al. 2002, Klein et al. 2007).

Our results provide insights into variation in colony behavior and the genetics and physiology underlying this behavior. The *foraging* gene is likely to provide a useful tool for further exploring how fire ant colonies vary in response to genetics and environment, and how colony-level behavioral differences are likely to impact the spread and ecological consequences of this important invasive insect (Ascunce et al. 2011). Moreover, because this gene appears to serve a convergent function in many social insects and affect behavior of a wide variety of organisms (Ben-Shahar 2005, Lucas et al. 2010a), these results are likely to have wide utility for future investigations in many fields.

CHAPTER V

COOPERATION VS. COMPETITION: GROUP COMPOSITION INFLUENCES THE SUCCESS OF GROUP-FOUNDING QUEENS IN THE RED IMPORTED FIRE ANT

5.1 Overview

Kin-selection predicts that individuals in groups will act in ways to maximize their inclusive fitness. Group-founding, or pleometrosis, is a widespread strategy among social insects in which foundress queens cooperate to establish new colonies. In bees and wasps foundress groups often have high relatedness, but in ants often found with less related individuals. The ability of foundress ants to perceive differences in group composition and adapt their cooperation and competition is largely unknown. Here, we use geographic distance as an estimate of relatedness, and compare the performance of fire ant foundress groups comprised of different numbers of queens collected from either the same site or geographically distant sites. Queens founding in groups showed clear evidence of intra-group conflict. Foundress groups comprised of queens from the same site produced more workers per group than solo or mixed-site queens. Mixed-site foundress groups did not perform noticeably better than queens founding alone, and produced significantly less workers per queen. Our results support the hypothesis that fire ant foundresses will be able to detect differences in co-foundress relatedness and alter their competitive behavior to maximize their inclusive fitness. These results have implications for predictions about foundress decision-making and the evolution of cooperative social behavior.

5.2 Introduction

According to kin-selection theory, group cooperation should be highest when individuals are closely related, and inclusive fitness benefits are high (Hamilton 1964). The cooperation of non-kin individuals can evolve in animals when the fitness benefits of group-living outweigh the costs of intraspecific competition (Higashi and Yamamura 1993, Nowak 2006). Social insects make excellent models for testing kin-selection theories. Group-founding, or pleometrosis, is a strategy employed across a wide variety of social insect taxa, in which often unrelated foundress queens cooperate in the establishment of new colonies (wasps: Itô 1993, halictine bees: Keller and Wilson 1993, termites: Roisin 1993, ants: Choe and Perlman 1997, thrips: Morris et al. 2002). Benefits accrue to the group through increased resources, sharing of dangerous or costly tasks, and faster colony growth leading to a higher chance of colony survival; while costs to individual queens may include reproductive sabotage, intra-group competition for resources, and decreased likelihood per queen of eventually producing daughter colonies (Balas and Adams 1996, Tsuji and Tsuji 1996, Bernasconi and Strassmann 1999, Kolmer and Heinze 2000, Tibbetts and Reeve 2003). In such situations, we expect selection to favor the ability of foundress queens to discriminate amongst more and less related queens and alter cooperative and competitive behavior based on inclusive fitness gained through co-foundresses. The significant effects of foundress relatedness are borne out in bees and wasps where in many species queens often found with sisters and foundress relatedness affects offspring sex ratios and inclusive fitness returns of subordinate foundresses (Metcalf and Whitt 1977, Frank 1985, Schwarz 1987). However, less is known about potential effects of relatedness on ant foundress groups. Although kin-discrimination and nepotistic behavior of workers in mature colonies has been demonstrated in multiple ant species (Page et al. 1989, Ratnieks and Reeve 1992, Hannonen and Sundström 2003), we know of no studies that have explicitly tested the ability of ant foundress groups to adapt their competitive and cooperative behavior based on group composition. In this study, we use an estimate of relatedness to create ant foundress groups of higher and lower probability of queen relatedness and compare their performance.

Ant foundresses provide an excellent model system to test hypotheses about cooperation among individuals related to different degrees. In bees and wasps, group-founding queens generally exhibit moderate relatedness, but in ants, mating flight behavior tends to reduce the probability of foundress relatedness, and foundress groups are often formed with unrelated individuals (Bernasconi and Strassmann 1999, Edenbrow 2011). These group-founding associations in ants are usually temporary, ending around the time the first workers emerge, when foundresses may fight to the death, be executed by workers or be evicted from the nest (Heinze 1993, Edenbrow 2011). Even in polygyne ants species, where mature colonies may maintain many unrelated queens, these queens often pay a fitness cost due to mutually inhibited fecundity or nepotistic worker care (Vargo and Fletcher 1989, Ratnieks and Reeve 1992, Hannonen and Sundström 2003). For group-founding to be evolutionarily favored, these substantial costs to queens must be balanced by equally substantial fitness gains.

One of the best early measures of group and queen performance is worker production. Newly-founded colonies compete strongly, and work force size correlates strongly with fitness in ant colonies and may give incipient colonies an advantage in early tasks like foraging and brood raiding (Tschinkel 1992, Gordon 1995, Palmer 2004). A non-additive increase in worker production with group size (reduced worker production per queen) is an indicator of selfish behavior or conflict within the group. Queens may behaviorally and pheromonally inhibit each other's egg-laying, may hoard resources and decrease their own energy expenditure on costly tasks, or may cannibalize brood to boost their own fitness (Choe and Perlman 1997, Bernasconi and Strassmann 1999, Cahan 2001). For example, Bernasconi et al. (1997) found that fire ant queens of different potential fighting abilities altered their egg-laying in order to maximize their survival probability.

Because queens with higher relatedness should receive more inclusive fitness from the other queens in the group, we expect groups with more related queens to have less competition and perform better than groups with less related queens. Here, we use geographic distance as an estimate of relatedness, and compare the performance of red imported fire ant queens (*Solenopsis invicta*) in founding groups comprised of queens from the same site of origin or from geographically distant sites of origin. We predict that larger founding groups will have higher total production of workers, but lower production of workers per queen. We further predict that founding groups of queens collected from the same location will have both higher total production of workers and higher production of workers per queen than founding groups comprised of queens from mixed locations.

5.3 Methods

5.3.1 Study system

The red imported fire ant, *S. invicta*, is an invasive pest species with significant ecological, economic, and health consequences for much of the southern United States and many areas around the world (Lofgren and Adams 1982, Ascunce et al. 2011). Fire ants have two genetically distinct social forms: monogyne, with a single queen, and polygyne, with multiple queens; which interbreed in variably sympatric populations (Ross and Fletcher 1985, Ross and Shoemaker 1993, Fritz and Vander Meer 2003). Alate sexual fire ant females mate monandrously in nuptial flights following heavy rainfalls. They then drop to the ground, remove their wings, and seek out a site to begin egg-laying (Tschinkel and Howard 1983, Tschinkel 2006). Queens practice claustral founding, sealing themselves into short burrows or crevices to rear their first complement of workers on resources stored in their own bodies. Both monogyne and polygyne queens may found colonies alone or in small groups (DeHeer et al. 1999, DeHeer 2002, Tschinkel 2006). Polygyne queens employ mixed dispersal strategies, and will also join established polygyne colonies and/or reproduce by budding from an established colony with a complement of workers (DeHeer et al. 1999). Tschinkel and

Howard (1983) surveyed fire ant foundress groups after seven mating flights and found that groups ranged in size from 1-17 queens, with average group size ranging from 1.1-3.4 queens.

Fire ant colonies are extremely vulnerable during establishment, and greater than 99% of all fire ant queens die during the founding period (Whitcomb et al. 1973). Groups with more queens and/or more workers are more likely to survive attacks and have a competitive advantage against other incipient colonies in tasks such as brood raiding (Tschinkel 1992, Adams and Tschinkel 1995, Jerome et al. 1998). Fire ant queens in groups mutually inhibit the reproduction of other queens via pheromones, cannibalize brood, and engage in direct conflict with other queens and workers (Vargo 1992, Tschinkel 1993, Balas and Adams 1996). Shortly after the first queen-raised workers have eclosed as adults, workers open the nest and begin foraging, brood care, and raids on other nests (Tschinkel 2006). In monogyne colonies, supernumerary queens are executed or expelled by the workers within about three weeks of eclosion (Balas and Adams 1996). Workers in experimentally manipulated colonies are as likely to execute a related as an unrelated queen, and aggression may be preferentially directed towards injured or less productive queens (Fletcher and Blum 1983, Balas and Adams 1996). In polygyne colonies the fate of foundress queens is poorly documented, but it seems likely foundress cohorts at least sometimes persist. There are no records on whether monogyne and polygyne fire ant foundresses ever found together, but dispersing foundresses of both social types are attracted to similar habitats (DeHeer et al. 1999).

5.3.2 Collection and assays

Following mating flights, we collected newly mated, dealate foundress queens from collection sites at least 2.5 km apart. Relatedness in fire ants tends to be spatially sorted, particularly in primarily polygyne populations (Ross and Shoemaker 1993, Shoemaker et al. 2006). This distance between collection sites is sufficient to ensure queens from primarily different colony sources. While winds may disperse queens great distances, a series of experiments found that the majority of fire ant queens landed with 400m of their source, and only from 3-15% of queens flew as far as 1.6 km from their source (Markin et al. 1974, reviewed in Tschinkel 2006). Our experiment was carried out in two blocks, first for a pair of sites in College Station, TX (5/9/12) and repeated for a pair of sites in Conroe, TX (5/12/12). We collected from 20-56 queens per site (n=181). Queens were individually weighed and cloistered into randomly selected founding groups within 24 hours of collection. Founding groups were established with different group compositions (Fig 1.): three group sizes (one, two, or four queens) and three group types (“solo”: one queen; “same”: all queens from the same site, or “mixed”: half queens from one site, half from the other). All applicable site combinations were represented in each group. Founding groups were cloistered in darkened nest tubes and incubated in standardized lab conditions (Banks et al. 1981)(Chapter II).

We observed the founding groups daily for 50 days and recorded to the days of first worker eclosion (molting from pupa to adult). We then recorded the number of eclosed workers daily for seven days following the first worker eclosion for each group.

Groups in which any queens died or in which no workers eclosed within 50 days were excluded from the analysis (final group n=71).

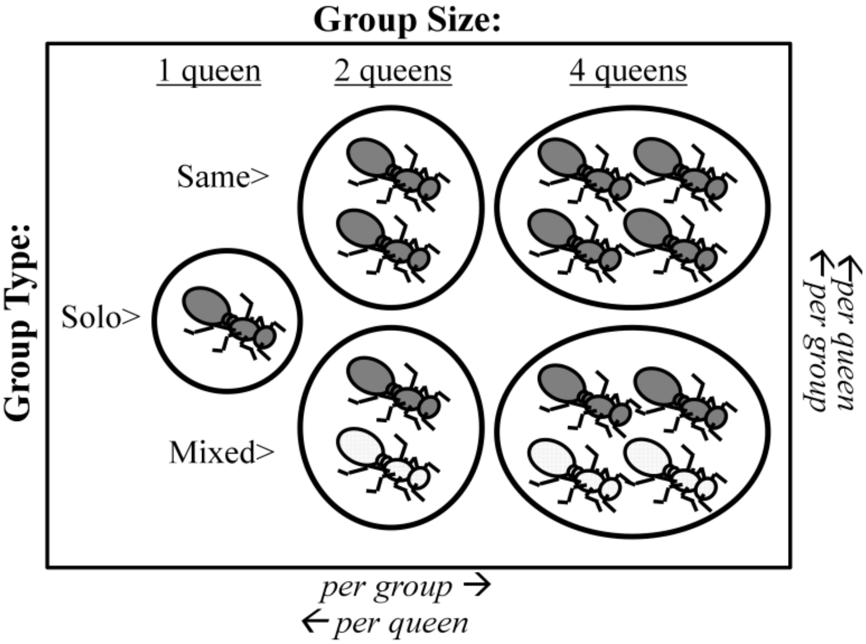

Figure 5.1: Experimental design showing experimental founding group composition. Newly mated foundress queens were collected immediately following mating flights and cloistered in founding groups of either 1, 2, or 4 queens, either singly (solo), from the same collection site (same) or half each from different collection sites (mixed). Arrows show predicted direction of increased worker production.

5.3.3 Analysis

We used one-way ANOVA and multiple regression analysis to test for the effects of founding group composition, founding queen number, and founding group type (solo, same, or mixed) on the days to first worker eclosion, the number of workers produced per group, and and the number of workers produced per queen, respectively. Average queen weight was included in the models as a covariate. We used contrast analysis to rank worker production per group and per queen for each founding group type (solo, same, or mixed). We saw no significant effect of block so this variable was excluded from the analyses. Solitary queens did not vary significantly in worker production based on collection site of origin, so data from all sites were collapsed into solo and same-type group categories, respectively, and considered together. We additionally tested for effects of group size and group type on the probability of queen death. All count data was square root transformed and days to first eclosion were log transformed for all analyses.

5.4 Results

Founding group composition significantly affected both the number of workers produced per group and the number produced per queen (Fig. 5.2, per group: $F_{4,65}=3.10$, $p=0.0213$; per queen: $F_{4,65}=13.32$, $p<0.0001$). The number of workers eclosed per group increased with group size in a non-additive manner. Groups with more queens produced significantly more workers per group, but less workers per queen (Fig. 5.3, per group: $F_{1,68}=4.69$, $p=0.0338$; per queen: $F_{1,68}=20.54$, $p<0.0001$). Founding groups with four queens produced on average only 1.6 times as many workers per group as founding groups with one queen. Queens in founding groups with three other queens produced 2.4 times less workers on average than queens founding alone.

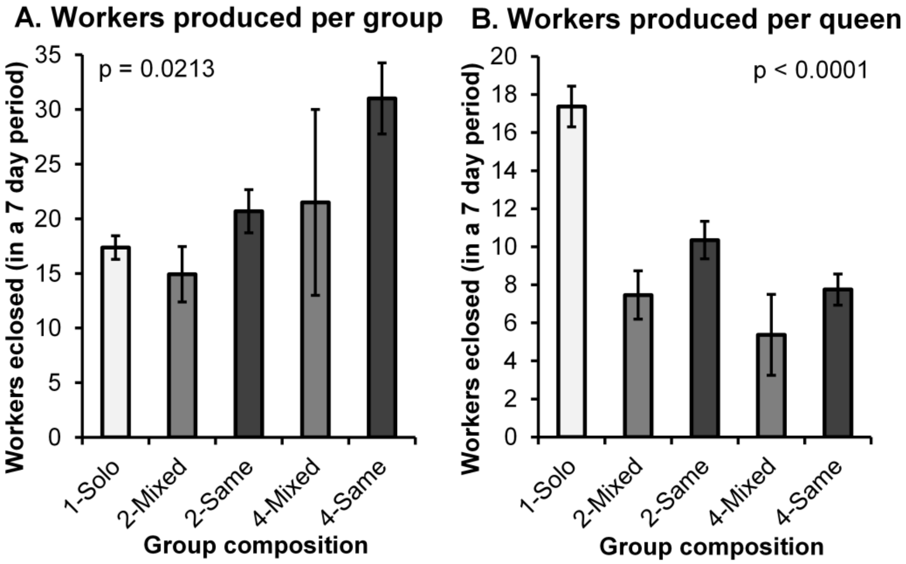

Figure 5.2: Graph of the average number of workers eclosed within seven days of first worker eclosion (A) per founding group; and (B) per queen. Bars show averages for founding groups sorted by number of queens (1, 2, 4) and group type (solo, mixed collection sites, same collection site). Error bars show standard error.

Figure 5.3: Graph of the average number of workers eclosed within seven days of first worker eclosion (A) per founding group; and (B) per queen, based on founding group size (1, 2, or 4 queens).

Founding group type also significantly affected both the number of workers produced per group and per queen within a seven day period. Queens in “solo” founding groups produced significantly less workers per group than queens in “same” type groups, while the number of workers produced per group by queens in “mixed” type groups was not significantly different from either other group type (Fig. 5.4A; solo vs. same: $F_{1,65}=6.72$, $p=0.0118$; solo vs. mixed: $F_{1,65}<0.01$, $p=0.9539$; same vs. mixed: $F_{1,65}=3.43$, $p=0.0686$). Queens in “solo” founding groups produced significantly less workers per queen than queens in “same” or “mixed” type groups (Fig. 5.4B; solo vs. same: $F_{1,65}=17.96$, $p<0.0001$; solo vs. mixed: $F_{1,65}=21.84$, $p<0.0001$; same vs. mixed: $F_{1,65}=2.10$, $p=0.1517$).

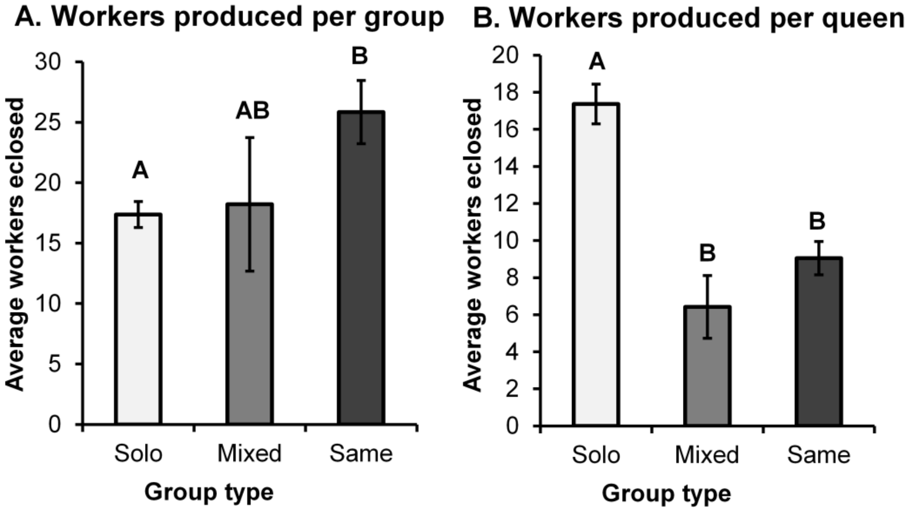

Figure 5.4: Graph of the mean (± standard error) of the number of workers eclosed within seven days of first worker eclosion (A) per founding group; and (B) per queen. Bars show unweighted averages for founding group types: solo (single queens), mixed (queens from two different collection sites) and same (queens from the same collection site). Letters show results for contrast analysis of significant differences.

On average, the first worker in a group eclosed 35 days after queens were cloistered. Neither the effect of founding group size nor group type were significant at the α=0.05 threshold (Fig. 5.5A-C; composition: $F_{4,65}$=1.43, p=0.2350; size: $F_{1,68}$=1.14, p=0.2895; type: solo vs. same: $F_{1,65}$=3.74, p=0.0574; solo vs. mixed: $F_{1,65}$=0.17, p= 0.6809; same vs. mixed: $F_{1,65}$=2.99, p=0.0885). However, the overall patterns in days to first worker eclosion among foundress groups of various compositions aligned with the patterns we observed for number of workers produced, with days to first worker eclosion tending to decrease with group size and in groups with queens from the same site of origin. Founding groups with higher average queen weights produced their first adult worker significantly faster than groups with smaller queens (Fig. 5.5D; $F_{1,65}$=8.52, p=0.0048). Of all the foundress queens collected, about 15% died prior to or within a week of the eclosion of any workers. Neither group size nor group type had any significant effect on the probability of queen death.

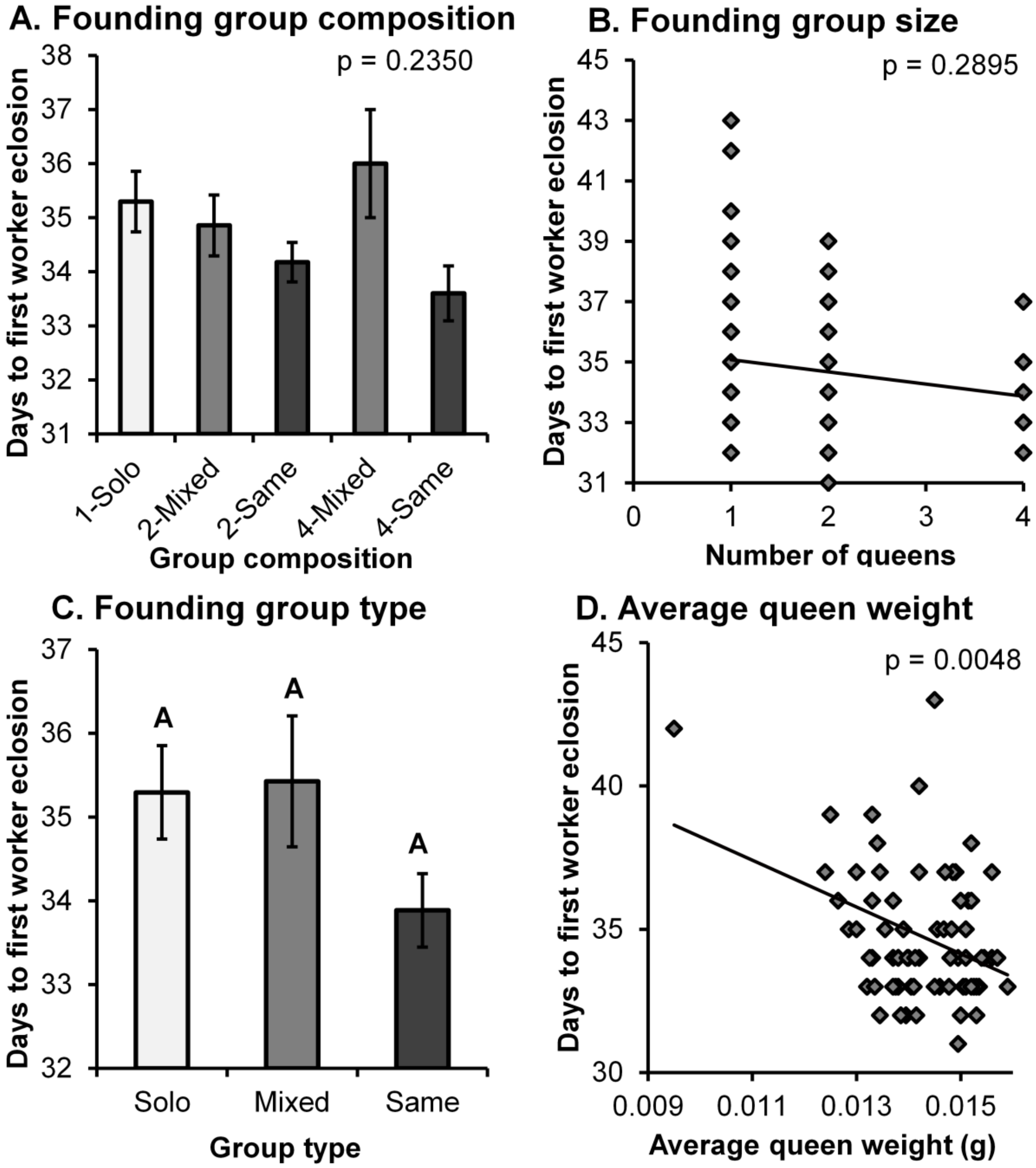

Figure 5.5: Effect of (A) founding group composition, (B) founding group size, (C) founding group type, and (D) average queen weight of founding group on the number of days until first worker eclosion. Founding groups were composed of 1, 2, or 4 queens in solo, mixed collection site, or same collection site group types. Error bars show standard error.

5.5 Discussion

Our study is the first to demonstrate that queen site of origin can alter the cooperative and competitive dynamics of ant foundress groups. As expected, fire ant foundress groups with more queens produced more workers per group but less workers per queen, indicating conflict in foundress associations. Foundress groups comprised of queens collected from the same site showed less signs of conflict than those comprised of queens from distant sites. In fact, foundress groups comprised of mixed-site queens did not produce significantly more workers than queens founding alone. This study demonstrates that foundress groups can perceive and respond to differences in group composition. Our results support the hypothesis that foundress groups with higher relatedness of queens will reduce conflict and increase cooperation compared to groups with less related queens (Hamilton 1964, Bourke 2011).

The differences in worker production we observed between foundress groups of same or mixed-site queens indicate that fire ant foundresses are able to perceive and respond to queen site of origin. In ants, nestmate recognition is primarily a function of olfactory detection of a subset of odors on the body surface, synthesized by individual ants and accumulated from the environment (Hölldobler and Wilson 1990, Tschinkel 2006). Colony members use “phenotype matching” to compare the odors of encountered individuals to a learned template of colony odors (Lacy and Sherman 1983, Gadagkar 1985). Both monogyne and polygyne fire ant workers respond to heritable and environmentally-acquired odors. Fire ant workers respond less aggressively to more genetically similar ants, as well as to ants fed on similar diets, and these effects are roughly additive (Obin et al. 1993). In our study, foundresses could have been responding to odor differences acquired from either differences in relatedness or differences in environmental history. Intriguingly, the net effect could be similar, as we would expect queens with similar environmentally-acquired odors (from diet, soil-type, and nestmates) to be more likely to be related. Because our experiments measured worker production for seven days following the first worker eclosion it is possible that the observed differences among groups could be primarily driven by newly eclosed workers: for example, nepotistic differences in brood-tending or cannibalism (Tschinkel 1993, Hannonen and Sundström 2003). However, the differences we observed in worker production subsequent to first eclosion aligned with the pattern of the first worker eclosion among foundress groups of different types. These group-level trends in worker eclosion speed, prior to the eclosion of any adult workers, lend strong credence to a queen-driven effect.

Our observations suggest several possible mechanisms by which these differences in foundress group worker production could be modulated by queens. Queens could reduce aggression towards queens perceived as more similar, decreasing stress and injuries as well as incentive to selfishly reduce energy expenditure on egg-laying or brood-feeding in order to boost individual fitness and fighting ability (Bernasconi et al. 1997). For example, Bernasconi et al. found that among pairs of fire ant foundresses, the queen that lost the least body mass tended to have more total offspring and was more likely to win fights. Cloistered foundress groups are closed systems, so all resources must come from metabolic stores in the queens' own bodies.

Alterations of how these resources are allocated could provide another mechanism by which conflict is modulated in foundress groups of different queen compositions. Queens deplete their fat reserves to feed larvae and newly-eclosed workers regurgitated liquid food and trophic (non-embryonated) eggs (Voss and Blum 1987, Tschinkel 2006). Solitary queens do not eat until workers begin foraging, but evidence suggests queens in foundress groups cannibalize brood and likely increase oophagy of trophic eggs (Tschinkel 1993, Bernasconi et al. 1997). Because previous studies have had no evidence that queens can distinguish their own brood, it has largely been assumed that more productive queens take advantage of the fact that random cannibalization will still leave a great proportion of their own brood untouched (Tschinkel 2006). However, in another recent study, we found that fire ant workers discriminate among related and unrelated brood and appear to preferentially cannibalize unrelated brood (McMichael et al., in prep.). It therefore seems likely that queens in foundress associations will also preferentially cannibalize unrelated brood. Under this assumption, queens from the same site of origin may be less able to distinguish their brood from the brood of co-foundresses and therefore reduce brood cannibalism. This hypothesis could be tested by supplementing the brood of foundress groups with marked brood from another source and tracking brood mortality to see if supplemental brood are consumed or otherwise vanish from the nest more frequently than native brood.

In order to understand the decision-making processes of foundress queens, studies of pleometrosis in social insects often seek to determine the optimal group size that will maximize a queen’s potential fitness, as well as to understand the selective factors that influence this number (Nonacs 1988, Tsuji and Tsuji 1996). Our work demonstrates that the optimal number of queens in a foundress association is likely to vary with the queen composition of the foundress groups. Groups with more related queens may be able to sustain higher queen numbers due to less intra-group conflict and have a competitive advantage over higher conflict foundress groups. It would be interesting to test how queen site of origin or relatedness affects foundress decision-making about group joining. In fire ants, the degree of pleometrosis increases with queen density, and foundresses also appear to be more selective as site saturation increases, clustering in nests non-randomly (Tschinkel and Howard 1983). Both nest quality and co-foundresses appear to affect fire ant queens' choice of nest: given a choice of identical nest holes, queens paired more frequently than would be expected by chance (Tschinkel 1998a). Queens that joined or were joined in foundress groups also had higher survivorship when reared individually than queens found alone, suggesting that queens evaluate their own condition or that of co-foundresses when making decisions about joining (Tschinkel 1998a). If queens make decisions about joining co-foundresses in order to maximize their fitness in groups, we would expect foundress queens collected from the same site to be more likely to co-found than queens from different sites. On the other hand, Tschinkel (1998a) found that randomly created foundress groups of queens collected in the Tallahassee, Florida area performed no better than queen-selected foundress groups, suggesting that queen selection of group members may not affect fitness. Since our results demonstrate that fire ant foundresses can adapt their competitive behavior in a context-dependent manner, this may actually help relieve selective pressures to be choosy about group type. Our study is the first to identify this novel source of group and individual-level differences in foundress associations of the red imported fire ant. Our results are likely to have broad implications for social insects and the evolution and ecology of cooperative group behavior in general. Future studies should test the genetic differentiation of ant foundresses, the relatedness of foundress associations, and queen decision-making behaviors.

CHAPTER VI

CONCLUSION

This study documents and quantifies colony-level variation in ecologically important behaviors of a widespread invasive social insect, demonstrates multitrophic ecological effects of this colony-level variation, and explores genetic factors that may affect and predict behavior at the colony-level. Although social insects function as a "superorganism" with much selection acting at the level of the colony, few studies have specifically examined among-colony variation in behavior (Smith et al. 2008, Hölldobler and Wilson 2009). Nevertheless, recent research has increasingly emphasized the importance of intraspecific variation in understanding the ecological effects of an organism (Smith et al. 2008, Bolnick et al. 2011, Jandt et al. 2014). I investigated colony-level variation in red imported fire ant behavior, quantifying the extent and heritability, the ecological consequences, and genetic factors affecting it. In addition, I explored the role of intraspecific variation in altering the behavior of fire ant foundress groups.

I first surveyed natural populations of fire ants from Texas and Mississippi and assayed their behavior across time and foraging habitat in order to quantify the extent of regional and colony-level variation (Chapter II). Fire ant colonies varied significantly in behaviors such as extra-nest activity, exploration, and resource discovery speed and recruitment effort. Moreover, these colony-level differences persisted over time and across foraging habitats. Fire ant colonies in the field can therefore be expected to interact with their environment in consistently and different ways. Incorporating these differences into ecological models will improve our ability to predict the spread and ecological consequences of this important invasive insect (Fox and Vasseur 2008, Bolnick et al. 2011, Laughlin et al. 2012).

These differences in fire ant behavior also have direct fitness consequences for the colonies. Fire ant colonies with higher recruitment to resources tended to collect more food, while colonies that maintained high extra-nest activity paid a cost in colony growth and had smaller final colony sizes. Although trade-offs associated with foraging behavior within colonies and among social insect species are well documented (Nonacs and Dill 1991, Bestelmeyer 2000, LeBrun and Feener 2006), there is surprisingly little work to demonstrate trade-offs among colonies (Gordon et al. 2013). The regional differences I observed further suggest that fitness-related behavioral differences among colonies may be selected for environmentally (Shine et al. 2011). For example, Gordon et al. (2013) found that more active harvester ant colonies had a fitness advantage only in drought conditions, while Bengston and Dornhaus (2014) found that the behavior of cavity-dwelling ants varied along a latitudinal gradient. Although direct assays of heritability are unpractical in this system, using single-lineage colonies reared in standardized environments I estimated broad-sense heritability of between 0.45 and 0.5 for the observed colony behaviors (Chapter II). These levels are comparable to evolutionarily significant levels of heritability observed in traits such as gyne mass in acorn ants and pollen-hoarding in honeybees (Hellmich et al. 1985, Linksvayer 2006).

Combined, the results of this study strongly indicate that colony-level differences in behavior are under selection and are likely to be evolving.

The colony-level behavioral variation I observed is sufficient to have significant ecological consequences. To test the ecological relevance of colony variation, I established microcosms in the laboratory and quantified the effect of colony foraging behavior on an insect herbivore and its host plant (Chapter III). Colony-level variation in fire ant foraging had significant direct and indirect effects on herbivores and plants. Fire ant colonies with higher foraging activity at carbohydrates significantly increased the rate of caterpillar mortality and reduced defoliation of cotton plants by as much as seven fold. Many studies have focused on nitrogen-limitation as the driving force in predator behavior (Denno and Fagan 2003), but these results support research that indicates the carbohydrates which fuel social insect workers may play the primary role in determining their ecological effects (Grover et al. 2007, Wilder and Eubanks 2010b, Wilder et al. 2011a). Intriguingly, colony differences in foraging activity at protein and lipid resources only improved predictions of fire ant predation rates when differences in carbohydrate foraging were first accounted for. These results provide the first documentation of multitrophic ecological effects of colony-level differences. These results are likely to be applicable to many social insects (Jandt et al. 2014), and have consequences for community structure, food web interactions, management efforts, and many other ecologically important factors (Morrison 2002, Styrsky and Eubanks 2007, Pintor et al. 2009, Eubanks and Finke 2014).

These colony differences correlate with expression of *foraging*, a gene that encodes a cGMP-dependent protein kinase which has been linked to differences in the foraging behavior of a wide variety of organisms (e.g. nematodes (Fujiwara et al. 2002), fruit flies (de Belle et al. 1989), grasshoppers (Lucas et al. 2010b), aphids (Tarès et al. 2013), honeybees (Ben-Shahar 2005), harvester ants (Ingram et al. 2011)). I quantified colony differences colony differences in the expression of the fire ant *foraging* gene (*sifor*) as well as colony-level differences in behavior for fire ant colonies collected from across 300km of Texas (Chapter IV). As expected, expression of the fire ant *foraging* gene (*sifor*) correlated with division of labor. On average, expression of *sifor* was more than three-fold higher in fire ant foragers than in fire ant workers in the interior of the nest. This fits with other recent work in fire ants (Lucas et al. 2015), and provides another data point in the growing picture of the convergent function of *foraging* orthologs across a wide variety of social insects (Ingram et al. 2005, Tobback et al. 2008, Kodaira et al. 2009, Lucas et al. 2010a). Notably, colony-level differences in *sifor* expression of foragers and interior workers correlated with colony behavior. Higher *sifor* expression in foragers correlated with higher foraging activity, exploratory activity, and recruitment to nectar in fire ant colonies. This study is the first to connect gene expression to colony-level behavior, and provides the first evidence that expression levels of a single gene can predict colony-level differences in ecologically relevant behaviors.

Finally, following intriguing observations made while collecting and rearing fire ant foundresses for previous experiments, I explored the hypothesis that fire ant foundress groups could maximize inclusive fitness benefits and alter cooperative and competitive behaviors in response to cues indicating higher relatedness of foundresses (Chapter V). Kin-selection theory predicts that cooperation is most advantageous when groups have high relatedness (Hamilton 1964). The number of workers produced per group increased with the number of queens in a non-additive fashion, confirming that fire ant foundress groups are in conflict. Intriguingly, groups composed of foundresses from distant sites, which were less likely to be related, produced no more workers as a group than queens founding alone. In contrast, groups composed of foundresses from the same site produced significantly more workers as a group. This suggests a that the optimal number of queens in social insect foundress associations is likely to vary with the queen composition of the foundress groups, a factor not previously documented in ants (Tschinkel and Howard 1983, Tsuji and Tsuji 1996). These results demonstrate that fire ant queens can perceive and respond to differences in group composition, and support the hypothesis that foundress groups with more related queens will reduce conflict and increase cooperation compared to groups with less related queens. These results are likely to have broad implications for social insects and the evolution and ecology of cooperative group behavior in general (Bernasconi and Strassmann 1999, Nowak 2006).

This study demonstrates that fire ants exhibit persistent, colony-level variation in foraging and other behaviors. This variation is likely heritable and has significant, multitrophic ecological consequences. Expression of the fire ant *foraging* gene correlates both with worker task group and with colony differences in behavior. Fire ant foundresses increase intra-group competitive behaviors when group-founding with queens less likely to be related to themselves. The conclusions of this study are broad and open up promising new avenues of research at several levels. First, at the mechanistic level, future studies should explore the genetic and physiological factors that influence colony-level behavioral variation. The expression of ants from different colony lineages should be compared to determine if there is genetic variation in expression levels. In several species of social insects, colony fitness has been shown to be improved by increased range of behaviors due to within-colony genetic variation (Cole and Wiernasz 1999, Mattila and Seeley 2007, Cole et al. 2008). In particular, as techniques for quantification of very low yield mRNA improve, it may become possible to quantify *sifor* expression in individual fire ant worker heads. This would allow the precise determination of how collective patterns of behavior emerge from the behavior and genetic expression of individual ants in a colony.

Second, at the organismal level, the selective and evolutionary forces affecting the fitness trade-offs we observed between colonies with higher and lower foraging activity should be explored. Higher activity may be most advantageous when resource distribution is patchy and sporadic, when competition for resources is higher and territories must be maintained, when conditions are harsh or when environmental disturbance is high (Tschinkel et al. 1995, King and Tschinkel 2008, Gordon 2013). Environmental manipulations in the lab and field will be useful in testing these hypotheses. Future studies should also quantify fire ant behavior along environmental gradients, particularly in relation to carbohydrate access and along an invasion gradient

(Shine et al. 2011, Bengston and Dornhaus 2014). Patterns of high activity and dispersal may link to invasive success (Pintor et al. 2009, Fogarty et al. 2011, Chapple et al. 2012, Knop et al. 2013). Further expanding the scope and contexts of behaviors tested may also allow the identification of behavioral syndromes among fire ant colonies (Jandt et al. 2014).

Third, at the level of the ecosystem, the impacts of colony-level behavioral variation on community structure, species interactions, and other ecological factors should be further explored. Colony *sifor* expression levels may prove a useful tool to categorize field colonies and make predictions about their behavior and ecological effects. Documenting colony-level variation in behaviors associated with the ecosystem functions provided by social insects will be critical to more accurately predict and potentially manage the ecological effects of these pervasive and critically important animals (Bolnick et al. 2011, Jandt et al. 2014). The convergent use of the *foraging* gene in social insect division of labor also provides an opportunity for studies to explore the intersection of genetics and ecology. For example, harvester ants upregulate and downregulate *foraging* expression in synchrony with their daily foraging cycles (Ingram et al. 2011), honeybees upregulate expression to trigger the age-dependent transition between interior and exterior tasks (Ben-Shahar 2005), and in big-headed ants both expression and worker task group are associated with specific polymorphic castes (Lucas and Sokolowski 2009). The *foraging* gene has been adapted to function in different ways based on the ecology of the social insect species. Fire ants, with both age-dependent polyethism and fluid polymorphism provide an intermediate data point.

Further determining how this gene is used in fire ant division of labor, as well as surveying its use in a wide variety of social insects species with different systems of division of labor, will allow tests of hypotheses about the relationship between ecology and gene function. The conclusions of this study have widespread implications for many social insects and their ecological interactions (Bolnick et al. 2011, Jandt et al. 2014). By further exploring these effects at the mechanistic, organismal, and ecological level we will improve our understanding of collective behavior, social evolution, and intraspecific variation.

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APPENDIX

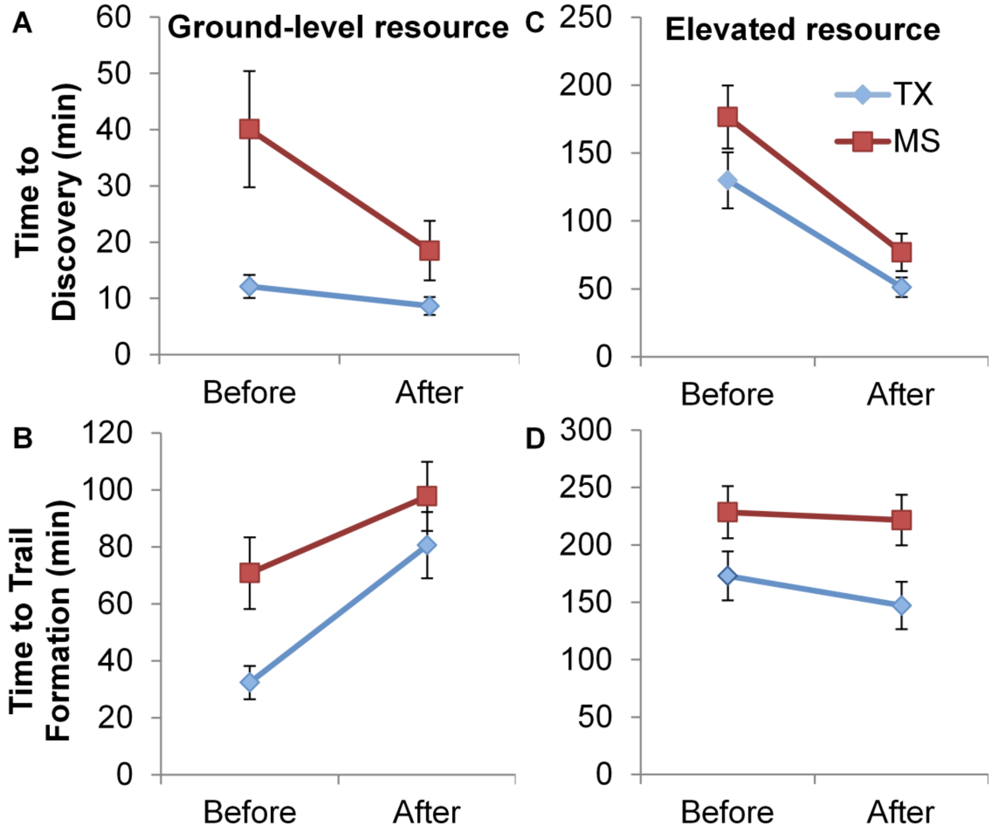

Figure A.1: Foraging habitat and regional effects on fire ant resource discovery and trail formation times. Graphs show average time to discovery for fire ants recruiting to (a) ground-level or (b) elevated foraging resources and average time to formation of a recruiting trail for (c) gound-level or (d) elevated foraging resources for standardized experimental colonies in standardized foraging habitats before and after being exposed to different foraging habitats for five weeks. Colonies are grouped by region of origin (Texas, light diamond vs. Mississippi, dark square). Error bars show standard error.

Table A.1: Analysis of fire ant resource discovery and trail formation times before and after exposure to different foraging habitats. Table summarizes repeated measures analysis of variance for standardized experimental colonies in standardized foraging habitats before and after being exposed to different foraging habitats for five weeks. Within subjects effects use multivariate analysis of variance; lambda is converted to the appropriate F value. Asterisks denote significance at $\alpha=0.05$.

Trait	Effect	p	F	df 1	df 2
Discovery time (elevated)	Foraging Habitat	0.6940	0.16	1	32
	Region	0.0058*	8.73	1	32
	Colony(Region)	0.0003*	3.60	31	32
	Time	<0.0001*	40.18	1	32
	Time*F.Habitat	0.6141	0.26	1	32
	Time*Region	0.4647	0.55	1	32
	Time*Colony(Region)	0.0734	1.69	31	32
Discovery time (ground-level)	Foraging Habitat	0.4479	0.59	1	32
	Region	0.0006*	14.45	1	32
	Colony(Region)	0.0582~	1.76	31	32
	Time	0.0661	3.62	1	32
	Time*F.Habitat	0.1047	2.79	1	32
	Time*Region	0.1782	1.89	1	32
	Time*Colony(Region)	0.9830	0.46	31	32
Trail formation (elevated)	Foraging Habitat	0.3007	1.11	1	32
	Region	0.0004*	15.49	1	32
	Colony(Region)	0.0015*	2.96	31	32
	Time	0.2861	1.18	1	32
	Time*F.Habitat	0.3383	0.95	1	32
	Time*Region	0.5353	0.39	1	32
	Time*Colony(Region)	0.0038*	2.65	31	32
Trail formation (ground-level)	Foraging Habitat	0.5015	0.46	1	32
	Region	0.0010*	13.20	1	32
	Colony(Region)	<0.0001*	4.29	31	32
	Time	<0.0001*	19.09	1	32
	Time*F.Habitat	0.5456	0.37	1	32
	Time*Region	0.2258	1.53	1	32
	Time*Colony(Region)	0.2766	1.24	31	32

Table A.2: Details of the collection sites of origin of the 21 fire ant colonies of analyzed in the experiment. The latitude and longitude of the site, as well as the county and Gould ecoregion are included.

Site (by County)	Colonies	latitude	longitude	Gould Ecogregion
Bandera	1	29.90127	-99.2576	Edwards Plateau
Bastrop	2	30.18539	-97.2863	Post Oak Savannah
Bexar	3	29.45875	-98.1267	Blackland Prairie
Brazos	4	30.61583	-96.3498	Post Oak Savannah
Grimes	2	30.47135	-96.0858	Blackland Prairie
Hays	2	30.29415	-98.113	Edwards Plateau
Lee	1	30.3933	-96.9028	Post Oak Savannah
Travis (Site A)	1	30.31473	-97.8549	Cross Timbers and Prairies
Travis (Site B)	2	30.29958	-98.0119	Edwards Plateau
Travis (Site C)	1	30.35744	-97.8128	Cross Timbers and Prairies
Williamson	2	30.63605	-97.894	Cross Timbers and Prairies