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ANT SYMBIOSES:

**COLONY-LEVEL EFFECTS OF ANTAGONISTIC AND MUTUALISTIC
INTERACTIONS IN TWO MODEL ANT SYSTEMS**

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COLONY-LEVEL EFFECTS OF ANTAGONISTIC AND MUTUALISTIC
INTERACTIONS IN TWO MODEL ANT SYSTEMS**

by

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Dissertation

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Dedication

For Dad, Mom, Shahnaz, and Farahnaz

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ANT SYMBIOSES:
COLONY-LEVEL EFFECTS OF ANTAGONISTIC AND MUTUALISTIC
INTERACTIONS IN TWO MODEL ANT SYSTEMS

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An ant colony can be considered a superorganism with the workers analogous to cells and the entire colony analogous to an organism. Few studies have taken a colony-level approach to understanding how ant societies function. This dissertation explores how ant colonies respond and adjust to changes in the environment brought about through symbiotic relationships. First, I focus on the antagonistic interaction between a specialized phorid fly parasitoid and its host, the red imported fire ant, *Solenopsis invicta*. Phorid flies, which inject their eggs into fire ant workers, have recently been introduced into the United States as a biological control agent of this invasive pest, but the impact of these flies on fire ant populations is yet unknown. I examine how phorids affect colony growth, foraging efficiency, and caste recruitment

of *S. invicta*. This study is the first to link indirect (behavioral modification of foraging) and direct (mortality to workers) effects of parasitic flies on fire ants. Second, I focus on the mutualistic interaction between ant farmers and their cultivated fungi. An intriguing dimension of complexity in the attine ant-fungus mutualism derives from potential conflict over the sex ratio, i.e., the ratio of male to female reproductives produced by a social insect colony. In hymenopteran societies, conflict over sex ratios is presumed to exist between the mother queen and her daughters (workers) due to differences in their genetic relatedness to the sexuals. The presence of heterospecific symbionts in a social insect colony (e.g., fungus-growing ants) complicates predictions of conflict resolution even furthermore. Three parties are potentially in conflict over sex ratios: the mutualistic fungus, the workers, and the queen. I studied the dynamics of sex-ratio conflict and ant-fungus cooperation in the primitive attine, *Cyphomyrmex muelleri*. I show that ants and native cultivars have coevolved to enhance mutualistic interactions. Despite these cooperative interactions, ant-cultivar conflict may have been revealed by the effect of cultivar switches on ant sex ratio and male survival. In sum, this work demonstrates that the integration of colony-level and individual-level investigations provides a comprehensive understanding of how ant societies (superorganisms) function and respond to their symbiotic partners.

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CHAPTER 1

Introduction

Symbiotic relationships may be classified along a continuum of interactions, ranging from parasitism at one extreme and mutualism at the other extreme (Bronstein 1994; Herre et al. 1999). However, such rigid classes may not best describe a close association between two species. Rather, a symbiosis may fall somewhere in between the two extremes of the continuum. Thus, any symbiosis may have aspects of cooperation and conflict.

This dissertation encompasses two distinct studies on ant symbioses. Each project represents the polar extremes along the continuum. The first half of this dissertation examines the effects of a specialized phorid fly parasitoid (*Pseudacteon tricuspis*) on colony growth, worker caste ratios, and task allocation of its host, the red imported fire ant (*Solenopsis invicta*). Female phorid flies use hypodermic needle-like ovipositors to inject their eggs into fire ant workers that are performing outside tasks, such as foraging. The mere presence of these flies causes fire ants to cease foraging and freeze into stationary defensive postures (Feener 1981; Feener and Brown 1992; Orr et al. 1995; Porter et al. 1995; Morrison 1999). Approximately 40 days after parasitism, adult flies emerge from the decapitated heads of their ant hosts.

The second half of this dissertation investigates aspects of cooperation and potential conflict in the attine ant-fungus mutualism. In this textbook case of a mutualism, fungus-growing ants in the tribe Attini obligately depend on fungus as

their major food source (Weber 1972; Hölldobler and Wilson 1990; Mueller et al. 2001) and in exchange provide nourishment in the form of vegetative substrate and protect the fungus from a number of pathogens (Currie et al. 1999). However, there is the potential for conflict between these two mutualistic partners (Mueller in press). One intriguing dimension of conflict may exist over the sex ratio, i.e., the ratio of male to female reproductives produced by the colony. Three parties are potentially in conflict over the sex ratio: the queen, the workers, and the mutualistic fungus. Due to differences in genetic relatedness to the sexuals, the queen should prefer an even sex ratio (male: female) because she is equally related to her sons and daughters; however, the workers should prefer female-biased sex ratios because they are more closely related to their sisters than to their brothers due to the haplodiploid sex-determination system of the Hymenoptera (Trivers and Hare 1976). In addition to the predicted sex-ratio interests of the queen and workers as stated above, the fungus should prefer exclusively females because only virgin queens propagate the fungus by using small pieces of the natal fungus to start their own garden (Ihering 1898; Huber 1905 a, b; Mueller et al. 2001; Mueller in press).

Despite these diverse examples of ant symbioses, a common link exists between the two projects. In both studies, I examined the colony-level effects of the symbionts on the two different ant species. In other words, I viewed the ants as a collection of individuals with common and sometimes diverse interests. For example, even though phorid flies attack only the sterile workforce, these parasitoids may also

indirectly affect the entire colony by reducing the amount of resources it collects for growth and survival (Feener 1981; Feener and Brown 1992; Orr et al. 1995; Porter et al. 1995; Morrison 1999; Mehdiabadi and Gilbert in press). To my knowledge, this is the first study to quantify the impacts of phorid fly parasitoids on colony growth of any ant species. In the second half of my work on fungus-growing ants, a colony-level approach was necessary in order to elucidate the benefits and possible costs of the mutualism between the fungus and the attine ants (Herre et al. 1999). For the first time, the evolutionary interests and leverages of the fungus are explored. Thus in the following chapters, a unifying theme of viewing the ants as a collective unit allowed for a more comprehensive understanding of how parasites and mutualists affect their eusocial hosts.

CHAPTER 2

Colony-level Impacts of Parasitoid Flies on Fire Ants

ABSTRACT

The red imported fire ant is becoming a global ecological problem, having invaded the United States, Puerto Rico, New Zealand, and most recently Australia. In its established areas, this pest is devastating natural biodiversity. Early attempts to halt fire ant expansion with pesticides actually enhanced its spread. Phorid fly parasitoids from South America have now been introduced into the United States as potential biological control agents of the red imported fire ant, but the impact of these flies on fire ant populations is currently unknown. In the laboratory, I show that an average phorid density of as little as one attacking fly per 200 foraging ants decreased colony protein consumption nearly two-fold and significantly reduced numbers of large-sized workers 50 days later. The high impact of a single phorid occurred mainly because ants decreased foraging rates in the presence of the flies. My experiment, the first to link indirect and direct effects of phorids on fire ants, demonstrates that colonies can be stressed with surprisingly low parasitoid densities. I interpret my findings with regard to the more complex fire ant-phorid interactions in the field.

INTRODUCTION

Biological invasions threaten global biodiversity and disrupt natural systems (Elton 1958; Pimm 1991; Vitousek et al. 1996; Mooney and Cleland 2001). Invasive social insects are particularly harmful (Vinson 1986; Williams 1994). The red imported fire ant, *Solenopsis invicta* Buren, native to South America, has established populations in two continents, North America (Vinson 1997) and, recently, Australia. In North America, the southern United States is significantly affected. Pesticides, which cost the United States millions of dollars annually (Thompson et al. 1995), have failed to control fire ants effectively (Summerlin et al. 1977). Because of its detrimental effects on native invertebrates and vertebrates (Wojcik et al. 2001), this invasive ant is also ecologically costly. One plausible reason why *S. invicta* is so successful in its introduced range may be due to the absence of nearly all of its natural enemies, which include parasitic phorid flies. Research therefore has increasingly focused on biocontrol agents, such as phorids.

Despite some successes (Williamson and Fitter 1996; Belloti et al. 1999), biological control has remained controversial. The careless introduction of one species to control another may not lessen, but actually exacerbate the problem (Louda et al. 1997). Therefore, understanding the potential impacts of a biocontrol agent is crucial to its success. Biological control of social insects, such as *S. invicta*, is complex because sterile workers of a eusocial insect colony are more like cells of a typical organism (Hölldobler and Wilson 1990). Thus, linking impacts of natural

enemies on individual workers to the entire colony (i.e., superorganism) is central in assessing the suitability of these organisms as biocontrol agents. Two species of phorid flies that are parasitoids of *S. invicta* have already been introduced into the southern United States, yet there has been no published study to date that has investigated whether phorids reduce colony growth of fire ants. Here, I provide the first quantification of the potential colony-level impacts of phorids on fire ants.

Several genera of the dipteran family Phoridae are specialized parasitoids of ant workers. These include *Pseudacteon*, most species of which specialize on *Solenopsis* spp. (Disney 1994). *Pseudacteon* females oviposit their eggs into ants performing activities outside of the nest, such as foraging and defense. About 20 days later, the host dies because the larva has consumed its internal tissues and has pupated in the decapitated ant's head, the site of adult fly emergence.

Previous work demonstrated that the behavioural responses of foragers to attacking phorids may substantially reduce diurnal food harvesting (Feener 1981; Feener and Brown 1992; Orr et al. 1995; Porter et al. 1995; Morrison 1999). Since the amount of food an ant colony collects is greatly influenced by competition with other species (Hölldobler and Wilson 1990), and since phorids modify such interactions (Feener 1981; Feener and Brown 1992; Orr et al. 1995; Porter et al. 1995; Morrison 1999), we included a native ant competitor in our experiments. *Forelius mccooki* is one of several native ants in Texas not reduced in overall range in fire ant invasion zones (Camilo and Phillips 1990). *Forelius mccooki* continues to coexist

with *S. invicta* possibly because of its greater heat tolerance and its ability to displace fire ants from food in the heat of the day. In the presence of *S. invicta*-specific phorids, *F. mccooki* might gain an overall competitive advantage.

I tested the effects of phorids on foraging success and colony growth of *S. invicta* by exposing laboratory fire ant colonies to the parasitoid, *Pseudacteon tricuspis* Borgmeier, and to an interspecific competitor, *F. mccooki*. Treatments included parasitism and competition independently and together, plus a control.

METHODS

Collection and splitting of colonies. I collected 12 polygynous (i.e., multiple-queen) colonies of *S. invicta* and 13 polygynous colonies of *F. mccooki* in Austin, Texas between April - August 2000. All field-collected *S. invicta* colonies were divided into four colony fragments for a total of 48 sub-colonies, each consisting of one mated queen, approximately 5,000 workers (roughly 4,250 minors, 400 small majors, and 350 large majors), and 2.5 g of brood. Minors and small and large majors were classified as workers weighing 0.5-1.5 mg, 1.5 -2.5 mg, and > 2.5 mg, respectively. For *S. invicta*, I used a blocking factor (i.e., each field-collected colony was divided into four sub-colonies, one for each of the four treatments) since workers from different colonies may vary in brood rearing capabilities (Porter and Tschinkel 1985). *Forelius mccooki* colonies had at least one queen, approximately 5,000 workers, and brood; I created as many sub-colonies from a field colony as possible. I chose to

equalize numbers, not biomass, of *F. mccooki* (which is a relatively small ant compared to *S. invicta*) for a realistic estimate of competition pressure.

Maintenance of colonies. Individual sub-colonies were housed in plastic nest boxes (43.2 cm by 27.9 cm by 7.6 cm), which were connected by transparent tubing to foraging arenas (55.9 cm by 43.2 cm by 7.6 cm). In treatments with both *S. invicta* and *F. mccooki*, colonies shared one foraging arena. All boxes were lined with Fluon™ to prevent ants from escaping. I reared colonies under a 12 hour light - 12 hour dark cycle at 30 °C. I placed water and sugar water test tubes in the nest boxes *ad libitum*.

Foraging observations. Foraging observations occurred on alternate days, two days per week, for 50 days. Each foraging period lasted approximately two hours. During foraging periods, I placed one freeze-killed cricket and a sugar water test tube in all the foraging arenas. Sub-colonies received sugar water in both the nest boxes and foraging arenas, whereas they received protein (crickets being the sole source in our experiments) only in the foraging arenas. All sub-colonies were protein-limited since resource limitation frequently mediates synergistic interactions between stress and parasitism in causing population declines in hosts (Sheldon and Verhulst 1996; Leung et al. 2001). At each foraging observation, I also introduced four female and two male phorids and removed the barrier between *S. invicta* and *F. mccooki* for the appropriate sub-colonies.

Data Collection and Analyses. I quantified total worker population size (numbers of surviving workers), numbers in each worker caste, and brood production for each fire ant sub-colony after 50 days. I also recorded phorid attack rates, abundance and rates of foraging ants, and mass of protein harvested during each feeding period. Phorid attack rates were collected three times throughout a two-hour foraging period, after 30 minutes, after one hour, and after two hours. I counted the number of attacking flies within a 30-second scan. Abundance of foraging ants was quantified at the end of the foraging observations; however, rate of foraging ants was measured after 30 minutes and after one hour by counting the number of workers crossing an arbitrary line to and from the crickets. Protein consumption was calculated by weighing crickets before and after foraging observations. Additional crickets were placed in boxes without ants in order to measure loss of cricket biomass due to desiccation. Crickets were dismembered before presentation to the sub-colonies.

Data were analysed with a mixed model ANOVA using SAS (Littell et al. 1996; Singer 1998). For analyses of protein consumption and foraging rates, the fixed effects were group (control, phorids, competition, phorids + competition) and observation (14 foraging periods), and the random effect was colony (each of the 12 field-collected colonies). For analyses of phorid pressure, the fixed and random effects were the same as above, except group contained only the control and phorid treatments. For analyses of worker population size, caste, and brood production, the fixed effects were group (all four treatments), seasonality (summer and fall), and

queen survival. Experimental treatments showed no significant effect on the last dependent variable. Seasonality was included in the analyses since all 12 replicates were not experimented on at the same time, and queen survival was included since some of the queens from sub-colonies died before the experiment ended. All P-values are reported using Tukey-Kramer adjustments to control for Type I error.

RESULTS

Solenopsis invicta sub-colonies from control groups harvested almost twice as much protein as *S. invicta* sub-colonies encumbered by parasitism, even though average parasite abundance was as little as 1 attacking fly per foraging observation (Fig. 1). (Protein consumption data are not reported for groups with competition and groups with phorids + competition since both *S. invicta* and *F. mccoocki* foraged on the cricket.) Interestingly, I detected an effect of phorids on fire ant foraging behavior even at times when phorids were not observed attacking the ants (Fig. 2). These effects on protein consumption were almost entirely behavioral, in that the rate of foraging ants decreased when phorids were present and even more so when phorids and competition were present (Fig. 3).

Exposure to phorid parasitoids affected colony growth. Total worker populations decreased in groups exposed to competition and in groups exposed to phorids + competition relative to controls (Table 1; Tukey-Kramer adjusted P-values: $t_{(32)} = -3.50$, $P = 0.0072$; $t_{(32)} = 3.08$, $P = 0.0208$). However, total worker population

size was not significantly reduced in groups with phorids relative to controls, after using Tukey-Kramer adjusted P-values (Table 1; $t_{(32)} = 2.46$, $P = 0.0860$).

Experimental treatments had the following impacts on the three different worker size classes. First, numbers of the smallest-sized workers ('minors') were reduced for groups in competition and for groups in phorids + competition compared to controls (Table 1; $t_{(32)} = 3.03$, $P = 0.0234$; $t_{(32)} = 2.71$, $P = 0.0495$). Once again, numbers of minors were not significantly different between groups with phorids and controls (Table 1; $t_{(32)} = 2.24$, $P = 0.1336$). Since minors make up the largest proportion of the colony, it is not surprising that we obtained similar results to those for total worker population size. Intermediate-sized workers ('small majors'), which *P. tricuspis* preferentially attack (Morrison et al. 1997), suffered higher mortality in groups with phorids, competition, and phorids + competition in comparison to controls (Table 1; $t_{(32)} = 2.90$, $P = 0.0325$; $t_{(32)} = -4.94$, $P = 0.0001$; $t_{(32)} = 4.64$, $P = 0.0003$). However, numbers of large majors were not significantly different among any of the four treatments.

DISCUSSION

My short-term experiment revealed two significant parasitoid impacts that could dramatically affect colony fitness in the long-term. First, the continued reduction in protein flow at the levels observed could decrease brood production because larvae require protein for growth (Sorenson et al. 1983). Moreover, if

phorid-harassed *S. invicta* abandon food resources, other predatory organisms, including competing ants, may forage, increase their colony growth, and thus intensify competitive effects on *S. invicta*. Indeed, in my experiments, phorid parasitoids of *S. invicta* seemed to provide a competitive advantage to *F. mccooki* (Mehdiabadi, unpublished results). Second, by killing small majors, both directly as well as indirectly (i.e., by lowering colony protein intake), phorids reduce worker populations. Protein-limited colonies may replace these costly, yet invaluable, majors as observed for other ants species (Hölldobler and Wilson 1990; Passera et al. 1996) when encountering severe environmental stresses (e.g., such as intense competition), but at the probable expense of investment in other castes, including reproductives. Conversely, if colonies do not replace these workers, they may be less likely to overcome such stresses (Hölldobler and Wilson 1990) and may lose task efficiency because this caste specializes in foraging and defense (Wilson 1978). My finding that numbers of large majors were not significantly impacted by any of the experimental treatments may be due to two reasons. First, large majors represent the smallest proportion of all worker size classes. Second, *P. tricuspis* does not prefer to attack this largest size class (Morrison et al. 1997). Thus, changes in colony composition were probably due to a combination of reduced colony food intake (through changes in foraging behavior or competition) and also due to mortality from phorids.

Competition with *F. mccooki* caused greater disruptions to worker population size than phorid fly parasitism (Table 1). One possible explanation is that the intense

interference competition observed between the two ant species resulted in quicker mortality of *S. invicta* workers compared to phorid fly parasitism (i.e., almost 20 days for fly larvae to kill hosts and potentially longer for phorids to decrease worker population size due to reduced protein intake).

Nevertheless, my findings that phorids alone were effective at reducing protein consumption and colony growth is important since *S. invicta* has successfully displaced many native ant species in parts of its introduced range (Wojcik et al. 2001), thereby decreasing potential competitors. *Pseudacteon tricuspis* is efficient in imposing a negative impact on *S. invicta* because one attacking fly per 193 foragers, on average, was capable of significantly reducing colony protein intake; for every ant attacked, other workers responded by posturing defensively or retreating to the nest, which diminished colony foraging rates. Abundance of flies in my experiments were similar to those experienced by colonies in their native range (Orr et al. 1995; Folgarait and Gilbert 1999); however, in South America, *S. invicta* is exposed to several phorid species that preferentially attack different worker castes (Morrison et al. 1997). These results suggest that achieving such densities of phorid attack in North America will vary by ecoregion and by phorid species. For example, *Pseudacteon* species in their native ranges vary in their seasonal phenologies (P. Folgarait, unpublished results) and in the degree to which they focus attack on ants at disturbed mounds versus foraging trails (Orr et al. 1997). The constant level of

phorid pressure in our experimental system may be achieved in nature only with a mix of complementary phorid species that orient to foraging trails.

Field estimates of native fire ant (*Solenopsis geminata*) worker infection rates in Texas indicate that no more than 2% of foragers (including minors and majors) will die of their host-specific *Pseudacteon* parasitoids (Morrison et al. 1997). Rather than direct mortality, it is the indirect behavioral responses of foraging *S. invicta* workers to phorid attack that is hypothesised to be most significant in altering colony fitness in the field. My experiments provide predictions of possible pathways that connect indirect (behavioral modification of fire ant foraging) and direct (mortality of workers) effects of phorids on imported fire ants. For example, a reduction in colony protein intake due to behavioral modifications in fire ant foraging may cause a reduction in the production of large-sized workers, which in turn could cause a reduction in the ability of affected colonies to forage effectively. Likewise, direct caste-specific mortality due to phorid infection may also affect foraging and the outcome of competition but on a different time scale.

Despite generations of relaxed selection in the absence of phorids, fly avoidance behaviours are intact in introduced fire ants. The aggressive behaviours of *S. invicta* are also intact in invasive populations, allowing this species to dominate native ant communities (Wojcik et al. 2001). Whereas non-pest native fire ants are also aggressive, they are encumbered by their own specific *Pseudacteon* parasitoids. Thus, freedom from the risk of phorids has been a favored hypothesis for the

ecological success of exotic fire ants and is the basis for current efforts to introduce *Pseudacteon* flies into North America prior to any direct field demonstration of their effectiveness (Feener 1981; Feener and Brown 1992; Orr et al. 1995; Porter et al. 1995; Morrison 1999; Porter 1998).

With few viable alternatives for the sustainable ecological control of imported fire ants, the introduction of host-specific *Pseudacteon* flies will continue. Thus, we need a quantitative basis for predicting the potential impacts of the flies on invasive fire ant populations. Quantifying and linking the indirect and direct effects of phorids using colonies from introduced fire ant populations allow for the evaluation of these parasitoids as biocontrol agents.

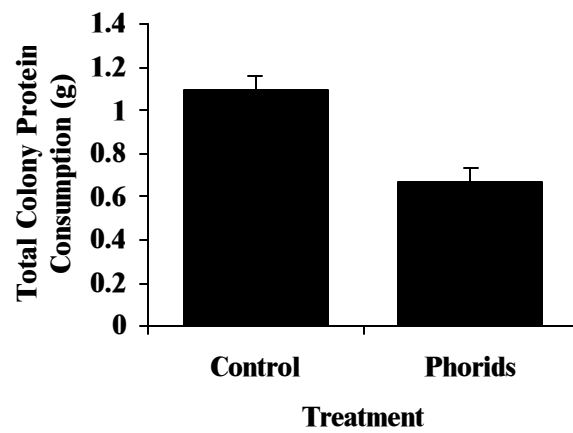


FIGURE 1 Mean total colony protein consumption (g; +SE) for controls and sub-colonies with phorids. Control sub-colonies harvested more protein than sub-colonies with phorids ($t_{(32)} = 6.47$; $P < 0.0001$).

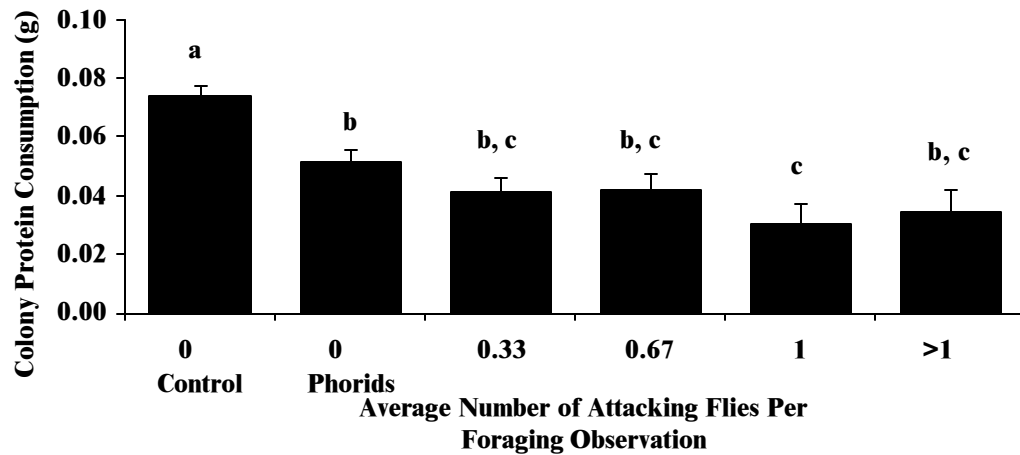


FIGURE 2 Effects of mean phorid pressure (average number of attacking flies per foraging observation; +SE) on colony protein consumption for sub-colonies with phorids in comparison to controls. Phorid pressure was calculated by averaging the three 30-second scans of numbers of attacking flies throughout a two-hour foraging period. Bars with different letters are statistically different using Tukey-Kramer adjusted P-values. (i) control vs. phorids (0), $t_{(31)} = 4.46$, $P = 0.0013$; (ii) control vs. 0.33, $t_{(31)} = 5.93$, $P < 0.0001$; control vs. 0.67, $t_{(31)} = 5.19$, $P = 0.0002$; control vs. 1, $t_{(31)} = 5.89$, $P < 0.0001$; control vs. >1, $t_{(31)} = 4.83$, $P = 0.0005$; phorids (0) vs. 1, $t_{(31)} = 3.17$, $P = 0.0370$.

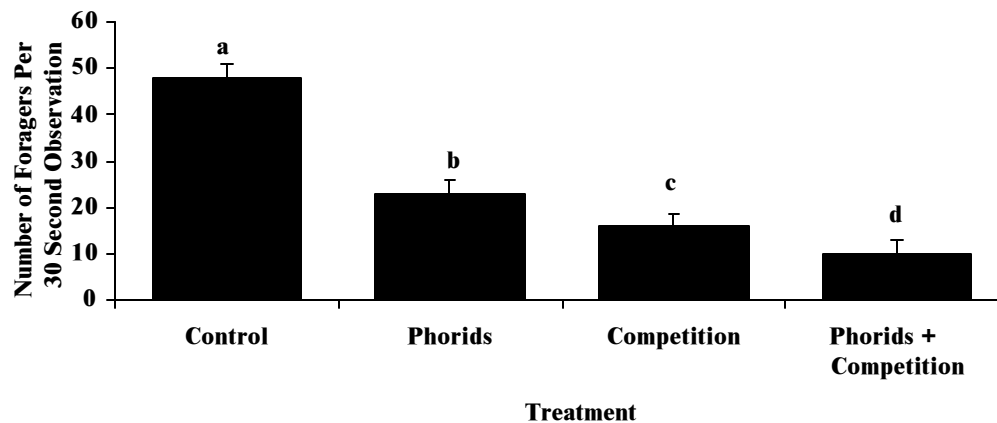


FIGURE 3 Mean foraging rates (average number of ants going to and from the protein food source in 30 seconds/foraging observation; +SE) for groups in all four treatments. Foraging rates were calculated by averaging the two 30-second scans of numbers of foraging ants crossing an arbitrary line to and from the crickets. (i) control vs. phorids, $t_{(653)} = 14.41$, $P < 0.0001$; control vs. competition, $t_{(653)} = -18.72$, $P < 0.0001$; control vs. phorids + competition, $t_{(653)} = -22.13$, $P < 0.0001$; phorids vs. competition, $t_{(653)} = -4.31$, $P = 0.0001$; phorids vs. phorids + competition, $t_{(653)} = -7.71$, $P < 0.0001$; competition vs. phorids + competition, $t_{(653)} = -3.41$, $P = 0.0039$.

TABLE 1. Mean numbers (\pm SE) of minors, small majors, large majors, and all workers for the four treatments.

Treatment	Number of	Mean	Mean	Mean	Mean
	sub-	(\pmSE)	(\pmSE)	(\pmSE)	(\pmSE)
	colonies	minors	small	large	all
			majors	majors	workers
Control	12	2,442	248	214	2,904
		(\pm 228)	(\pm 24)	(\pm 22)	(\pm 253)
Phorids	12	2,075	201	199	2,475
		(\pm 233)	(\pm 25)	(\pm 23)	(\pm 253)
Competition	12	1,946	168	181	2,295
		(\pm 233)	(\pm 25)	(\pm 23)	(\pm 259)
Phorids + Competition	12	1,998	172	196	2,368
		(\pm 233)	(\pm 25)	(\pm 23)	(\pm 259)

CHAPTER 3

Independent and Simultaneous Effects of Parasitism and Competition on Foraging Success of an Invasive Ant

ABSTRACT

Biological invasions disrupt the functioning of natural communities by interfering with an intricate web of native species interactions, including altering competitive hierarchies. The ecological mechanisms that allow invaders to alter species interactions are poorly understood. Furthermore, few studies have experimentally demonstrated whether potential control methods impact these mechanisms. Phorid fly parasitoids are currently being introduced from South America as a potential biological control agent of the red imported fire ant, *Solenopsis invicta*, a devastating pest of the southeastern United States that competitively displaces native ants. In the laboratory, I investigated the independent and simultaneous effects of phorid fly parasitism and interspecific competition on foraging efficiency and caste recruitment of *S. invicta* over a total of 50 days. My results show that parasitism, competition, and both biotic processes together differentially affected the numbers of ants traveling to and from two types of bait, the mean abundance of ants at those baits, and the ratios of castes involved in foraging. For example, phorid fly parasitism affected the rates of travel, but not the abundance, of foraging fire ants at a protein food source; however,

the presence of the flies reduced both the rate and abundance of *S. invicta* foragers at a carbohydrate food source. In contrast, competition and the two biotic processes together negatively impacted both measures of foraging efficiency at each food type. Treatment also affected the caste of workers recruited to forage. For instance, recruitment of large-sized workers (majors) was disrupted more by phorids than by an interspecific competitor. Although majors specialize in foraging, *S. invicta* colonies exposed to phorids had lower traveling rates of majors than did controls. This may be because *Pseudacteon tricuspis*, the phorid species used in our study, preferentially attacks large-sized workers. Interestingly, traveling rates of majors, which also specialize in defense, were not significantly different between competition groups and controls. Thus, *S. invicta* colonies seem to be modifying foraging strategies differentially according to the food type and the nature of the biotic stress: parasitism or competition.

INTRODUCTION

Biological invasions can alter natural communities by disrupting a complex network of species interactions (Mooney and Cleland 2001). The ecological mechanisms that allow invaders to interfere with these interactions are poorly understood (Petren et al. 1993; D'Antonio 1993; Holway et al. 1998). The red imported fire ant, *Solenopsis invicta* Buren, is a devastating pest of the southeastern United States that has altered competitive hierarchies in native ecosystems (Porter

and Savignano 1990). In its established areas, this invasive species is depleting natural biodiversity (Wojcik et al. 2001). Since early attempts to control fire ants with pesticides have failed (Summerlin et al. 1977), recent research has focused on biological control, such as phorid fly parasitoids.

I have shown (Chapter 1, Mehdiabadi and Gilbert In press) that phorid fly parasitoids (*Pseudacteon tricuspis*) reduced protein consumption of *S. invicta* by approximately 50% and significantly reduced numbers of large-sized workers 50 days later. Most of the reductions in colony growth were likely caused by a combination of mortality from phorids and reduced colony food intake (through changes in foraging behavior). For example, phorid fly parasitoids are observed to cause host ants to cease foraging and to assume a defensive posture (Feener 1987; Porter 1995) or simply to return to the nest. These phorid-avoidance behaviors, in turn, allow other ant species to have a competitive advantage at shared food items. Therefore, the introduction of phorid fly parasitoids as biocontrol agents into ant communities invaded by *S. invicta* may result in substantial restructuring of these communities in response to the combined influences of parasitism (Feener 2000) and interspecific competition among ants (Feener 1981; Feener and Brown 1992; Orr et al. 1995; Porter et al. 1995; Morrison 1999).

The goal of the present study was to examine the foraging strategies of the red imported fire ant in the presence and absence of two other species: a recently-introduced, specialized phorid fly parasitoid and an interspecific ant competitor. For

this experiment, I chose the dolichoderine, *Forelius mccooki*, as the ant competitor. This monomorphic, polygynous species is one of several native ants in Texas not reduced in overall range in fire ant invasion zones (Camilo and Phillips 1990). The following questions were addressed in this study: (1) What independent and combined effects do parasitism and competition have on the rates and abundance of foraging fire ants at protein and carbohydrate food sources? (2) Since *S. invicta* is a polymorphic species, do these biotic factors affect the caste of workers recruited to forage? (3) Is there temporal variation in fire ant foraging due to parasitic and competitive effects?

METHODS

The independent and simultaneous effects of phorid fly parasitism and interspecific competition on the foraging success and caste recruitment of *S. invicta* were tested in a fully-factorial design by exposing laboratory fire ant colonies to the parasitoid fly, *Pseudacteon tricuspis* Borgmeier, and to the competing ant, *Forelius mccooki*. The four treatments included parasitism and competition separately and combined, plus an untreated control.

Collection, splitting, and maintenance of colonies

Twelve polygynous (multiple-queen) colonies of *Solenopsis invicta* were collected in Austin, Texas between April-August 2000. Each field-collected colony was divided into four sub-colonies, one for each of the four treatments. This block

design was used because workers from different colonies may vary in task capabilities (Porter and Tschinkel 1985). Each of the 48 sub-colonies consisted of one mated queen, approximately 5,000 workers (roughly 4,250 minors and 750 majors), and 2.5 grams of brood. Minors and majors were classified as workers weighing 0.5-1.5 mg and >1.5 mg, respectively.

Thirteen polygynous colonies of *Forelius mccooki* were collected in Austin, Texas between April-August 2000. A field colony was divided into as many sub-colonies as possible. Each sub-colony had at least one queen, approximately 5,000 workers, and brood.

Individual sub-colonies of both species were housed in plastic nest boxes (43.2 cm by 27.9 cm by 7.6 cm). A petri dish (diameter: 90mm; height: 15 mm) with moistened plaster on the bottom was placed in each nest box to maintain humidity. All sub-colonies were provided with water and sugar water *ad libitum* in the nest boxes. Each nest box was connected by transparent tubing to a foraging arena (55.9 cm by 43.2 cm by 7.6 cm). In treatments with both species, colonies shared one foraging arena. All boxes were lined with Fluon™ to prevent ants from escaping. Colonies were reared under a 12 hour light – 12 hour dark cycle at 30 °C.

Foraging Observations

Experiments were conducted between May – September 2000. One freeze-killed cricket (the only source of protein in this experiment) and a sugar water test tube were placed in each foraging arena two days per week (on alternating days) over

a total of 50 days. Each of these foraging periods lasted approximately two hours. At the beginning of this two-hour period, four female and two male phorids were introduced into the foraging arenas of each of the 12 sub-colonies with parasitism and barriers between the two ant species were removed in the 12 sub-colonies with competition. In the 12 sub-colonies with parasitism + competition both treatments were applied. The 12 control sub-colonies were untreated.

The abundance, traveling rate, and caste of foraging ants were recorded at both food sources (cricket and sugar water) during each feeding period. The abundance of *S. invicta* foragers was quantified by counting the number of ants on or near each food source at the end of each foraging period. Traveling rates of *S. invicta* were quantified twice in each foraging period (after 30 minutes and 1 hour) by drawing an arbitrary line perpendicular to the track of the ants to and from each food source and counting the number of ants crossing that line to and from each food source within a 30-second time interval. In addition, I distinguished the caste (minors versus majors) of recruited ants for the above data.

Statistical Analysis

All statistical analyses were performed with a univariate, repeated measures mixed model ANOVA using SAS (Littell et al. 1996; Singer 1998). In this study, the fixed effects were treatment (control, phorid, competition, phorids + competition) and observation (14 feeding periods over 50 days for each *S. invicta* sub-colony), and the

random effect was colony (each of the 12 field-collected colonies of *S. invicta*). All P-values are reported using Tukey – Kramer adjustments to control for Type I error.

RESULTS

Traveling rates vs. abundance of foragers

Compared to controls, traveling rates of *S. invicta* to and from the protein source (crickets) significantly decreased by 52% when phorids were present, by 67% when a native ant competitor was present, and by 79% when both phorids and competitors were present (Table 1; Fig. 1A). Furthermore, all possible pairwise comparisons were significantly different from each other (Table 1; Fig. 1A). Treatment effects on traveling rates of fire ants to and from the carbohydrate source (sugar water test tube) differed from effects on traveling rates to the protein source. All experimental treatments reduced traveling rate, but only on the protein did the combined treatment (parasitoids plus competition) reduce foraging more than either of the single treatments. On the protein bait, presence of competitors had a significantly stronger impact than presence of parasitoids, while on the sugar bait, the reverse was the case (Table 1; Fig. 1B).

When effects were measured as abundance of foragers actually on the baits rather than the numbers traveling back and forth, presence of parasitoids had no effect at all at the protein bait (Table 2; Fig. 2A). However, groups with competition and groups with parasitism + competition had a 44% and a 58% reduction, respectively,

in the abundance of foragers relative to controls (Table 2; Fig. 2A). The abundance of foragers differed between the two food sources. At the sugar water, all three experimental groups had significantly fewer numbers of *S. invicta* foragers relative to controls (Table 2; Fig. 2B). Effects of competition and of parasitoids were similar (Table 2; Fig. 2B).

Caste of Recruited Foragers

Dividing workers into two size classes (minors and majors) yielded the following results on *S. invicta* traveling rates and abundance of foragers. Sub-colonies exposed to phorids had a two-fold reduction in traveling rates of minors and majors on the protein source relative to controls (Table 1; Fig. 3A, 4A). Interestingly, competition only reduced the rate of small-sized foragers and not the rate of large-sized foragers compared to controls (Table 1; Fig. 3A, 4A). In groups with both phorids and competitors, traveling rates of minors and majors to and from the protein source were approximately five times and four times lower, respectively, than traveling rates of minors and majors from controls (Table 1; Fig. 3A, 4A). In addition, groups with only phorids had higher traveling rates of minors at the crickets in comparison to groups with both phorids and competition (Table 1; Fig. 3A). Similar to the above results at the protein food source, sub-colonies exposed to phorids also had a two-fold reduction in traveling rates of minors and majors at the carbohydrate food source relative to controls (Table 1; Fig. 3B, 4B). Once again, competition only reduced the number of small-sized workers and not the number of large-sized workers

in comparison to controls (Table 1; Fig. 3B, 4B). These same trends were observed for groups with both parasitism and competition (Table 1; Fig. 3B, 4B). In addition, traveling rates of majors were three times higher for groups with competition in comparison to groups with phorids (Table 1; Fig. 4B).

When the abundance data were analyzed according to caste, phorids caused no significant decline in the number of minors or majors at the protein source (Table 2 ; Fig. 5A, 6A). In contrast, groups with competition and those with both phorids and competition reduced the abundance of minors, but not the abundance of majors, at the crickets relative to controls (Table 2; Fig. 5A, 6A). Groups with phorids had twice as many minors at the protein source relative to groups with competition and groups with both phorids and competition (Table 2; Fig. 6A). The abundance of *S. invicta* minors at the carbohydrate food source showed similar trends to those at the protein food source. However, there were a few notable differences in the abundance of foraging majors at the two food sources. Groups with competition had twice as many majors at the sugar water in comparison to groups with phorids, and groups with parasitism + competition were not significantly different than groups exposed to each biotic process on its own (Table 2; Fig. 6B).

Temporal effects of parasitism and competition on foraging behavior

Over the 14 foraging periods, there was temporal variation in *S. invicta* traveling rates and abundance of foragers depending on treatment and food type. Traveling rates of *S. invicta* to and from the protein source (observation * group: $F_{(3,$

$_{653}) = 16.41, P < 0.0001)$ and carbohydrate food source (observation * group: $F_{(3, 653)} = 2.64, P = 0.0489)$ varied across time. At the protein source, all groups seemed to increase traveling rates over time; however, this increase appeared highest for controls, followed by groups with parasitism, groups with competition, and groups with both parasitism and competition, respectively (Fig. 7). In contrast, *S. invicta* traveling rates across the 14 feeding periods seemed to show different trends at the sugar water (Fig. 8). Control sub-colonies appeared to increase traveling rates over time, whereas sub-colonies with both parasitism and competition seemed to decrease traveling rates over time. Furthermore, groups with phorids appear to show a slight increase in traveling rates over time; however, the exact opposite seems to occur for groups with competition (Fig. 8).

Interestingly, the abundance of foragers at the protein source varied over time (observation * group: $F_{(3, 653)} = 18.84, P < 0.0001)$ (Fig. 9), whereas the difference across time was constant at the sugar water for all four treatments (observation * group: $F_{(3, 653)} = 0.56, P = 0.6436)$. At the protein source, the abundance of *S. invicta* foragers seemed to increase for controls and groups with phorids. However, groups with competition appeared to show only a slight increase, while groups with both phorids and competitors appeared to show a slight decrease, in the abundance of foragers over the 14 feeding periods.

DISCUSSION

Traveling rates vs. abundance of foragers

I set out to investigate the mechanisms responsible for the known twofold reduction in protein consumption by groups exposed to phorids (Chapter 1, Mehdiabadi and Gilbert In press). My results showed a strong effect of phorids on the rate at which ants travel between the food and the nest, but a weak or nonexistent effect on the abundance of ants at the baits. Therefore I suggest that the effect of phorids on protein consumption is principally mediated by effects that are detectable as changes in rate of traveling. I observed that when phorids hovered nearby most of the ants feeding at the baits stopped foraging and assumed stationary defensive postures. In other words, even though the ants were on the food baits, which was how we defined the abundance of foragers, these workers were not actually foraging because of harassment by phorids. This delay on the baits would result in a reduction of traveling rate such as the effect that I found.

Caste of Recruited Foragers

Treatment seemed to differentially affect the caste of recruited foragers at the carbohydrate food source. Compared to controls, traveling rates of majors were lower for groups with phorids, yet not significantly different for groups with *F. mccooki*. A likely hypothesis to why phorids reduced the rates of foraging majors in comparison to the controls is that *P. tricuspis* preferentially attacks this size class (Morrison et al.

1997). However, traveling rates of majors were not significantly different between groups with competition and controls possibly because majors specialize in defense (Wilson 1978). Interestingly, the results on the abundance of foraging majors at the sugar water yielded the same trends as above.

In the ergonomic phase of growth, ant colonies invest energy in the production of workers, so that they may perform tasks necessary for colony maintenance and survival. In polymorphic ant colonies, such as *S. invicta*, large workers, or majors, specialize in activities involving defense and foraging, whereas small workers, or minors, perform a broader range of activities, including brood care, nest maintenance and foraging (Wilson 1980; Porter and Tschinkel 1985).

Previous work shows that majors and minors differ in their performance and in the net energetic yield of their behavioral repertoires (Wilson 1980; Porter and Tschinkel 1985). This is the result of several factors. First, worker longevity differs between the castes. On average, majors tend to live for six months and minors tend to live for three months (Porter and Tschinkel 1985). Second, although both majors and minors forage, food items collected by minors are much smaller than those taken by majors, primarily due to physiological limitations (Wilson 1978). Third, although majors live longer and forage on larger prey compared to minors, large individuals cost more in terms of the energy required for their production and maintenance.

At both types of food, all three experimental treatments reduced traveling rates of minors relative to controls. Since minors make up the largest proportion of

the colony, it was not surprising that we obtained similar trends to those for traveling rates of total workers.

Temporal effects of parasitism and competition on foraging behavior

In the control group, *S. invicta* traveling rates and abundance of foragers seemed always to increase over time (Fig. 7-9). This is in accordance with our predictions since these sub-colonies had no threat from either parasitoid flies or an interspecific competitor. However, *S. invicta* sub-colonies in the experimental groups seemed to have less of an increase, and in some cases a decrease, in foraging over the 14 feeding periods (Figs. 7-9). In almost all cases, *S. invicta* sub-colonies exposed to both *P. tricuspis* and *F. mccooki* had the greatest disruptions in foraging. Nevertheless, the combined threat of both parasitism and interspecific competition was not necessarily more effective at disrupting foraging compared to each biotic process on its own (see Figs. 1, 3-6). Another observed trend was that phorids had no significant effect on the abundance of foraging minors at each food source in comparison to controls (Fig. 5). In addition, there was no significant difference in the abundance of foraging minors when comparing groups with competition and those with parasitism and competition (Fig. 5), suggesting that the cause of disruption to foraging in groups exposed to both biotic processes simultaneously was only due to competition.

Conclusion

This study provides insights into the effectiveness of potential biocontrol methods for *S. invicta*. Because phorid fly parasitoids have already been introduced into the southern United States as a biological control agent of *S. invicta*, it is crucial that their potential impacts are understood (Mehdiabadi and Gilbert In press). My results indicate that phorids do seem to impose a negative effect on *S. invicta* (see also Feener 1981; Feener and Brown 1992; Orr et al. 1995; Porter et al. 1995; Morrison 1999), and they also reveal the mechanism by which these flies cause a disruption to fire ant foraging: that is, by reducing traveling rates of foragers. Whether or not these negative impacts to red imported fire ants are sufficient to reduce invasive fire ant populations is yet to be known. Nevertheless, these types of studies will allow for the evaluation of phorids as biocontrol agents, and in general, these experiments will elucidate more precisely the behavioral and ecological responses of organisms to introduced species interactions, which in turn improves our understanding of the role that those interactions play in community structure.

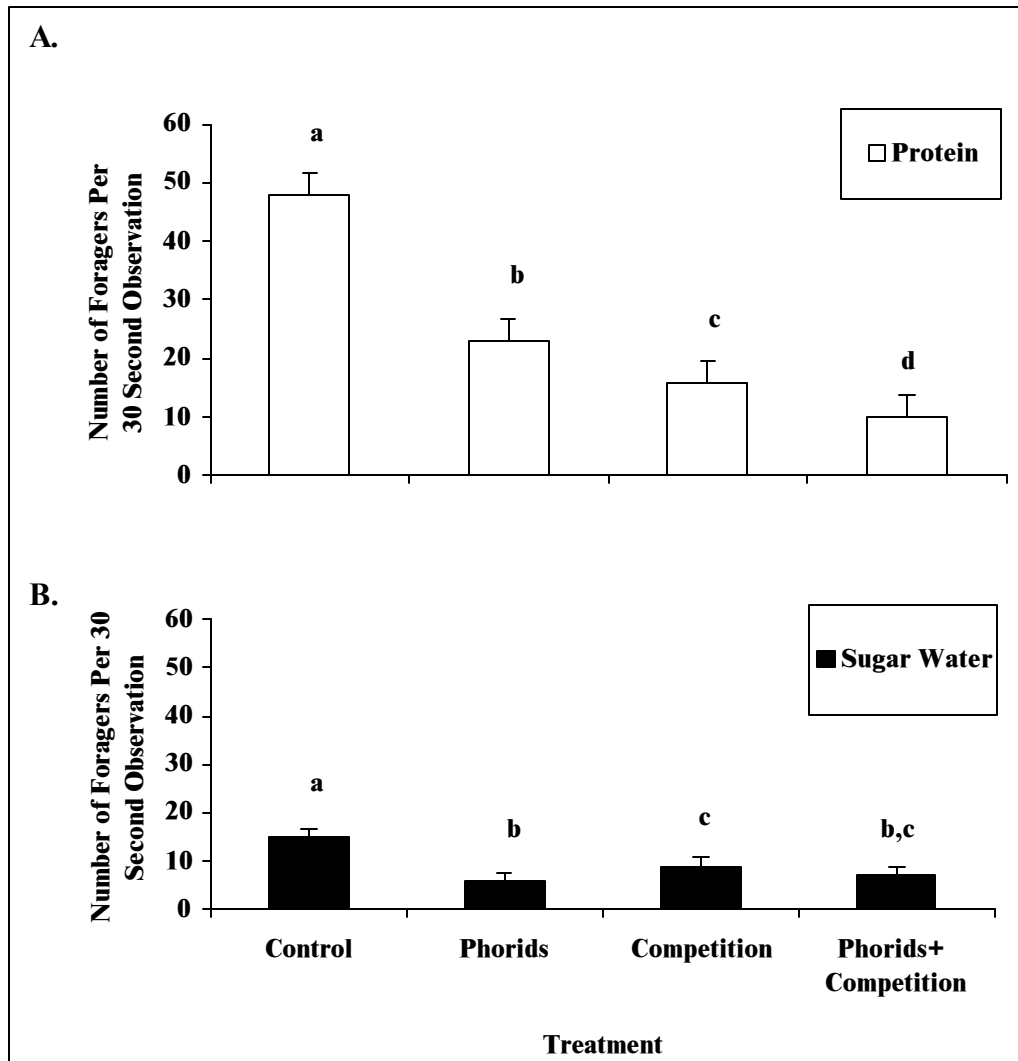


FIGURE 1 Mean traveling rates (average number of ants traveling to and from the food source in 30 seconds per foraging observation; +SE) for groups in all four treatments at the (A) protein and (B) sugar water. Traveling rates were calculated by averaging two 30-second scans of numbers of foraging ants crossing an arbitrary line to and from each food source throughout each of the 14 two-hour foraging periods.

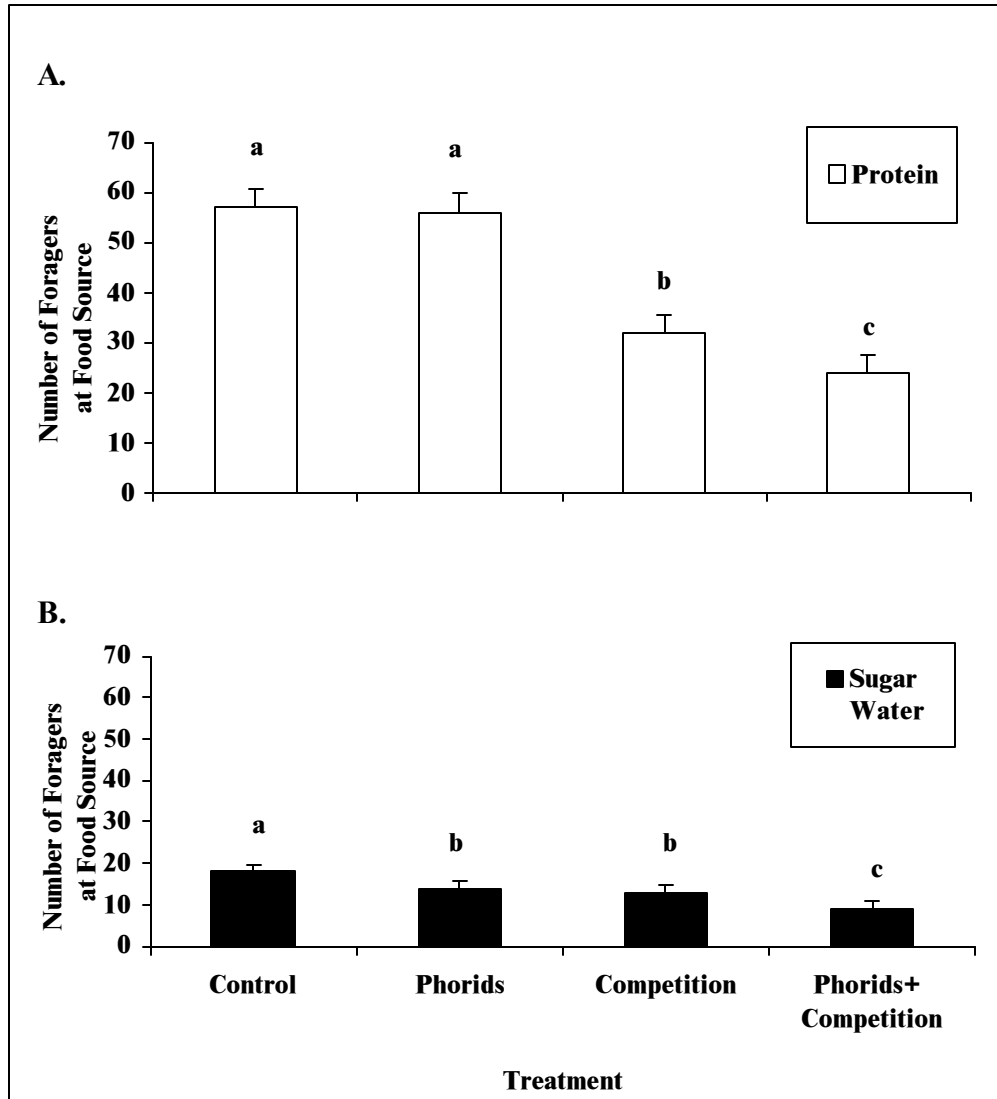


FIGURE 2 Mean abundance of foragers (average number of ants on or near the food source per foraging observation; +SE) for groups in all four treatments at the (A) protein and (B) sugar water. Abundance of foragers was measured at the end of each of the 14 two-hour foraging periods.

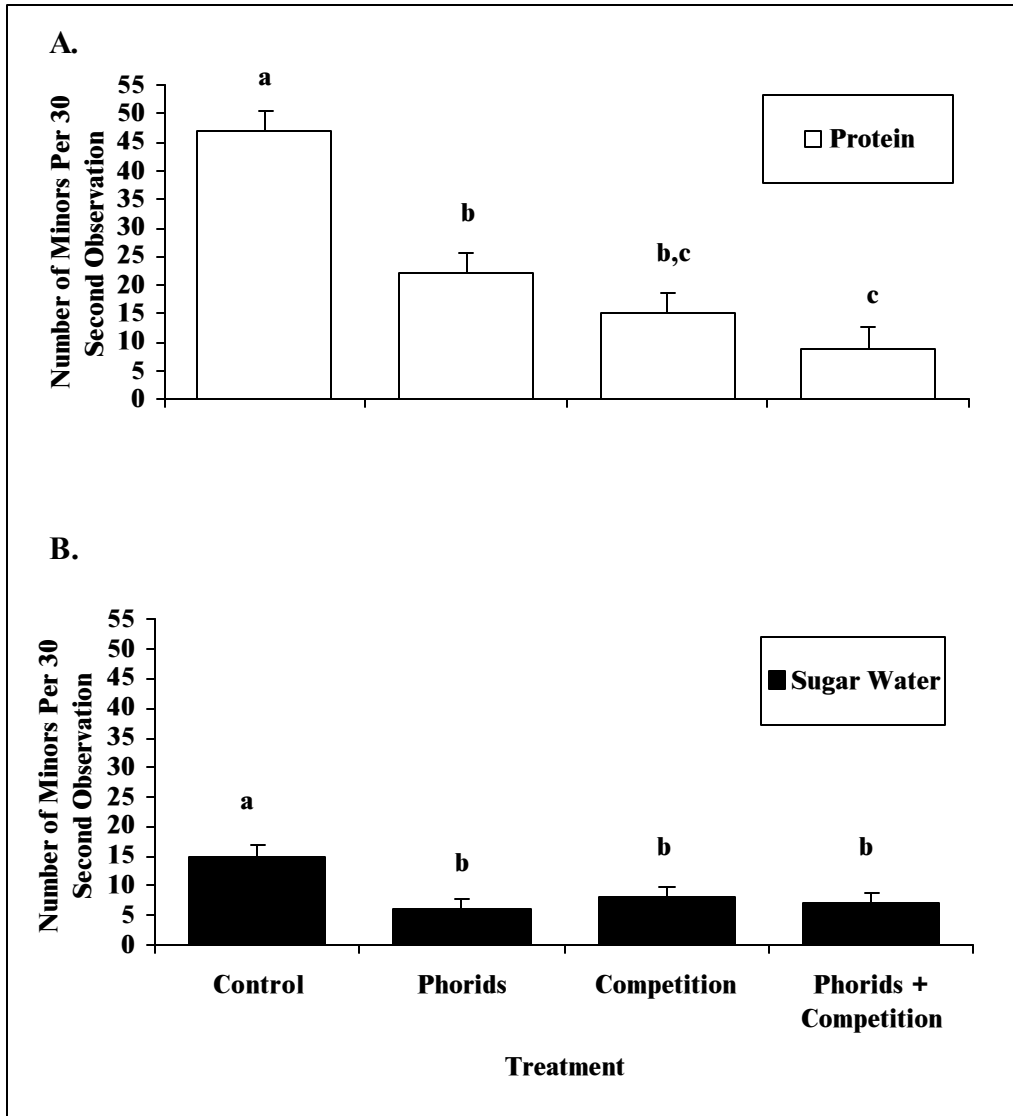


FIGURE 3 Mean rates (average number of foragers traveling to and from each food source in 30 seconds per foraging observation; +SE) of foraging minors at the (A) protein and (B) sugar water for all four groups. Traveling rates were measured twice, after 30 minutes and after one hour, during each of the 14 foraging observations.

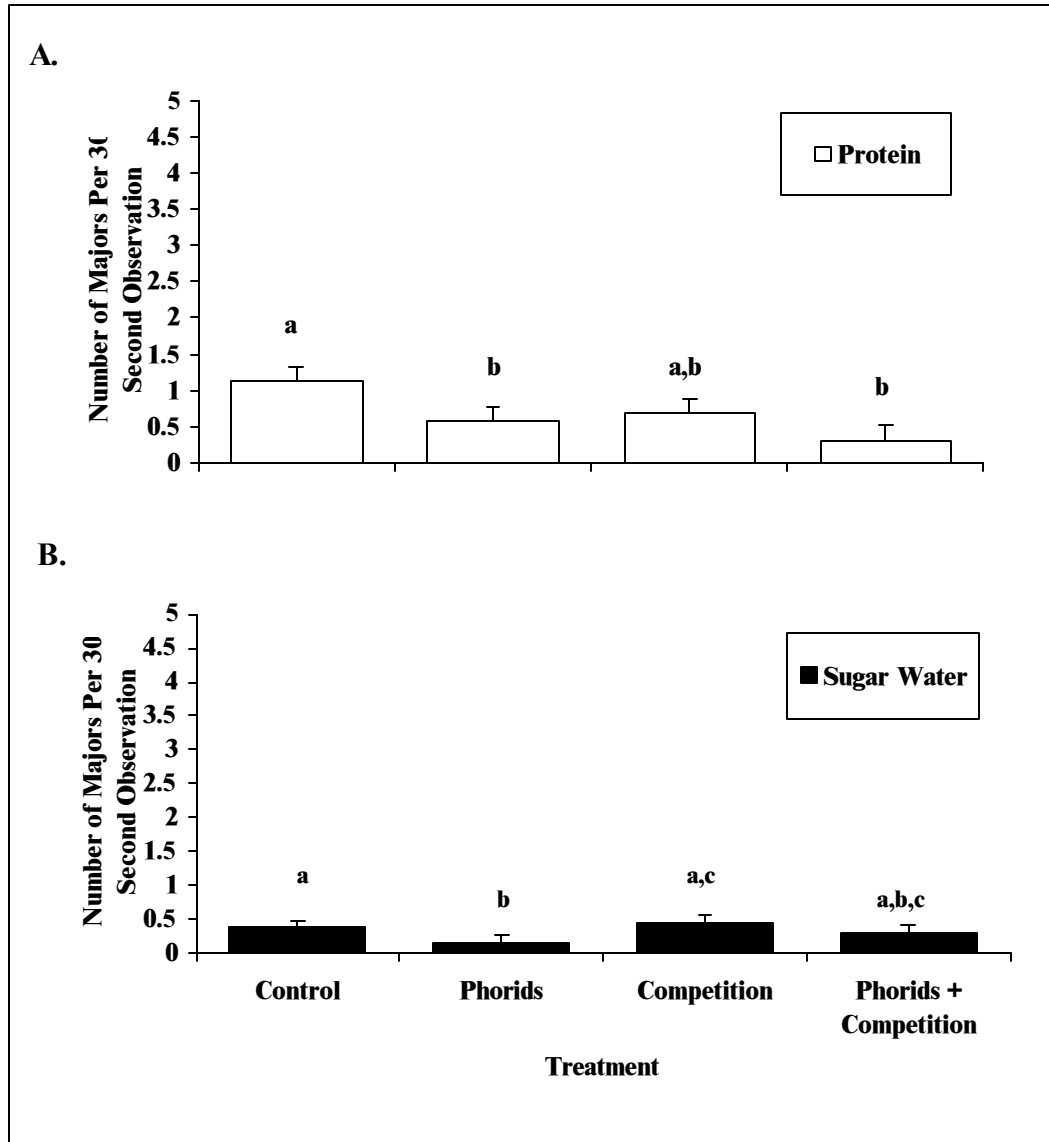


FIGURE 4 Mean rates (average number of foragers traveling to and from each food source in 30 seconds per foraging observation; +SE) of foraging majors at the (A) protein and (B) sugar water for all four groups. Traveling rates were measured twice, after 30 minutes and after one hour, during each of the 14 foraging observations.

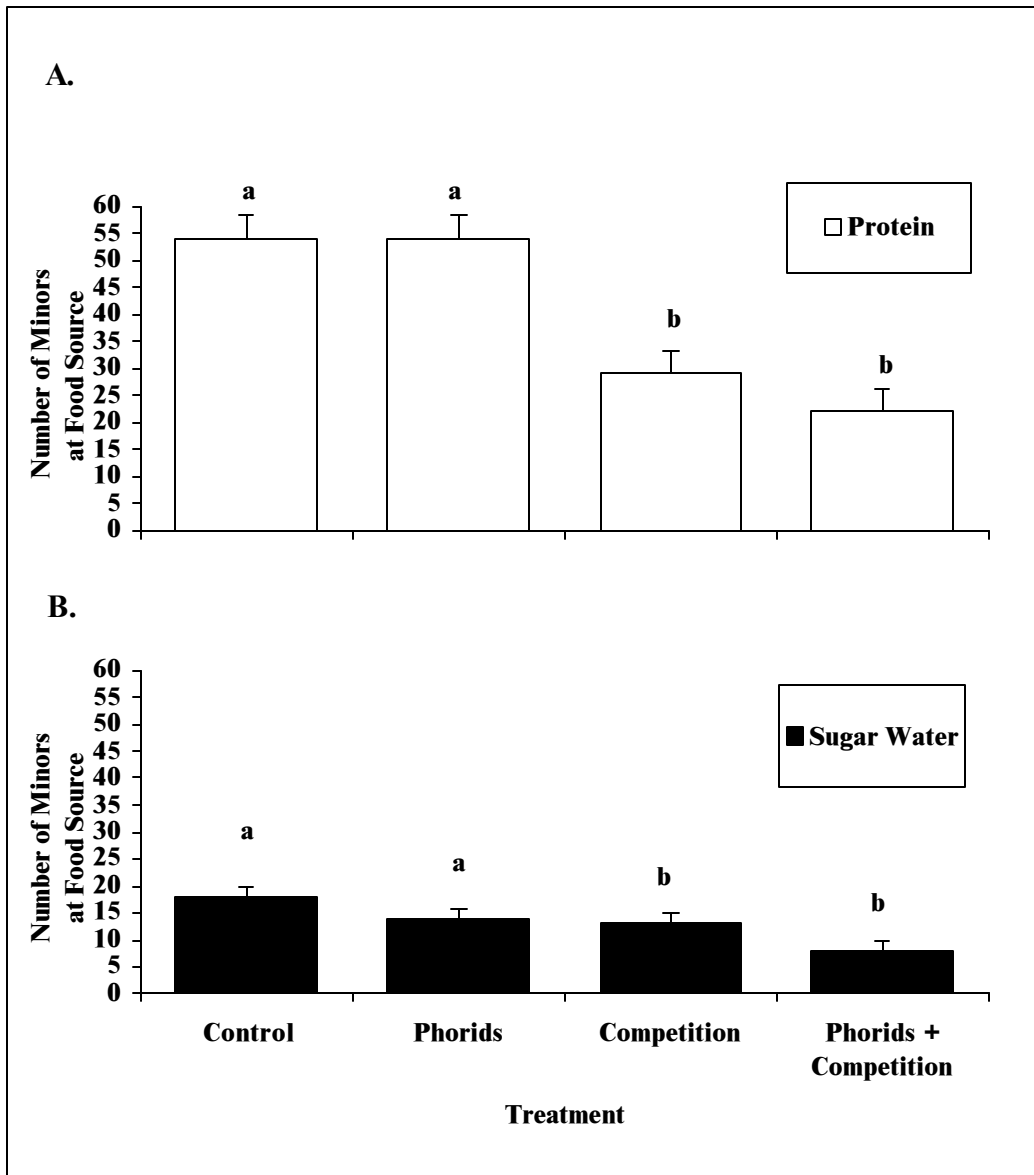


FIGURE 5 Mean abundance (average number of foragers on or near each food source per foraging observation; +SE) of foraging minors at the **(A)** protein and **(B)** sugar water for all four groups. Abundance of foragers was measured at the end of each foraging observation.

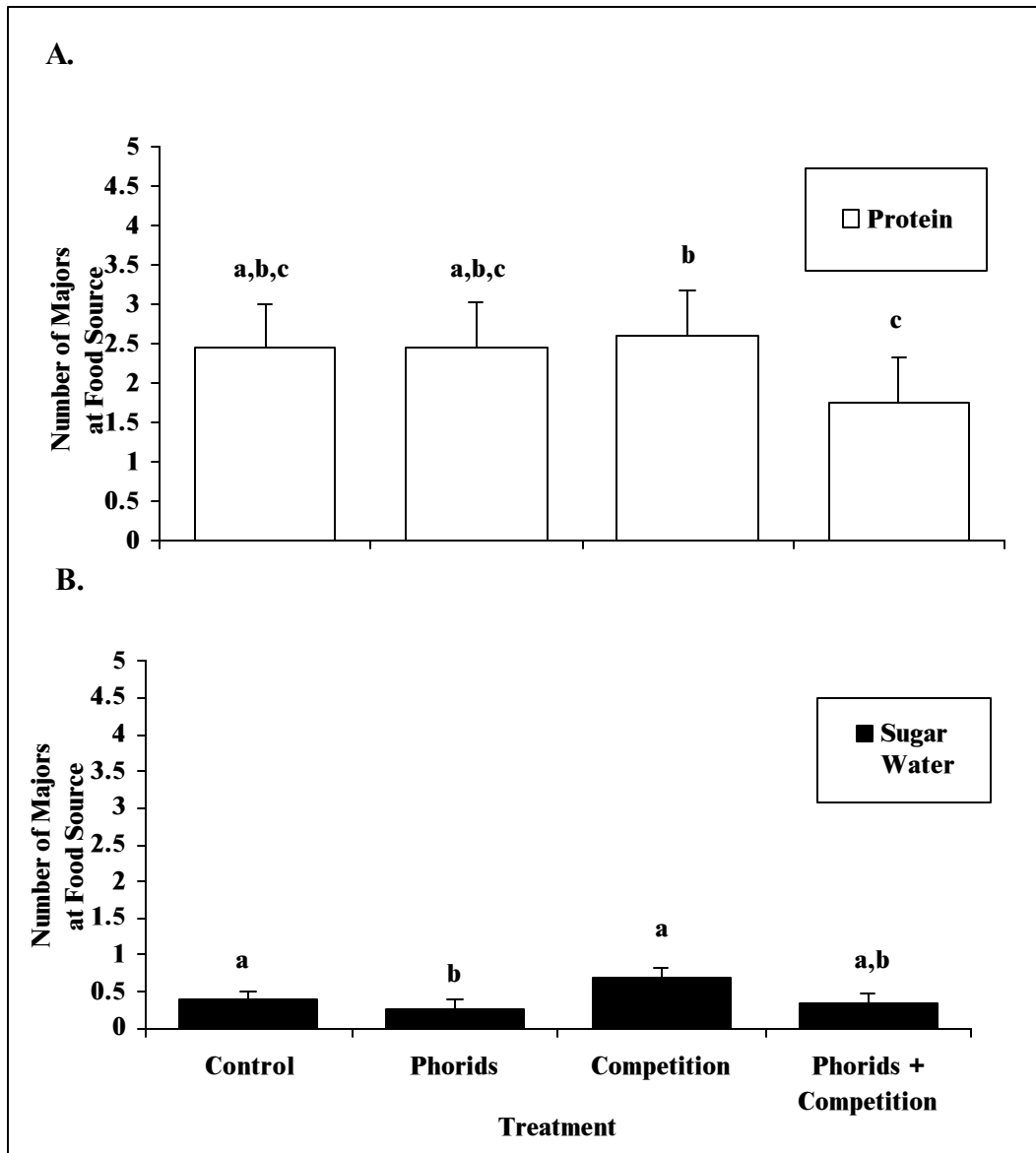


FIGURE 6 Mean abundance (average number of foragers on or near each food source per foraging observation; +SE) of foraging majors at the **(A)** protein and **(B)** sugar water for all four groups. Abundance of foragers was measured at the end of each foraging observation.

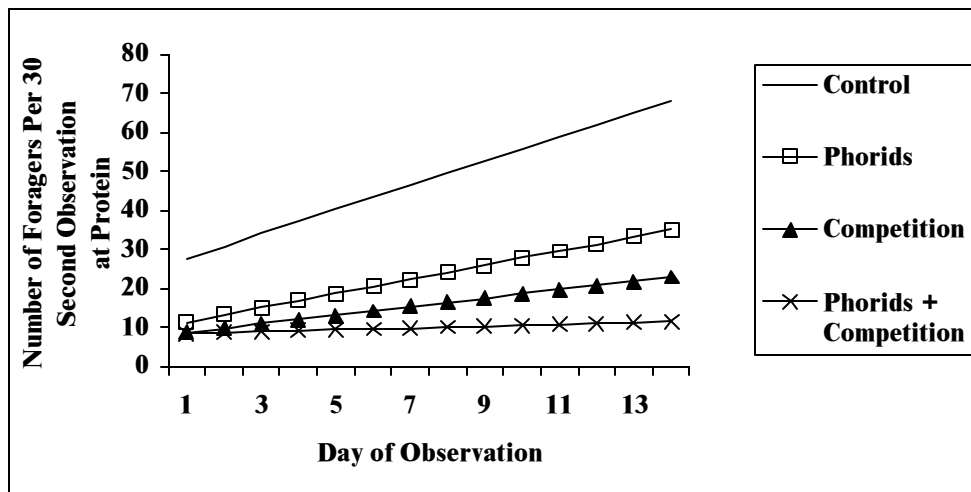


FIGURE 7 Traveling rates of workers (minors and majors) over time at the protein food source (crickets) for controls, groups with phorids, groups with competition, and groups with phorids + competition. Data are clumped according to treatment. Traveling rates are shown for each of the 14 foraging periods.

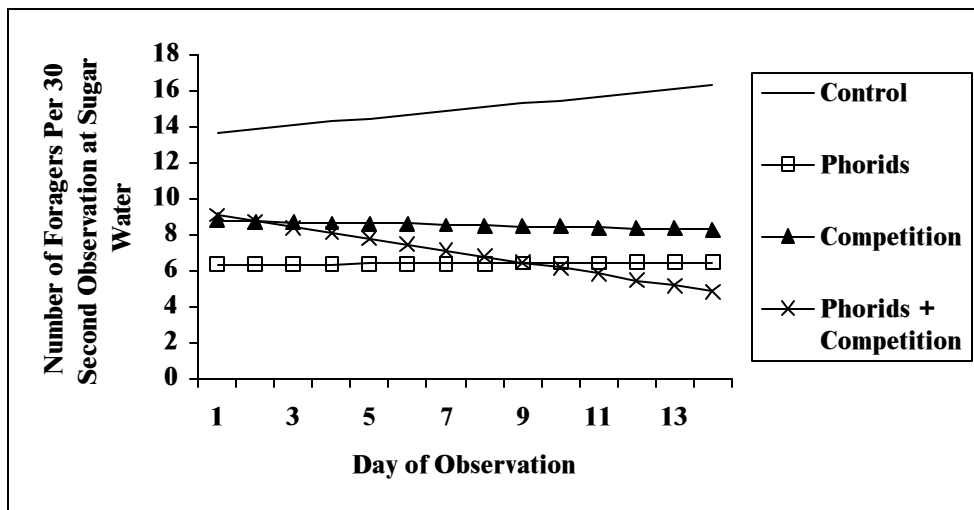


FIGURE 8 Traveling rates of workers (minors and majors) over time at the carbohydrate food source (sugar water) for controls, groups with phorids, groups with competition, and groups with phorids + competition. Data treated the same as in Figure 7.

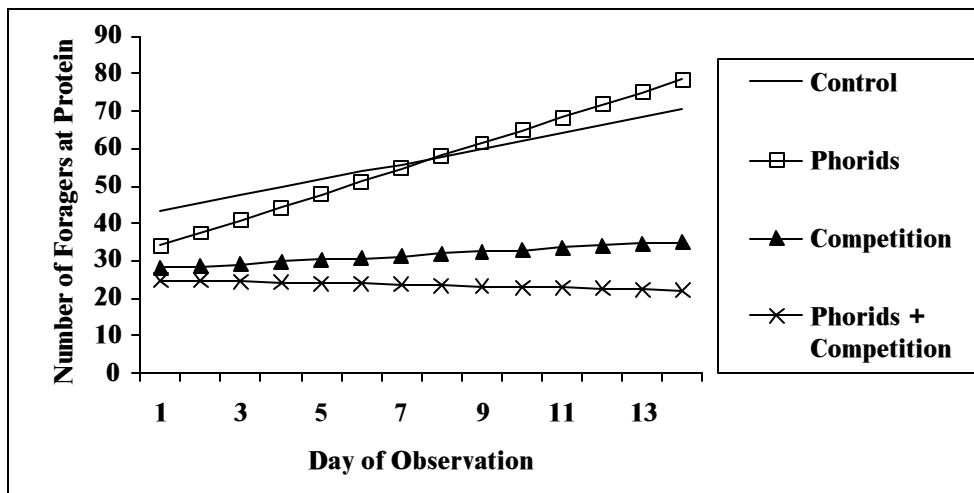


FIGURE 9 Abundance of workers (minors and majors) over time at the protein food source (crickets) for controls, groups with phorids, groups with competition, and groups with phorids + competition. Data are clumped according to treatment. Abundance of foragers is shown for each of the 14 foraging periods.

TABLE 1. All possible pairwise comparisons for traveling rates and caste (minors and majors) of fire ants at each food source (protein and sugar water) using Tukey-Kramer adjusted P-values. Cn= Control; P= Phorids; C= Competition; P + Cm = Phorids + Competition; Prot= Protein; SW= Sugar Water.

	Cn vs. P	Cn vs. Cm	Cn vs. P + Cm	P vs. Cm	P vs. P + Cm	Cm vs. P + Cm
Prot; Total	$t_{(653)} =$ 14.41 P<0.0001	$t_{(653)} =$ -18.72 P<0.0001	$t_{(653)} =$ -22.13 P<0.0001	$t_{(653)} =$ -4.31 P=0.0001	$t_{(653)} =$ -7.71 P<0.0001	$t_{(653)} =$ -3.41 P=0.0039
Prot; Minors	$t_{(620)} =$ 5.72 P<0.0001	$t_{(620)} =$ -7.49 P<0.0001	$t_{(620)} =$ -8.78 P<0.0001	$t_{(620)} =$ -1.78 P=0.2858	$t_{(620)} =$ -3.06 P=0.0121	$t_{(620)} =$ -1.29 P=0.5704
Prot; Majors	$t_{(620)} =$ 3.04 P=0.0133	$t_{(620)} =$ -2.47 P=0.0657	$t_{(620)} =$ -4.52 P<0.0001	$t_{(620)} =$ -0.57 P=0.9417	$t_{(620)} =$ -1.49 P=0.4471	$t_{(620)} =$ -2.05 P=0.1701
SW; Total	$t_{(653)} =$ 11.20 P<0.0001	$t_{(653)} =$ -8.41 P<0.0001	$t_{(653)} =$ -10.46 P<0.0001	$t_{(653)} =$ 2.79 P=0.0281	$t_{(653)} =$ 0.73 P>0.05	$t_{(653)} =$ -2.05 P>0.05
SW; Minors	$t_{(620)} =$ 4.49 P<0.0001	$t_{(620)} =$ -3.31 P=0.0055	$t_{(620)} =$ -4.26 P=0.0001	$t_{(620)} =$ 1.19 P=0.6350	$t_{(620)} =$ 0.23 P=0.9958	$t_{(620)} =$ -0.96 P=0.7730
SW; Majors	$t_{(623)} =$ 2.67 P=0.391	$t_{(623)} =$ 0.79 P=0.8576	$t_{(623)} =$ -1.80 P=0.2732	$t_{(623)} =$ 3.46 P=0.0032	$t_{(623)} =$ 1.66 P=0.3470	$t_{(623)} =$ -1.80 P=0.2732

TABLE 2. All possible pairwise comparisons for foraging abundance and caste (minors and majors) of fire ants at each food source (protein and sugar water) using Tukey-Kramer adjusted P-values. Cn= Control; P= Phorids; C= Competition; P + Cm = Phorids + Competition; Prot= Protein; SW= Sugar Water.

	Cn vs. P	Cn vs. Cm	Cn vs. P + Cm	P vs. Cm	P vs. P + Cm	Cm vs. P + Cm
Prot; Total	$t_{(653)} =$ 0.26 P=0.9936	$t_{(653)} =$ -11.88 P<0.0001	$t_{(653)} =$ -15.67 P<0.0001	$t_{(653)} =$ -11.62 P<0.0001	$t_{(653)} =$ -15.41 P<0.0001	$t_{(653)} =$ -3.79 P=0.0009
Prot; Minors	$t_{(620)} =$ 0.12 P=0.9994	$t_{(620)} =$ -5.30 P<0.0001	$t_{(620)} =$ -6.80 P<0.0001	$t_{(620)} =$ -5.18 P<0.0001	$t_{(620)} =$ -6.68 P<0.0001	$t_{(620)} =$ -1.50 P=0.4363
Prot; Majors	$t_{(623)} =$ -0.06 P=0.9999	$t_{(623)} =$ 0.52 P=0.9544	$t_{(623)} =$ -2.12 P=0.1492	$t_{(623)} =$ 0.46 P=0.9669	$t_{(623)} =$ -2.17 P=0.1324	$t_{(623)} =$ -2.64 P=0.0427
SW; Total	$t_{(656)} =$ 4.06 P=	$t_{(656)} =$ -5.31 P<0.0001	$t_{(656)} =$ -10.87 P<0.0001	$t_{(656)} =$ -1.25 P=0.5967	$t_{(656)} =$ -6.81 P<0.0001	$t_{(656)} =$ -5.56 P<0.0001
SW; Minors	$t_{(623)} =$ 1.98 P=0.1954	$t_{(623)} =$ -2.86 P=0.0225	$t_{(623)} =$ -5.47 P<0.0001	$t_{(623)} =$ -0.88 P=0.8161	$t_{(623)} =$ -3.49 P=0.0029	$t_{(623)} =$ -2.61 P=0.0455
SW; Majors	$t_{(623)} =$ 1.03 P=0.7320	$t_{(623)} =$ 2.50 P=0.0607	$t_{(623)} =$ -0.39 P=0.9795	$t_{(623)} =$ 3.53 P=0.0025	$t_{(623)} =$ 0.64 P=0.9198	$t_{(623)} =$ -2.89 P=0.0205

CHAPTER 4

Queens versus Workers:

Sex-ratio Conflict in Eusocial Hymenoptera

ABSTRACT

Studies of sex-ratio conflict in the eusocial Hymenoptera (ant, bees, wasps) have provided the most rigorous tests of kin-selection theory. The hymenopteran haplodiploid system of sex determination generally renders workers more closely related to their sisters than to brothers, and kin-selection theory therefore predicts that resource allocation into male or female reproductives is a source of parent-offspring (queen-worker) conflict. Under the traditional assumption of worker control, sex ratios should evolve towards female-bias, shifting away from the queen's optimum, an even sex ratio. Three decades of research on sex-ratio conflict largely supported worker control, but recent studies revealed queen-controlled sex ratios even in societies previously thought to operate under worker control. Recent studies further documented that queen-worker sex-ratio conflict is modulated by other within-colony conflicts, such as conflicts over colony growth or worker reproduction. Shared-control, multi-conflict models are now needed to encompass the dynamic balance between queen and worker power over the colony sex ratio.

INTRODUCTION

Kin selection theory is the key hypothesis for explaining a wide variety of evolutionary phenomena, including the origin and maintenance of eusociality (Hamilton 1964). True eusociality occurs in vertebrate and invertebrate social systems, yet the extreme forms are found in hymenopteran insects – the ants, bees, and wasps. Workers of social insect colonies usually forgo reproduction and help the mother queen(s) to produce collateral kin (siblings), thus augmenting each worker's inclusive fitness. As a consequence of the hymenopteran haplodiploid system of sex determination, workers are generally more closely related to sisters than to brothers (in the extreme case, three times more related to sisters than to brothers), whereas the queen is equally related to sons and daughters (Hamilton 1964). This difference in relatedness generates conflict over sex allocation (investment into male to female reproductives produced by a colony). Thus, in addition to the kinship-mediated evolution of cooperation of hymenopteran insect societies, relatedness asymmetry also creates a potential for within-colony conflict.

In 1976, Trivers and Hare (1976) outlined a theoretical framework specifying the differing sex-ratio optima for a queen and her workers. This seminal work integrated and extended both sex-ratio theory, as developed by Fisher (1958), and kin selection theory, as developed by Hamilton (1964, 1972). Trivers and Hare (1976) argued that relatedness asymmetries generate selection favoring workers that behave more nepotistically toward sisters than toward brothers. In particular, workers should

bias the colony investment sex ratio (Fisher 1958) toward their more closely related sisters and away from their more distantly related brothers, up to a point where the fitness increase accrued through investment in the more closely related females is offset by the increased mean reproductive value (mating success) of males in a female-biased population. In contrast, reproductive queens are symmetrically related to their male and female offspring, and selection should favor queens that counter any female-bias induced by the workers. According to Trivers and Hare (1976), sex allocation in eusocial Hymenoptera is therefore characterized by parent-offspring (queen-worker) conflict over the colony sex ratio.

To test the hypothesis of queen-worker conflict, Trivers and Hare (1976) expanded on Fisher's sex-ratio theory (Fisher 1958) and derived the sex ratios expected if either the queen or workers had complete control over the allocation of resources in the two sexes. Because empirical data on population-wide sex ratios of ants closely matched the female-biased sex ratios expected under worker control, Trivers and Hare (1976) argued that the queen-worker conflict in colonies of ants is resolved in favor of workers. Using an augmented data set of ant sex ratios, Nonacs (1986) later confirmed the population-wide female bias among ants. Since then, numerous studies on various hymenopterans have supported this view that workers often control sex-investment ratios (Bourke and Franks 1995). However, recent work has provided surprising examples of queen control over sex ratios, urging the

development of shared-control models that encompass the dynamic power balance between queen and worker influences on colony sex ratios.

PRIMARY VERSUS SECONDARY SEX RATIOS

Sex-investment ratios may be measured at different life stages. Primary sex ratios refer to proportions of haploid (male) and diploid (female) reproductive-destined eggs laid by a queen. Secondary sex ratios, on the other hand, refer to proportions of male and female reproductives at later developmental stages (e.g., pupal or adult colony sex ratio). Queens presumably control primary sex ratios during oviposition, whereas workers traditionally have been assumed to control secondary sex ratios because they rear and feed the brood. Thus, control over sex allocation is likely to vary according to the developmental stage at which sex-investment ratios are measured. The precise quantification of sex-investment ratios has proved challenging (Boomsma 1989) and only recently, have empirical studies begun to examine and compare both ratios (Aron et al. 1994, 1995; Keller et al. 1996; Sundström et al. 1996). These experiments provide support for queen control over the primary sex ratio, and worker control over the secondary sex ratio, implying that workers altered the sex ratio of queen-laid eggs by selectively eliminating male brood ratios (Aron et al. 1994, 1995; Keller et al. 1996; Sundström et al. 1996). Thus, these studies strongly support the idea that the queen and workers have different sex ratio optima and that both parties have at least some leverage in the resulting conflict.

SEX-RATIO BIASING MECHANISMS

The mechanisms that allow queens and workers to control sex allocation have only recently received attention (Bourke and Franks 1995; Queller and Strassman 1998; Chapuisat and Keller 1999). Each party may bias sex ratios through either direct or indirect tactics.

Direct mechanisms

Because workers rear and feed the brood, they may adjust sex-investment ratios by selectively eliminating males (i.e., fratricide) (Aron et al. 1994, 1995; Keller et al. 1996; Sundström et al. 1996; Passera and Aron 1996; Chapuisat et al. 1997), preferentially feeding/rearing females (Helms et al. 2000), facultatively manipulating female caste fate (i.e., queen or worker) (Hammond et al. 2002), or even by imprisoning males and thus preventing male access to food (Starks and Poe 1997). Such manipulations assume that workers are capable of distinguishing between the sexes (which may be difficult at early developmental stages); otherwise, these tactics would be costly, for example, because of occasional misdirected harm to females (Nonacs 1993).

Mechanisms by which queens control sex allocation are less well known (Pamilo 1982; Bourke and Franks 1995; Seger 1996; Helms 1999), but queen-control mechanisms are implicated by recent studies of hymenopterans with split sex ratios (i.e., population-wide bimodal sex-ratio distributions with colonies that specialize in the production of either male or female reproductives). For example, in monogynous

(single-queen) colonies of the fire ant *Solenopsis invicta*, queens seem to control sex ratios by limiting the number of female eggs laid, thus forcing workers to rear males in male-specialist colonies (Passera et al. 2001). In the ant *Pheidole desertorum*, queens from male-specialist colonies appear to manipulate sex ratios by producing worker-biased, instead of reproductive-biased, female eggs (i.e., by affecting female caste determination) (Helms 1999). Nevertheless, both *P. desertorum* and *S. invicta* workers are capable of biasing sex ratios toward females (Aron et al. 1995; Helms et al. 2000), implying overt queen-worker conflict over the sex ratio with both queens and workers having some leverage over the colony sex ratio. As hypothesized also for *P. desertorum*, bumblebee (*Bombus terrestris*) queens apparently control female caste determination; *B. terrestris* queens seem to chemically inhibit diploid larvae from developing into female sexuals (Cnaani et al. 2000). However, it is important to note that the chemical control of caste in male-specialist colonies of *B. terrestris* does not necessarily imply that the queen is manipulating the brood; she merely may be chemically signaling her presence to female brood as an incentive for the brood not to develop into reproductives but into workers (which maximize inclusive fitness when helping a mother queen) (Keller and Nonacs 1993). Interestingly, the population investment sex ratio is at the queen optimum of 1:1 for both *P. desertorum* and *B. terrestris* (Bourke 1997; Helms 1999), and because male- and female-specialist colonies do not differ in relatedness asymmetry or productivity, queen control therefore has been hypothesized to explain the evolution of split sex ratios in these

two species (Helms 1999; Bourke and Ratnieks 2001). That is, queens completely control the sex ratio in some colonies, and such colonies therefore become male-specialists because only male and worker-destined eggs are produced. Through a process called sex-ratio compensation, other colonies in the same population are selected to become female-specialists, because the value of males relative to that of females decreases with an increasing frequency of male-specialist colonies in the population (Pamilo 1982).

Potential mechanisms of queen control have also been examined in honeybee (*Apis mellifera*) colonies. Honeybee queens adjust sex ratios by regulating egg fertilization (Ratnieks and Keller 1993) according to the nutritional status of the colony and intrinsic seasonal factors (Sasaki and Obara 2001). Ratnieks and Keller (1998) were able to demonstrate the precise control that a honeybee queen has over the fertilization of the eggs she lays because each sex is reared in different-sized cells (Seeley 1985). Because in *A. mellifera* the queen and her workers have similar sex-ratio optima due to extreme multiple mating by queens, workers should not have been favored to suppress or modify such mechanisms of apparent queen control (Bourke and Franks 1995). Nevertheless, honeybee queens should be selected to control egg fertilization because if males are reared in smaller cells, instead of larger cells, then these males have reduced mating success (Berg et al. 1997).

In sum, a variety of direct sex-ratio biasing mechanisms have been demonstrated for both queens and workers. Some of the queen mechanisms probably

operate in the absence of conflict with workers, but other mechanisms implicate some degree of sex-ratio control by queens even when in conflict with the numerically superior workers.

Indirect mechanisms

Indirect sex-ratio biasing mechanisms regulate sex ratios through worker manipulation of colony conditions (e.g., nutrient supplements) that induce queens to change the sex-ratio output at oviposition. In indirect mechanisms, workers influence queen behavior by providing the queen with incentives for the production of females, effectively making it a queen's optimal strategy to overproduce females. Indirect mechanisms thus are an alternative to direct influences such as those discussed above, but the indirect nature of these mechanisms makes them harder to study empirically.

One indirect tactic involves workers providing the queen with incentives to lay fertilized eggs. For example, female fitness may increase faster with investment than male fitness (Trivers and Willard 1973; Crozier and Pamilo 1996); hence, in mass-provisioning bees, presenting the queen with a large investment in a cell (provisioning mass on which an offspring is reared) would induce the queen to lay a fertilized egg (Boomsma 1991). Bulmer (1981) suggested that queens in annual colonies have their own indirect leverages to control the sex ratio (e.g., by laying male eggs only after a prolonged phase of female production) and thereby win the sex-ratio war. Obviously, indirect mechanisms of manipulation are more subtle and thus more difficult to detect than the overt direct mechanisms (e.g., fratricide), but the

large number of untested hypotheses make indirect mechanisms a particular promising area of future study.

INTEGRATING SEX-RATIO CONFLICT WITH OTHER QUEEN - WORKER CONFLICTS

Even though sex-ratio conflict according to Trivers and Hare (1976) strictly pertains to investment into queen-produced sexuals, recent research has demonstrated that sex-ratio conflicts that are entangled with other intra-colonial conflicts. Ultimately, a theory of the simultaneous resolution of all conflicts is necessary for a full understanding of the properties of eusocial hymenopteran societies.

Conflict over male production

The queen and workers' optimal allocation ratio changes with the proportion of a colony's males that are sons of workers (rather than sons of the queen). Worker reproduction (generally limited to sons because workers in many species are unfertilized) thus influences the sex-allocation conflict (Crozier and Pamilo 1996). Chemical cues and various ritualized behaviors provide queens with potential mechanisms to limit worker reproduction. For example, as shown in other hymenopterans, recent work on the ponerine ant *Pachycondyla apicalis* (Dietemann and Peeters 2000) implicates that queens emit a pheromone that inhibits workers from producing males. However, as mentioned above, such inhibition does not necessarily entail conflict, because workers may use the queen pheromone as an indicator of the

presence of a healthy mother queen and thus a higher payoff for rearing full sisters than for rearing their own sons and nephews (full sisters' sons) (Keller and Nonacs 1993). In addition to chemical cues, ritualized interactions between queens and workers may also allow for queen control over reproduction. In two Australian species of stingless bees, the queens appear to suppress worker reproduction by performing a series of ritualized behaviors during oviposition (Drummond et al. 1999). Such displays are consistent with conflict between the queen and the workers, because such conventional displays are expected when interactants are signaling relative fighting or competitive ability (Reeve 1997).

Another social mechanism for controlling worker reproduction is worker policing. If the effective mating frequency of the queen exceeds two, then the sons of queens are genetically more valuable than worker-produced males. Thus, according to kin selection theory, workers are expected to police each other's reproduction in colonies with multiply-mated queens because they are more closely related to males produced by the queen (brothers) than to those produced by other workers (nephews) (Crozier and Pamilo 1996). Using the vespine wasp, *Dolichvespula saxonica*, Foster and Ratnieks (2000) found support for this prediction by showing that worker policing occurs in colonies with a multiply-mated queen but not in those with a singly-mated queen. Thus, queen mating frequency affects both worker policing and queen-worker sex ratio conflict, and a queen may be able to promote both her optimum sex ratio and her control of male production by increasing her mating

frequency. It becomes a puzzle as to why multiple mating is not more common in Hymenoptera. While there are many factors that undoubtedly influence the evolution of multiple mating, (e.g., sexually transmitted diseases, predation risks), single mating may be so prevalent in the social Hymenoptera because male mates should tend to avoid mating with already-mated queens as the latter would tend to specialize in producing sons when there are split sex ratios, and only daughters genetically benefit the queen's mates (Boomsma 1996).

Worker policing of male production also occurs in polyandrous honeybees (Barron et al. 2001), and is absent in monandrous stingless bees (Peters et al. 1999), in accordance with theory. However, worker policing also occurs in a variety of other hymenopterans, including the common wasp (Foster and Ratnieks 2001), hornets (Foster and Ratnieks 2000; Foster et al. 1999; Foster et al. 2002), and queenless ants (Gobin et al. 1999; Kikuta and Tsuji 1999; Liebig et al. 1999), even though theory does not predict worker policing for these species (where workers are more closely related to nephews than to brothers). Worker policing in such cases may be a byproduct of sex-ratio manipulation by workers when the latter have constrained abilities to recognize sex (Foster and Ratnieks 2001).

Conflict over reproductive partitioning among queens

Conflicts among queens over the partitioning of reproduction (reproductive skew) can be connected to queen-worker sex-ratio conflict. For example, Bourke (2001) showed recently that, when workers cause a female-biased population sex

ratio, a dominant queen has added incentive to share reproduction with a subordinate queen, thereby reducing the reproductive skew. This sharing is favored because shared reproduction by multiple queens reduces the workers' relatedness asymmetry, causing the workers to be more likely to invest in males, and the latter benefits the dominant queen because the female-biased population sex ratio makes sons especially valuable to her.

Conflict over life history decisions (colony growth)

One major theoretical development is the extension of queen-worker conflict over sex allocation to life history allocation, that is, the relative investment of resources into colony growth (production of workers) versus colony reproduction (production of sexuals) (Crozier and Pamilo 1996). The applicability and scope of this type of conflict in various kin-structured hymenopteran societies has recently stimulated controversy. Bourke and Chan (1999) argued against the proposed generality of queen-worker conflict over life-history decisions (Pamilo 1991) and suggested it should be absent in colonies with the simplest social organization (i.e., characterized by a monogynous, monandrous queen, sterile workers, and independent mode of colony foundation) because only queens can produce reproductives for these societies, and thus, workers and queens have aligned interests in maximizing colony fitness. Herbers et al. (2001) and Reuter and Keller (2001) contributed to the theoretical resolution of this controversy by developing models that link conflict over life-history (colony growth) decisions to conflict over sex ratios. Herbers et al. (2001)

concluded that there is potential for queen-worker conflict over life-history allocation (i) only when sex-ratio conflict occurs and (ii) only when there is variation in population-wide sex ratios (i.e., split sex ratios). Reuter and Keller (2001) showed that queen-worker conflict over colony growth occurred only if neither party had complete control over sex allocation.

CONCLUSIONS AND FUTURE DIRECTIONS

Two major advances arising from recent empirical and theoretical work on sex-ratio conflict include (i) the synthesis of sex-ratio conflict with other queen-worker conflicts and (ii) the elucidation of cases of queen control over the sex ratio. The integration of multiple queen-worker conflicts not only allows a more realistic approach to the study of conflict evolution, but also should lead to new predictions about the resolution of conflicts in eusocial Hymenoptera. The original paradigm of worker-controlled sex ratios clearly has been an oversimplification. This is especially evident now in societies where the queen controls the sex ratio while workers control relative investment in worker broods versus reproductive females (Reuter and Keller 2001). What is needed is a framework when neither the queen nor the workers have complete control over the sex ratio, that is, in situations where it is unclear a priori when and how potential conflict over the sex ratio becomes realized as actual conflict. One approach for modeling the resolution of sex ratio conflicts is to co-opt incomplete control or “tug-of-war” models on the resolution of conflicts over

reproductive skew. These models are likely to generate new predictions for explaining variation in sex ratios in nature. For example, incomplete control models can account for split sex ratios in species without variation in relatedness asymmetry, the tendency of smaller ant colonies to produce more males, and previously unanticipated variability in colony sex ratios. Thus, the study of the resolution of queen-worker sex-ratio conflicts has barely begun.

CHAPTER 5

Ant vs. Fungus vs. Mutualism:

Sex-ratio Conflict in the Attine Ant - Fungus Symbiosis

ABSTRACT

An intriguing complexity in the attine ant-fungus mutualism is potential sex-ratio conflict between ant farmers and their cultivated fungi. Three parties are presumably in conflict over ant sex ratios, the mutualistic fungus, the workers, and the queen. Fungal cultivars are selected to bias the colony sex ratio towards 100% investment in females because only virgin queens disperse the cultivar by using pieces of the natal fungus to start their own garden. In contrast, under monogyny and monandry, workers and queens prefer, respectively, 75% and 50% investment in females, following theoretical predictions derived originally by Trivers and Hare. I studied the dynamics of sex-ratio conflict and ant-fungus cooperation in the monogynous, monandrous attine ant *Cyphomyrmex muelleri*. Using a block design, I standardized numbers of workers and fungal biomass for each of 10 pairs of *C. muelleri* laboratory colonies reared on either their host fungus (control condition) or the cultivar of the sister ant species *Cyphomyrmex longiscapus* (switch condition). *Cyphomyrmex muelleri* colonies that cultivated their native fungus increased in biomass (garden + ants) and fitness (number of reproductives) over time, whereas those that cultivated the switched fungus did not. The greater ant fitness in association with the original

host fungus indicates that ants and native cultivars have coevolved to enhance mutualistic interactions. Nevertheless, ant-cultivar conflict may have been revealed by the effect of cultivar switches on ant sex ratio and male survival. Sex ratio was female-biased for colonies reared on their native, coevolved cultivar, but male-biased for those reared on the novel, non-coevolved cultivar. Interestingly, males had higher survivorship on the novel cultivar compared to their native cultivar, yet the opposite was true for female survivorship. The native cultivar thus may manipulate ant sex ratios by compromising male survivorship. Such cultivar switch experiments may emerge as a major new research tool for unraveling the evolutionary dynamics of conflict and cooperation between mutualistic partners in the attine ant-fungus symbiosis.

INTRODUCTION

Fungus-growing ants in the tribe Attini obligately depend on fungus as their major food source (Weber 1972; Hölldobler and Wilson 1990) and in exchange provide nourishment in the form of vegetative substrate and protect the fungus from a number of pathogens (Currie et al. 1999). This classic example of a mutualism, as well as others, is well known as a reciprocally beneficial relationship. However, current theory suggests that mutualisms should be regarded as reciprocal exploitations whereby each partner receives net benefits from the other (Maynard Smith and Szathmáry 1995; Leigh and Rowell 1995; Nowak et al. 1994; Doebeli and Knowlton

1998). Thus, any symbiosis may have aspects of both cooperation and conflict, and here, I aim to identify and explore the benefits and costs of the association between the fungus and attine ants.

Ant-cultivar conflict is likely to evolve because recent phylogenetic and population genetic analyses contradicted the century-long assumption of strict vertical inheritance of fungi from maternal to offspring colony; instead, cultivars are frequently horizontally transmitted, and at least some cultivars appear to have close links to free-living fungal populations (Mueller et al. 1998; Green et al. 2002). Horizontal cultivar transmission erodes alignment of reproductive interests between ants and cultivars, creating novel types of ant-cultivar conflict and exacerbating conflicts existing even under strict vertical transmission.

An intriguing dimension of complexity in the attine ant-fungus mutualism derives from potential conflict over the sex ratio, i.e., the ratio of male to female reproductives produced by a social insect colony. In hymenopteran societies, including those with and without heterospecific symbionts, conflict over sex ratios is presumed to exist between the mother queen and her daughters (the workers) due to differences in their genetic relatedness to the sexuals. Assuming the simplest case of a single, once-mated queen, the queen should prefer a 1:1 sex ratio (male: female) because she is equally related to her sons and daughters; however, workers should prefer a 1:3 sex ratio since they are more closely related to their sisters ($r= 0.75$) than to their brothers ($r= 0.25$) based on the haplodiploidy sex determination system of

hymenopterans (Trivers and Hare 1976). Predicting and quantifying sex-ratio conflict in social insect colonies with more than one queen and/or with multiply mated queens becomes more complex (Bourke and Franks 1995; Crozier and Pamilo 1996). The presence of heterospecific symbionts in a social insect colony (e.g., fungus-growing ants) complicates predictions of conflict resolution even furthermore. Three parties are potentially in conflict over sex ratios: the mutualistic fungus, the workers, and the queen. Along with the theoretical predictions of the sex ratio interests of the queen and workers as explained above, the fungus should prefer 100% production of female sexuals since only virgin queens disperse the fungus by using small pieces of the natal fungus to start their own garden (Ihering 1898; Huber 1905 a, b; Mueller et al. 2001). Viewed from the evolutionary interests of the cultivar, males are therefore a “waste” of colony resources. The goal of this laboratory study was to elucidate the dynamics of sex-ratio conflict and ant-fungus cooperation in the attine agricultural symbiosis.

Fungus-growing ants in the strictly Neotropical tribe Attini comprise a monophyletic group of about 200 described species (Schultz and Meier 1995). Depending on the ant species, gardens are manured with a variety of live and dead vegetative substrates. Ants in the seven genera of “lower attines” are inconspicuous and frequently cryptic; they utilize dead vegetative substrate and thus do not attack plants like the leafcutter ants, a subgroup of the derived “higher attines”. Lower

attines have received little attention, and their ecologies and life histories are largely unknown.

For this study, I used the primitive attines, *Cyphomyrmex longiscapus* and *C. muelleri* (Mueller and Wcislo 1998). Population genetic analyses using allozyme and microsatellite markers revealed that the previously recognized *Cyphomyrmex longiscapus sensu lato* actually consists of two cryptic species (named *C. longiscapus sensu stricto* and *C. muelleri*; Schultz et al. in press). Reproductive isolation between the two species is indicated by the absence of heterozygotes at several microsatellite and allozyme loci (i.e., fixed alternate alleles in each species) (Schultz et al. in press). Each of these cryptic species cultivates only one cultivar type, and these two cultivar types are distantly related (Mueller et al. 1998). Because the two cryptic ant species are clearly of recent origin, yet they cultivate distantly related fungi, these patterns suggest that a recent switch to a novel, distantly related fungus may have caused the ancestral *C. longiscapus s.l.* to differentiate into two, reproductively isolated lineages.

METHODS

Collection and set-up of colonies. I used 20 queenright colonies (i.e., colonies with a queen) of *Cyphomyrmex muelleri* and *C. longiscapus* that were collected in the Republic of Panama in September 1999. *Cyphomyrmex muelleri* colonies were reared on either their native fungus (control condition) or a novel cultivar from the sister ant

species *C. longiscapus* (switch condition). Using a block design, I standardized worker number and garden biomass for each of 10 colony pairs. Worker size ranged from 2 to 61 workers and garden biomass ranged from 67 to 455 mg across the ten replicates. (That is, both the control and switch colonies from replicate one had two workers and 67 mg of garden biomass and both the control and switch colonies from replicate ten had 61 workers and 455 mg of garden biomass and so on.) At the beginning of the experiment, I removed brood and alates from all colonies.

Maintenance of colonies. Individual colonies were housed in plastic square nest boxes (side: 7.5 cm; height: 3.0 cm), which were connected by transparent Tygon tubing (diameter: 5/16 in) to dump chambers of similar size. Once per week, I moistened the plaster on the bottom of the nest boxes and placed UV-sterilized (i.e., free of contaminants) organic oats, *ad libitum*, in the dump chambers for use as garden substrate. I reared colonies under an approximate 8 hour light - 16 hour dark cycle at room temperature.

Data Collection and Analyses. For all colonies, I quantified ant mortality weekly and garden biomass monthly. To minimize disturbance to colonies, I kept all ants on their gardens during garden biomass measurements. (Ant biomass was negligible relative to fungal biomass.) After 8 months, I counted the number of live and dead sexuals (i.e., alates) produced by each colony. Biomass data were analyzed with a univariate mixed model ANOVA using SAS (Littell et al. 1996; Singer 1998). The fixed effects were treatment (control and switch conditions) and time (6 monthly

observations for each colony), which served as a repeated measure. The random effect was colony (each of the 10 colony pairs standardized according to garden biomass and colony size). Analysis of number of reproductives was done with a generalized linear model, assuming a Poisson distribution for the dependent variable, a log link function to linearize the data, and a GEE (Generalized Estimating Equations) method to account for non-independence within colony (Stokes et al. 2002). In addition, sex ratio data were analyzed with a chi-square test in SAS. Categories included male-biased sex ratios (< 50%), female-biased sex ratios (> 50%), and even sex ratios (= 50%).

RESULTS AND DISCUSSION

Ants and native cultivars seem to have coevolved to enhance mutualistic benefits. First, mean garden biomass increased over time for control groups, but not for groups with the switched fungus ($F_{(1, 62)} = 5.77$; $P = 0.0193$; Fig. 1). Second, *C. muelleri* colonies reared on their native, coevolved cultivar produced significantly more reproductives compared to those reared on the novel, non-coevolved cultivar ($Z_{(1, 17)} = 3.19$; $P=0.0014$; Fig. 2). Nevertheless, ant-cultivar conflict may have been revealed by the effect of cultivar switches on ant sex ratio and on male survival. Sex ratio was female-biased for colonies reared on their native, coevolved cultivar, but male-biased for those reared on the novel, non-coevolved cultivar ($X^2_{(2)} = 6.0$; Asymptotic $P= 0.0498$; Fisher Exact $P= 0.0571$; Fig. 3). Interestingly, males had

higher survivorship on the novel cultivar compared to their native cultivar, yet the opposite was true for female survivorship (Fig. 4).

One hypothesis for the observed trend is that, since mean biomass was lower for *C. muelleri* colonies on the non-native cultivar compared to colonies on the native cultivar, the overall smaller, switched colonies may have invested in the energetically cheaper sex (i.e., males). However, our analyses of alate production controlled for the variation due to worker population size and garden biomass. A second hypothesis for the observed trend is fungal manipulation of the ants. Due to its long association with *C. muelleri*, the native cultivar is presumably “better” at manipulating the ants to produce a female-biased sex ratio. The mechanism by which the fungus might manipulate the ant sex ratio is unknown; however, the mortality data suggest that feeding on the fungus is harmful to males, but not to females. Specifically, (i) the native fungus might produce chemicals which are harmful to males but not to females; or (ii) the native fungus might provide an optimal diet for females but a sub-optimal diet for males. Additional experiments (e.g., rearing male and female reproductives on native and non-native cultivar in the absence of workers) are needed in order to determine the underlying mechanism of possible fungal manipulation.

The attine ant-fungus symbiosis historically has been viewed as a textbook case of mutualism, yet theory predicts the existence of conflicts between the partners even in this highly derived and integrated mutualism. Indeed, my results reveal that the possibility of conflict between these two mutualistic partners deserves further

exploration. The recent findings of cultivar exchanges already overturns the century-long assumption of strict vertical inheritance of fungi from maternal to offspring colony (Mueller et al. 1998; Green et al. 2002), and thus predicts some level of ant-cultivar conflict. However, similar to sex-ratio biasing mitochondria in some plants (Werren and Beukeboom 1998), sex-ratio conflict between ants and their cultivars is predicted even under strict vertical transmission. Horizontal transmission will create novel conflicts, and may intensify the one over the sex ratio existing even under strict vertical transmission.

Sex-ratio conflict in attines involves three players: the mutualistic fungus, the queen, and the workers. If other eusocial Hymenoptera also have maternally inherited symbionts (e.g., *Wolbachia*), then the three-party conflict examined here in the attine ant-fungus mutualism for the first time may be widespread among social insects. This implies that traditional sex-ratio predictions (Trivers and Hare 1976) ignoring the influence of microbial third parties, may have been based on oversimplifying assumptions, and therefore, much of the previous sex-ratio work now needs to be re-examined within a novel theoretical framework that incorporates a third player (Reeve et al. In preparation). Because of the ability to experimentally manipulate and substitute mutualistic partners, the attine ant-fungus symbiosis is poised at the threshold of an entirely new conceptual approach to conflict resolution.

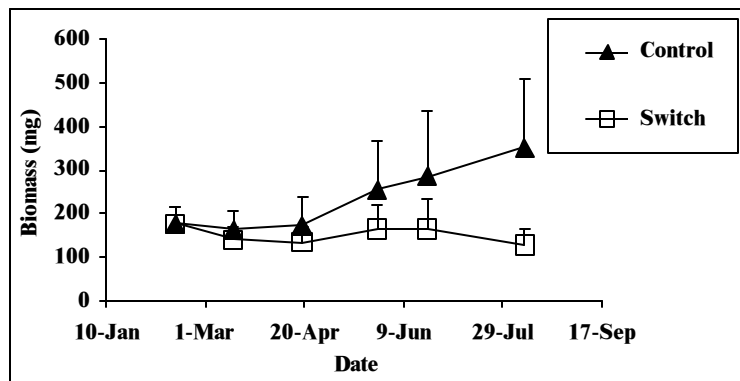


FIGURE 1 Mean biomass (fungus and ants) over time for *C. muelleri* colonies that cultivated their native fungus (control condition) and those that cultivated a novel fungus from the sister species *C. longiscapus* (switch condition). Biomass increased more rapidly in the colonies with the native fungus.

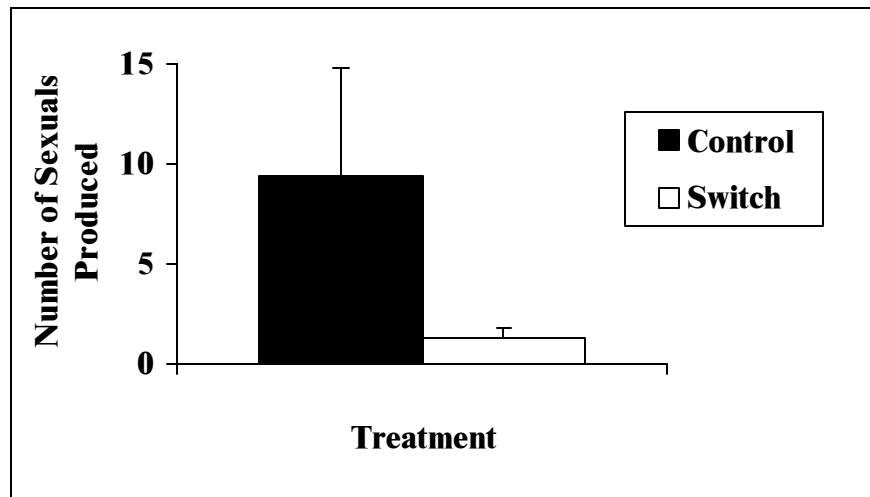


FIGURE 2 Mean number of sexuals for control and switched colonies. Control colonies produced more reproductives than switched colonies.

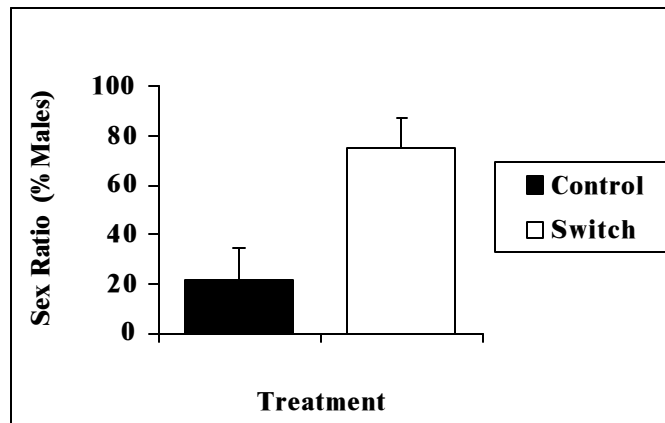


FIGURE 3 Mean sex ratio (% males) for control and switched colonies. Control colonies had female-biased sex ratios; however, switched colonies had male-biased sex ratios.

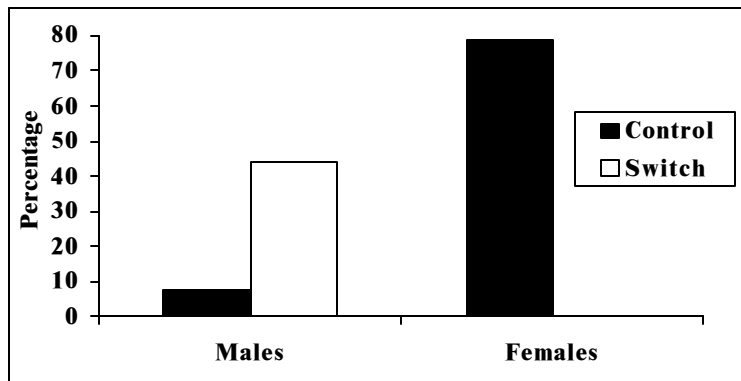


FIGURE 4 Percentage of surviving sexuals in control and switched colonies. Each percentage represents the proportion of live reproductives of a given sex relative to the total number of reproductives (live + dead) produced of that sex. Male survival rate was lower in the colonies with the native fungus relative to colonies with the switched fungus.

BIBLIOGRAPHY

- Aron, S., Passera, L., and Keller, L. 1994. Queen-worker conflict over sex ratio: A comparison of the primary and secondary sex ratios in the Argentine ant, *Iridomyrmex humilis*. *Journal of Evolutionary Biology* **7**: 403-418.
- Aron, S., Vargo, E. L., and Passera, L. 1995. Primary and secondary sex ratios in monogyne colonies of the fire ant. *Animal Behaviour* **49**: 749-757.
- Barron, A., Oldroyd, B. P., and Ratnieks, F. L. W. 2001. Worker policing and anarchy in *Apis*. *Behavioural Ecology and Sociobiology* **50**: 199-208.
- Bellotti, A. C., Smith, L., and Lapointe, S. L. 1999. Recent advances in cassava pest management. *Annual Review of Entomology* **44**: 343-370.
- Berg, S., Koeniger, N., Koeniger, G., and Fuchs, S. 1997. Body size and reproductive success of drones (*Apis mellifera* L.) *Apidologie* **28**: 449-460.
- Boomsma, J. J. 1989. Sex-investment ratios in ants: Has female bias been systematically overestimated? *American Naturalist* **133**: 517-532.
- Boomsma, J. J. 1991. Adaptive colony sex ratios in primitively eusocial bees. *Trends in Ecology and Evolution* **6**: 92-95.
- Boomsma J.J. 1996. Split sex ratios and queen-male conflict over sperm allocation. *Proceedings of the Royal Society of London, Series B* **263**: 697-704.
- Bourke, A. F. G. 1997. Sex ratios in bumblebees. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* **352**: 1921 –1933.
- Bourke A.F.G. 2001. Reproductive skews and split sex ratios in the Hymenoptera.

- Evolution **55**: 2131-2136.
- Bourke, A. F. G., and Chan, G. L. 1999. Queen-worker conflict over sexual production and colony maintenance in perennial social insects. American Naturalist **154**: 417-426.
- Bourke, A. F. G., and Franks, N. R. 1995. Social Evolution in Ants. Princeton Press, New Jersey.
- Bourke, A. F. G., and Ratnieks, F. L. W. 2001. Kin-selected conflict in the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). Proceedings of the Royal Society of London, Series B **268**: 347-355.
- Bronstein J. L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution **9**: 214-217.
- Bulmer, M.G. 1981. Worker-queen conflict in the annual social Hymenoptera. Journal of Theoretical Biology **83**: 239-252.
- Camilo, G. R., and Phillips, Jr., S. A. 1990. Evolution of ant communities in response to invasion by the fire ant *Solenopsis invicta*. Pages 190-198 in R. K. Vander Meer, K. Jaffe, and A. Cedeno, editors. Applied Myrmecology: a World Perspective. Westview Press, Boulder, Colorado.
- Chapuisat, M., and Keller, L. 1999. Testing kin selection with sex allocation data in eusocial Hymenoptera. Heredity **82**: 473-478.

- Chapuisat, M., Sundström, L., and Keller, L. 1997. Sex-ratio regulation: The economics of fratricide in ants. *Proceedings of the Royal Society of London, Series B* **264**: 1255- 1260.
- Cnaani, J., Robinson, G. E., Bloch, G., Borst, D., and Hefetz, A. 2000. The effect of queen-worker conflict on caste determination in the bumblebee *Bombus terrestris*. *Behavioural Ecology and Sociobiology* **47**: 346-352.
- Crozier, R. H., and Pamilo, P. 1996. *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. Oxford University Press, Oxford.
- Currie, C. R., Scott, J. A., Summerball, R. C., and Malloch, D. 1999. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature (London)* **398**: 701-704.
- Dietemann, V., and Peeters, C. 2000. Queen influence on the shift from trophic to reproductive eggs laid by workers of the ponerine ant *Pachycondyla apicalis*. *Insectes Sociaux* **47**: 223-228.
- Disney, R. H. L. 1994. *Scuttle Flies: The Phoridae*. Chapman and Hall, London.
- Doebeli, M., and Knowlton, N. 1998. The evolution of interspecific mutualisms. *Proceedings of the National Academy of Sciences, U.S.A.* **95**: 8676-8680.
- Drumond, P. M., Oldroyd, B. P., Dollin, A. E., and Dollin, L. J. 1999. Oviposition behaviour of two Australian stingless bees, *Austroplebeia symei* Rayment and *Austroplebeia australis* Friese (Hymenoptera: Apidae: Meliponini). *Australian Journal of Entomology* **38**: 234-241.

- Elton, C. S. 1958. *The Ecology of Invasions by Animals and Plants*. Wiley, New York.
- Feener, Jr., D. H. 1981. Competition between ant species outcome controlled by parasitic flies. *Science* **214**: 815-817.
- Feener, Jr., D. H. 1987. Size-selective oviposition in *Pseudacteon-crawfordi* Diptera Phoridae a parasite of fire ants. *Annals of the Entomological Society of America* **80**: 148-151.
- Feener, Jr., D. H. 2000. Is the assembly of ant communities mediated by parasitoids? *Oikos* **90**: 79-88.
- Feener, Jr., D. H., and Brown, B. V. 1992. Reduced foraging of *Solenopsis geminata* (Hymenoptera: Formicidae) in the presence of parasitic *Pseudacteon* spp. (Diptera: Phoridae). *Annals of the Entomological Society of America* **85**: 80-84.
- Fisher, R. A. 1958. *The Genetical Theory of Natural Selection*. Dover, New York.
- Folgarait, P. J., and Gilbert, L. E. 1999. Phorid parasitoids affect foraging activity of *Solenopsis richteri* under different availability of food in Argentina. *Ecological Entomology* **24**: 163-173.
- Foster, K., and Ratnieks, F. L. W. 2000. Facultative worker policing in a social wasp. *Nature* **407**: 692-693.
- Foster, K. R., and Ratnieks, F. L. W. 2001a. The effect of sex allocation biasing on the evolution of worker policing in hymenopteran societies. *American Naturalist* **158**: 615-623.

- Foster, K., and Ratnieks, F. L. W. 2001b. Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proceedings of the Royal Society of London, Series B* **268**: 169-174.
- Foster, K. R., Seppä, P., Ratnieks, F. L. W., and Thorén, P. A. 1999. Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behavioural Ecology and Sociobiology* **46**: 252-257.
- Foster, K. R. 2002. Worker policing in the European hornet, *Vespa crabro*. *Insectes Sociaux* **49**: 41-44.
- Gobin, B., Billen, J., and Peeters, C. 1999. Policing behaviour towards virgin egg layers in a polygynous ponerine ant. *Animal Behaviour* **58**: 1117-1122.
- Gotelli, N. J., and Arnett, A. 2000. Biogeographic effects of red fire ant invasion. *Ecology Letters*. **3**: 257-261.
- Green A.M., Adams, R.M.M., and Mueller, U.G. 2002. Extensive exchange of fungal cultivars between sympatric species of fungus-growing ants. *Molecular Ecology* **11**: 191-195.
- Hamilton, W. D. 1964. The genetical evolution of social behaviour, I and II. *Journal of Theoretical Biology* **7**: 1-52.
- Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. *Annual Review of Ecology and Systematics* **3**: 193-232.

- Hammond, R. L., Bruford, M. W., and Bourke, A. F. G. 2002. Ant workers selfishly bias sex ratios by manipulating female development. *Proceedings of the Royal Society of London, Series B* **269**: 173-178.
- Helms, K. R. 1999. Colony sex ratios, conflict between queens and workers, and apparent queen control in the ant *Pheidole desertorum*. *Evolution* **53**: 1470-1478.
- Helms, K. R., Fewell, J. H., and Rissing, S. W. 2000. Sex ratio determination by queens and workers in the ant *Pheidole desertorum*. *Animal Behaviour* **59**: 523-527.
- Herbers, J., DeHeer, C. J., and Foitzik, S. 2001. Conflict over sex allocation drives conflict over reproductive allocation in perennial social insect colonies. *American Naturalist* **158**: 178-192.
- Herre, E. A., Knowlton N., Mueller, U. G., and Rehner, S. A. 1999. The evolution of mutualisms: Exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution*. **14**: 49-53.
- Hölldobler, B., and Wilson, E. O. 1990. *The Ants*. Belknap Press, Cambridge, Massachusetts.
- Huber, J. 1905a. Über die Koloniegründung bei *Atta sexdens*. *Biologisches Centralblatt* **25**: 606-619.
- Huber, J. 1905b. Über die Koloniegründung bei *Atta sexdens*. *Biologisches Centralblatt* **25**: 625-635.

- Ihering, R. von. 1894. Die Ameisen von Rio Grande do Sul. Berliner Entomologische Zeitschrift **39**: 321-446.
- Keller, L., and Nonacs, P. 1993. The role of queen pheromones in social insects: queen control or queen signal? *Animal Behaviour* **45**: 787-794.
- Keller, L., Aron, S., and Passera, L. 1996. Inter-nest sex-ratio variation and male brood survival in the ant *Pheidole pallidula*. *Behavioral Ecology* **7**: 292-298.
- Kikuta, N., and Tsuji, K. 1999. Queen and worker policing in the monogynous and monandrous ant, *Diacamma* sp. *Behavioural Ecology and Sociobiology* **46**: 180-189.
- Leigh, E. G., Jr., and Rowell, T. E. 1995. The evolution of mutualism and other forms of harmony at various levels of biological organization. *Ecologie* **26**: 131-158.
- Leung, B., Forbes, M. R., and Baker, R. L. 2001. Nutritional stress and behavioural immunity of damselflies. *Animal Behaviour* **61**: 1093-1099.
- Liebig, J., Peeters, C., and Hölldobler, B. 1999. Worker policing limits the number of reproductives in a ponerine ant. *Proceedings of the Royal Society of London, Series B* **266**: 1865-1870.
- Littell, R. C., Milliken, G. A., Stroup, W. W., and Wolfinger, R. D. 1996. SAS system for mixed models. SAS Institute, Inc., Cary, North Carolina.
- Louda, S. M., Kendall, D., and Simberloff, D. 1997. Ecological effects of an insect introduced for the biological control of weeds. *Science* **277**: 1088-1090.

- Maynard Smith, J., and Szathmáry, E. 1995. *The Major Transitions in Evolution*. W. H. Freeman.
- Mehdiabadi, N. J., and Gilbert, L. E. In press. Colony-level impacts of parasitoid flies on fire ants. *Proceedings of the Royal Society of London, Series B*.
- Mooney, H. A., and Cleland, E. E. 2001. The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences, U.S.A.* **98**: 5446-5451.
- Morrison, L. W. 1999. Indirect effects of phorid fly parasitoids on the mechanisms of interspecific competition among ants. *Oecologia* **121**: 113-122.
- Morrison, L. W., Dall'Aglia-Holvorcem, C. G., and Gilbert, L. E. 1997. Oviposition behavior and development of *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). *Environmental Entomology* **26**: 716-724.
- Mueller, U. G. In press. Ant versus fungus versus mutualism: Ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. *American Naturalist*.
- Mueller, U.G., Rehner, S., and Schultz, T.R. 1998. The evolution of agriculture in ants. *Science* **281**: 2034-2038.
- Mueller, U.G., Schultz, T.R., Currie, C., Adams, R. M. M., and Malloch, D. 2001. The origin of the attine ant-fungus symbiosis. *Quarterly Review of Biology* **76**: 169-197.
- Mueller, U. G., and Wcislo, W. 1998. Nesting biology of the fungus-growing ant

- Cyphomyrmex longiscapus* Weber (Attini, Formicidae). *Insectes Sociaux* **45**: 181-189.
- Nonacs, P. 1986. Ant reproductive strategies and sex allocation theory. *Quarterly Review of Biology* **61**: 1-21.
- Nonacs, P. 1993. Male parentage and sexual deception in the social Hymenoptera. Pages 384-401 in D. L. Wrensch, and M. A. Ebbert, editors. *Evolution and Diversity of Sex Ratio in Insects and Mites*, Chapman and Hall, London.
- Nowak, M. A., Bonhoeffer, S., and May, R. M. 1994. Spatial games and the maintenance of cooperation. *Proceedings of the National Academy of Sciences, U.S.A.* **91**: 4877-4881.
- Orr, M. R., Seike, S. H., Benson, W. W., and Gilbert, L. E. 1995. Flies suppress fire ants. *Nature* **373**: 292-293.
- Orr, M. R., Seike, S. H., and Gilbert, L. E. 1997. Foraging ecology and patterns of diversification in dipteran parasitoids of fire ants in South Brazil. *Ecological Entomology* **22**: 305-314.
- Pamilo, P. 1982. Genetic evolution of sex ratios in eusocial Hymenoptera: Allele frequency simulations. *American Naturalist* **119**: 638-656.
- Pamilo, P. 1991. Evolution of colony characteristics in social insects. I. Sex allocation. *American Naturalist* **137**: 83-107.

- Passera, L., and Aron, S. 1996. Early sex discrimination and male brood elimination by workers of the Argentine ant. *Proceedings of the Royal Society of London, Series B* **263**: 1041-1046.
- Passera, L., Aron, S., Vargo, E. L., and Keller, L. 2001. Queen control of sex ratio in fire ants. *Science* **293**: 1308-1310.
- Passera, L., Roncin, E., Kaufmann, B., and Keller, L. 1996. Increased soldier production in ant colonies exposed to intraspecific competition. *Nature* **379**: 630-631.
- Peters, J. M., Queller, D. C., Imperatriz-Fonseca, V. C., Roubik, D. W., and Strassman, J. E. 1999. Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proceedings of the Royal Society of London, Series B* **266**: 379-384.
- Pimm, S. L. 1991. *The Balance of Nature? Ecological Issues in the Conservation of Species and Communities*. University of Chicago Press, Chicago.
- Porter, S. D. 1998. Biology and behaviour of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). *Florida Entomologist* **81**: 292-309.
- Porter, S. D., Pesquero, M. A., Campiolo, S., and Fowler, H. G. 1995. Growth and development of *Pseudacteon* phorid fly maggots (Diptera: Phoridae) in the heads of *Solenopsis* fire ant workers (Hymenoptera: Formicidae). *Environmental Entomology* **24**: 475-479.

- Porter, S. D., and Tschinkel, W. R. 1985. Fire ant polymorphism: the ergonomics of brood production. *Behavioral Ecology and Sociobiology* **16**: 323-336.
- Porter, S. D., Vander Meer, R. K., Pesquero, M. A., Campiolo, S., and Fowler, H. G. 1995. *Solenopsis* (Hymenoptera: Formicidae) fire ant reactions to attacks of *Pseudacteon* flies (Diptera: Phoridae) in southeastern Brazil. *Annals of the Entomological Society of America* **88**: 570-575.
- Queller, D. C., and Strassman, J. E. 1998. Kin selection and social insects. *Bioscience* **48**: 165-175.
- Ratnieks, F. L. and Keller, L. 1998. Queen control of egg fertilization in the honeybee. *Behavioural Ecology and Sociobiology* **44**: 57-61.
- Reeve H.K. 1997. Evolutionarily stable communication between kin: A general model. *Proceedings of the Royal Society of London, Series B* **264**: 1037-1040.
- Reeve, H. K., Mehdiabadi, N. J., and Mueller, U. G. In preparation. Sex-ratio conflict as a tug-of-war.
- Reuter, M., and Keller, L. 2001. Sex ratio conflict and worker production in eusocial Hymenoptera. *American Naturalist* **158**: 166-177.
- Sasaki, K., and Obara, Y. 2001. Nutritional factors affecting the egg sex ratio adjustment by a honeybee queen. *Insectes Sociaux* **48**: 355-359.
- Schultz, T. R., and Meier, R. 1995. A phylogenetic analysis of the fungus-growing ants (Hymenoptera: Formicidae: Attini) based on morphological characters of the larvae. *Systematic Entomology* **20**: 337-370.

- Schultz, T. R., Solomon, S. A., Mueller, U. G., Boomsma, J. J., Villesen, P., Adams, R. M. M., and Norden, B. In press. Cryptic speciation in the fungus-growing ant *Cyphomyrmex "longiscapus"* s.l. (Formicidae: Attini), a model organism for the attine agricultural symbiosis. *Insectes Sociaux*.
- Seeley, T. D. 1985. *Honeybee Ecology*. Princeton University Press, Princeton.
- Seger, J. 1996. Exoskeletons out of the closet. *Science* **274**: 941.
- Sheldon, B. C., and Verhulst, S. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11**: 317-321.
- Singer, J. D. 1998. Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. *Journal of Educational Behavioral Statistics* **24**: 323-355.
- Sorenson, A. A., Busch, T. M., and Vinson, S. B. 1983. Behaviour of worker subcastes in the fire ant, *Solenopsis invicta*, in response to proteinaceous food. *Physiological Entomology* **8**: 83-92.
- Starks, P., and Poe, E. 1997. Male stuffing in wasp societies. *Nature* **389**: 450.
- Stokes, M. E., Davis, C. S., and Koch, G. G. 2000. *Categorical data analysis using the SAS system*, Second Edition. SAS Institute, Inc., Cary, North Carolina.
- Summerlin, J. W., Hung, A. C. F., and Vinson, S. B. 1977. Residues in nontarget ants, species simplification and recovery of populations following aerial applications of mirex. *Environmental Entomology* **6**: 193-197.

- Sundström, L., Chapuisat, M., and Keller, L. 1996. Conditional manipulation of sex ratios by ant workers: A test of kin selection theory. *Science* **274**: 993-994.
- Thompson, L. C., Jones, D. B., Semevski, F. N., and Semenov, S. M. 1995. Fire ant economic impact: Extending Arkansas' survey results over the South. Pages 155-156 *in* S. B. Vinson, and B. M. Drees, editors. Proceedings of the Fifth International Pest Ant Symposia and the 1995 Annual Imported Fire Ant Conference, San Antonio, TX.
- Trivers, R. L., and Hare, H. 1976. Haplodiploidy and the evolution of the social insects. *Science* **191**: 249-276.
- Trivers, R. L., and Willard, D. E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179**: 90-92.
- Vinson, S. B. 1986. Economic Impact and Control of Social Insects. Praeger, New York.
- Vinson, S. B. 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae): Spread, biology, and impact. *American Entomologist* **43**: 23-39.
- Vitousek, P. M., D'Antonio, C. M., Loope, L. L., and Westbrooks, R. 1996. Biological invasions as global environmental change. *American Scientist* **84**: 468-478.
- Weber N. A. 1972. Gardening Ants: The Attines. American Philosophical Society, Philadelphia.
- Werren, J. H., and Beukeboom L. W. 1998. Sex determination, sex ratios, and genetic

- conflict. *Annual Review of Ecology and Systematics* **29**: 233-261.
- Williams, D. F. 1994. *Exotic Ants: Biology, Impact, and Control of Introduced Species*. Westview, Boulder, Colorado.
- Williamson, M., and Fitter, A. 1996. The varying success of invaders. *Ecology* **77**: 1661-1666.
- Wilson, E. O. 1978. Division of labor based on physical castes in fire ants (Hymenoptera: Formicidae: *Solenopsis*). *Journal of the Kansas Entomological Society* **51**: 615-636.
- Wilson, E. O. 1980. Caste and division of labor in leafcutter ants (Hymenoptera: Formicidae: *Atta*), II. The ergonomic phase optimization of leaf cutting. *Behavioral Ecology and Sociobiology* **7**: 143-156.
- Wojcik, D. P., Allen, C. R., Brenner, R. J., Forys, E. A., Jouvenaz, D. P., and Lutz, R. S. 2001. Red imported fire ants: impact on biodiversity. *American Entomologist* **47**: 24-50.

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