DISSERTATION

OBLIGATE AND FACULTATIVE SLAVE-MAKING ANTS: RAIDING BEHAVIOR, HOST-PARASITE COEVOLUTION, AND THE EVOLUTION OF SLAVE-MAKING BEHAVIOR

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JEREMY M. BONO ENTITLED: OBLIGATE AND FACULTATIVE SLAVE-MAKING ANTS: RAIDING BEHAVIOR, HOST-PARASITE COEVOLUTION, AND THE EVOLUTION OF SLAVE-MAKING BEHAVIOR, BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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ABSTRACT OF DISSERTATION

OBLIGATE AND FACULTATIVE SLAVE-MAKING ANTS: RAIDING BEHAVIOR, HOST-PARASITE COEVOLUTION, AND THE EVOLUTION OF SLAVE-MAKING BEHAVIOR

Slave-making ants are social parasites that exploit the labor of workers from a closely related host species by incorporating them into the slave-maker colony as slaves. The unique life-history of slave-making ants makes them suitable for studying a variety of questions ranging from host-parasite coevolution to the evolution of sex allocation strategies. My research focuses on filling two major gaps in our understanding of slave-making ant systems. First, although factors contributing to the evolution of slave-making behavior have been considered for well over a century, the process remains poorly understood. The social and colonial structure of ancestral populations of slave-makers and their hosts is undoubtedly relevant, but we lack information on these traits for a number of slave-making ants and their hosts. Here, I report on the social and colonial structure of an obligate slave-making ant, *Polyergus breviceps* and two sympatric hosts, *Formica subsericea* and *F.* near *argentea.* My research also moves toward a better understanding coevolution between slave-making ants and their hosts in complex systems that include multiple slave-makers and multiple hosts. My study system includes two hosts, *F. subsericea* and *F.* near *argentea*, which are parasitized by the obligate slave-makers *P. breviceps* and two facultative slave-makers, *F. puberula* and *F. gynocrates*. Here, I compare slave-raiding behavior of the

slave-makers and characterize interactions between slave-makers and hosts that have significance for coevolution.

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Chapter I

Introduction to Dissertation

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Social parasitism occurs when one species is parasitically dependent on another for raising its young. Social parasites are found in birds, fish, and are particularly common in the insect order Hymenoptera (Davies *et al.* 1989). Among the Hymenoptera, social parasitism is most common in ants. Ant social parasites range in their degree of specialization from temporary parasites that found new colonies by invading established nests of another species but mature to be independent, to inquilines which are permanent parasites that have lost the worker caste altogether (Hölldobler & Wilson 1990).

Slave-making ants represent an intermediate stage of social parasitism in which slave-makers are dependent, to varying degrees, upon workers of another species. The life-cycle of slave-making ants includes two components: parasitic colony founding by queens, and slave-raiding by workers. A newly mated slave-maker queen is unable to initiate a new colony independently, and instead must enter a nest of its host, kill the resident queen(s), and either expel or gain acceptance by the workers. She then commences laying eggs, which develop into workers that replenish the slave supply each year by stealing brood during raids on neighboring host colonies. Slave-making ants represent varying degrees of specialization, ranging from facultative slave-makers, whose workers maintain a normal behavioral repertoire and can be found without slaves, to obligate slave-makers, which are entirely dependent on slaves because workers are unable to perform normal worker duties (Hölldobler & Wilson 1990).

Although only about 50 of 10,000 ant species are slave-makers, this behavior has probably evolved at least 10 times independently in two ant subfamilies, Myrmicinae and Formicinae. Slave-makers are patchily distributed within these two

subfamilies, with particular hot spots in the myrmicine tribe, Formicoxenini, and the formicine tribe, Formicini (D'Ettorre & Heinze 2001). Despite the relatively high number of independent origins of this parasitic behavior, slave-making ants share a number of morphological and behavioral adaptations that are a product of convergent evolution (D'Ettorre & Heinze 2001). For example, *Polyergus* and *Strongylognathus* share the trait of sickle shaped mandibles (used to pierce the heads of other ants) despite the fact that they are distantly related. Moreover, chemical weaponry used to facilitate entrance to host nests during slave-raiding and colony take-over has independently evolved in some myrmicine and formicine slave-makers (e.g. *Polyergus* and *Harpagoxenus*). Nevertheless, certain traits are quite variable among slave-makers, including colony size, recruitment tactics (tandem running vs. group recruitment), and mating strategies (D'Ettorre & Heinze 2001; Hölldobler & Wilson 1990; Mori *et al.* 2001).

The unique life-history of slave-making ants makes them well suited for studies of the evolution and ecology of social behavior, reproductive strategies, and host-parasite coevolution. Detailed studies on slave-raiding behavior date back almost two centuries (Huber first described the behavior in *P. rufescens* in 1810), while more recent studies have focused on questions concerning the evolution of slave-making behavior, sex allocation, chemical strategies during colony take-over and slave-raiding, and, most recently, coevolution between slave-makers and their hosts (D'Ettorre & Heinze 2001).

My study system is comprised of two hosts, *Formica subsericea* and *F.* near *agrentea*, and three of their parasites: the highly specialized, obligate slave-maker *P. breviceps*, and two facultative slave-makers from the *F. sanguinea* group, *F. puberula* and *F. gynocrates*. The genus *Polyergus* includes five obligate slave-

making species: *P. rufescens* of Eurasia, *P. samurai* from Japan, *P. nigerrismus*, of the former Soviet Union, *P. lucidus* of eastern North America, and *P. breviceps* of western North America. The *Formica sanguinea* group consists of 12 slave-makers, one from Europe and 11 from North America. Most are assumed to be facultative slave-makers, with the exception of *F. subintegra* (Savolainen & Deslippe 1996; Savolainen & Deslippe 2001), but few have been studied in any detail (Hölldobler & Wilson 1990).

My research addresses what I consider to be two major gaps in our current understanding of slave-making ant systems. First, mechanisms that could drive the evolution of slave-making behavior have been considered since Darwin's discussion of the subject in *The Origin of Species by Means of Natural Selection* (1859), but the process generally remains unresolved. The social and colonial structure of ancestral populations that gave rise to slave-making ants are undoubtedly relevant to the evolution of this behavior (see next section), but we lack information on these traits for a number of slave-making ants and their hosts. These data, along with phylogenetic data, should help us identify which traits are ancestral in taxa that have evolved slave-making. My research includes the analysis of social and colonial structure for the two hosts in my population as well as for the obligate slave-maker, *P. breviceps.*

The second gap that my work attempts to fill is the paucity of information on complex systems that include multiple slave-makers and multiple hosts. Despite the fact that formicine slave-makers often occur sympatrically and enslave the same hosts, little attention has been focused on such systems . When multiple parasites and hosts occur in sympatry we must consider the outcome of all possible interactions (slave-makers and hosts, slave-makers themselves, host themselves) if

we are to fully understand how slave-making ants and their hosts coevolve. My study system, which includes three slave-makers and two hosts, provides an ideal opportunity to examine the complexity of interactions in this type of system.

Evolution of slave-making behavior

Darwin (1859) offered the first hypothesis concerning the evolution of slave-making behavior. He envisioned that the ancestors of slave-makers were predators of other ants that would frequently steal brood from neighboring colonies for food. If, by chance, some of these brood were overlooked when brought back to the predator's nest, they could complete development and become incorporated as adults into the predator's nest. If these "slaves" behaved normally and provided a selective advantage to colonies of the predator, then the predators could presumably evolve to steal developing brood from neighboring nests for purposes of slavery. This hypothesis has since been largely disregarded. First, it accounts only for the evolution of slave-raiding behavior; any hypothesis on the origin of slavery must also account for the evolution of parasitic colony founding, because all slave-makers found new colonies parasitically. Second, slave-making has not evolved in taxa that are specialized predators on other ants, and some taxa that have evolved this behavior repeatedly, for example the formicoxenines, are not predatory (Hölldobler & Wilson 1990).

Buschinger (1970) proposed the first hypothesis that accounted for the evolution of slave-making behavior and parasitic colony founding. He argued that slave-makers probably evolved from ancestors who were polygynous (multiple queens per colony) and polydomous (a single colony occupies multiple nests). In most cases, polygyny results when established colonies readopt new queens back

into their natal nest. This behavior could be exploited by "parasitic" queens who secured acceptance into alien colonies. Frequent transport of brood between nest units of a polydomous colony could evolve into slave-raiding if this habit was extended to unrelated colonies.

While Buschinger's (1970) account of the origin of parasitic colony founding seems reasonable, it is more difficult to imagine how the transport of brood among nests belonging to the same colony could evolve into more violent acts of slave-raiding. Thus, Alloway (1980) and Stuart and Alloway (1982, 1983) extended this hypothesis to include territoriality in addition to polygyny and polydomy. Slave-raiding behavior could have evolved from frequent territorial battles among polygynous and polydomous ants that included the robbing of brood from the weaker colony.

Support for the importance of territoriality in the evolution of slave-making is indeed strong. Territoriality with brood raiding is common in *Leptothorax* species, which are common hosts to obligate slave-makers. In fact, Wilson (1975) showed that larger colonies of *L. curvispinosus* (which are hosts to obligate slave-makers) attacked smaller colonies and brought brood back to their nest where some eclosed and were incorporated in the colony. *Leptothorax ambiguus* (also hosts of obligate slave-makers) colonies likewise attacked smaller *L. curvispinosus* colonies and allowed some adults that emerged from raided pupae to survive in their nest for a few days. Alloway (1980) later discovered mixed colonies of *L. ambiguus*, *L. curvispinosus*, and *L. longispinosus* in Canada and the northern United States. Moreover, he was able to produce such mixed colonies in the laboratory by placing colonies close enough that they would engage in territorial raids. Finally, Stuart and Alloway (1983) demonstrated that the slave-raiding behavior of the obligate slave-

maker, *Harpagoxenus canadensis*, closely resembled the territorial behavior of its host *L. muscorum*.

Pollock and Rissing (1989) proposed a hypothesis that emphasized territoriality in the evolution of slave-making behavior, but also argued that slave-making behavior is only possible when "host" colonies are polydomous. Nests of polydomous species, they argued, are replaceable because colonies frequently emigrate, establishing new nests spontaneously. For an evolving slave-maker, host nests would be a renewable resource, thereby allowing for specialization in brood raiding. Otherwise, "slave-makers" would quickly deplete their potential slave supply. Furthermore, queen movement between polydomous nest units could result in queens mistakenly entering a foreign nest, thus setting the stage for parasitic colony founding.

These hypotheses are not mutually exclusive, and all emphasize some combination of polygyny and polydomy as important precursors for the evolution of slave-making behavior. Yet, support for these hypotheses from analysis of social and colonial structure of slave-makers and their hosts is equivocal. Polygyny and polydomy are common, but not exclusive in both the formicini and formicoxenini. Hosts of the obligate slave-maker, *Protomagnathus americanus* are polygynous and polydomous (Foitzik & Herbers 2001a; Herbers & Stuart 1998), but most hosts of *Challepoxenus* and *Epimyrma* are monogynous and monodomous (D'Ettorre & Heinze 2001). Many hosts of the formicine slave-makers are polygynous and polydomous (Pamilo 1982) , but others are monogynous and presumably (*F. subanescens, F. argentea,* and *F. hewitti*, Bennett 1987). Most slave-makers are themselves monogynous, with a few exceptions particularly in the formicines (Buschinger 1990; D'Ettorre & Heinze 2001).

My research investigates the social and colonial structure of the obligate slave-maker *P. breviceps* and two hosts, *F. subsericea* and *F.* near *argentea*. *P. breviceps*, like other slave-makers, was monogynous. *Formica.* near *argentea* was polygynous and polydomous, but *F. subsericea* was monogynous and presumably monodomous. Although my data therefore do little to support or refute current hypotheses on the evolution of slavery, such data are important for determining which traits are ancestral in clades that evolved slave-making behavior should phylogenetic data become available in the future.

Analysis of complex systems including multiple slave-makers and hosts

The other major objective of my research was to provide data on complex systems of slave-makers and hosts that include interactions among multiple species. Parallels between slave-making ants and avian brood parasites (Davies *et al.* 1989) have prompted a recent surge in research on coevolution between slave-makers and their hosts. Like avian brood parasites, slave-making ants are closely related to their host species and have similar generation times and population sizes (Davies *et al.* 1989). Thus, slave-making ant systems are especially appropriate for applying models of stepwise coevolution, where hosts evolve resistance to parasite adaptations for manipulation, and vice versa (Blatrix & Herbers 2003; Davies *et al.* 1989; D'Ettorre & Heinze 2001; Foitzik *et al.* 2001; Foitzik *et al.* 2003; Foitzik & Herbers 2001b; Hare & Alloway 2001; Zamora-Munoz *et al.* 2003). Recent research has revealed clear evidence of ongoing arms races between slave-making ants and their hosts, which vary in intensity across different populations of a particular host-parasite pair (Blatrix & Herbers 2003; Foitzik *et al.* 2001; Foitzik *et al.* 2003; Foitzik & Herbers 2001b; Hare & Alloway 2001; Zamora-Munoz *et al.* 2003). Host defenses

can in some cases arise from relatively simple shifts in behavior that make colonies more resistant to slave-raids (Foitzik *et al*. 2001).

To date, most studies of coevolution between slave-making ants and their hosts have considered only a particular host-parasite pair, or sometimes one parasite that enslaves more than one host. In fact, populations often include multiple slave-makers who enslave the same hosts, especially in populations of formicine slave-makers in North America. The evolution of host defenses should be more difficult when hosts are enslaved by more than one parasite, particularly if optimal defensive strategies differ for different species of slave-makers. My study system, which includes one obligate slave-maker, two facultative slave-makers, and two hosts, provides a unique opportunity to compare the impacts of different slave-makers (both facultative and obligate) on their host populations. Since obligate slave-makers are completely dependent on their hosts, we might expect that they have evolved to minimize negative impacts on host fitness. My work reveals that slave-makers have different slave-raiding styles, with facultative slave-makers being generally more aggressive during raids. Differences in raiding style should make it difficult for hosts to evolve defenses that are efficient against multiple slave-makers, and, in fact, I found no evidence for hosts that were resistant to more than one slave-maker. I also provide evidence that slave-maker colony raiding activities are spatially restricted to exclusive areas that overlap minimally with areas utilized by neighboring slave-makers. This is, to my knowledge, the first analysis of interactions among slave-makers themselves.

Future directions

What I consider to be one of the most promising areas of future research on slave-making ants is the possibility of cryptic speciation or host race formation due to colony level host specificity. Although *P. breviceps* enslaved both *F. subsericea* and *F.* near *argentea* in my study population, individual colonies specialized on one host. Host specificity has also been reported for other slave-makers including *P. rufescens* (Mori *et al.* 1994), *P. lucidus* (Goodloe & Sanwald 1985; Goodloe *et al.* 1987), and *Chalepoxenus muellerianus* (Schumann & Buschinger 1995). New queens of these species almost exclusively usurp colonies of the host species present in their natal nest, and workers raid colonies of the host species present when the slave-maker nest was initiated.

Host specificity in slave-making ants strongly resembles the process by which the common cuckoo forms host-specific races (Gibbs *et al.* 2000; Marchetti *et al.* 1998). Cuckoos parasitize other species by laying eggs in their nests. They parasitize multiple hosts, and the species is divided in host specific races (gentes); females of each race lay eggs that closely resemble the eggs of its host. Gentes are restricted to female lineages and speciation is prevented because male cuckoos mate with females from different gentes (Gibbs *et al.* 2000; Marchetti *et al.* 1998). Host specificity in slave-making ants also appears to be maternally transmitted because new queens are more successful in usurping host colonies of the host species present in the natal nest. Moreover, this appears to be genetically mediated because slave-makers tend to have hydrocarbon profiles (which are used in species and colony recognition) that are typical of their host species (D'Ettorre *et al.* 2002). If mating is restricted to males and females from the same host lines, then lines would

become genetically isolated and speciation should ensue. This would be particularly likely if the recognition of suitable mates relies in part on hydrocarbon profiles.

This possibility could be tested easily by comparing levels of genetic differentiation for different classes of genetic markers. If maternal lineages are host specific, we would expect strong genetic differentiation for mitochondrial markers among sympatric slave-makers that enslave different hosts. If nuclear markers show similarly high levels of differentiation then we would have evidence for genetic isolation of host strains and incipient speciation. However, if nuclear markers showed low levels of differentiation then we could assume that males mate with females from all host lines, and host specificity is limited to maternal lineages.

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Chapter II

Slave-raiding behavior of one obligate (*Polyergus breviceps*) and two facultative (*Formica puberula* and *F. gynocrates*) slave-making ants

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ABSTRACT

Slave-making ants are social parasites that exploit the labor of workers from closely related host species by enslaving them in the slave-maker nest. They vary in their degree of specialization, ranging from obligate slave-makers that cannot survive without slaves, to facultative slave-makers that must initiate colonies dependently, but can mature to become independent. Our study system included one obligate slave-maker, *Polyergus breviceps*, two facultative slave-makers, *Formica puberula* and *F. gynocrates*, and two hosts, *F. subsericea* and *F. near argentea*. We observed all slave-raids conducted during two raiding seasons by seven *P. breviceps* colonies, two *F. puberula* colonies, and two *F. gynocrates* colonies. We found intercolonial differences among *P. breviceps* colonies for the number of raids conducted and for average raid distance. Although we predicted that *P. breviceps* colonies would raid more often than *F. puberula* or *F. gynocrates* colonies, we found that some colonies of the facultative slave-makers raided as frequently or more frequently than *P. breviceps* colonies. Thus, the impact of facultative slave-makers on host populations is likely to be similar to that for obligate slave-making ants.

INTRODUCTION

Ant slavery is a form of social parasitism in which colonies of one species exploit the labor of workers from closely related species by incorporating them into the slave-maker colony as slaves. Slave-making is rare in ants. While only about 50 of 10,000 ant species are slave-makers, this behavior has evolved at least 10 times independently, and is particularly common in the myrmicine tribe Formicoxenini and the formicine tribe Formicini (D'Ettorre & Heinze 2001).

The life-history of slave-making ants includes two key components: parasitic colony founding by new queens and slave-raiding by workers. A newly mated queen is unable to establish colonies independently and instead must enter a free-living host nest, execute the resident queen(s), and either expel or gain acceptance from the host workers. The invading queen then begins laying eggs that are cared for by the host workers. Each year, slave-making workers raid neighboring host colonies to steal developing brood, which eclose in the slave-making nest and perform normal worker duties.

Slave-making ants are either obligate or facultative depending on the degree to which they depend on their hosts. Obligate slave-makers often have morphological and behavioral specializations that enhance their efficiency as slave-makers while compromising their ability to perform normal worker tasks. Thus, they are completely dependent on slaves for survival. In contrast, facultative slave-maker workers often retain a normal behavioral repertoire and colonies can mature to become independent.

Our study system included one obligate slave-maker, *Polyergus breviceps* and two less specialized slave-makers from the *Formica sanguinea* group (*F. gynocrates* and *F. puberula*). The genus *Polyergus* includes five highly specialized obligate slave-makers: *P. samurai* (Japan), *P. nigerrimus* (former Soviet Union), *P. rufescens* (Europe), *P. lucidus* (eastern North America), and *P. breviceps* (western North America). The *Formica sanguinea* group consists of 12 species, one in Eurasia and 11 in North America, which are generally considered to be facultative slave-makers (Holldobler and Wilson, 1990). However, at least one North American species, *F. subintegra*, is regarded as an obligate slave-maker (Savolainen & Deslippe 1996; Savolainen & Deslippe 2001).

Our study had two main objectives. First, we wanted to compare slave-raiding behavior of *P. breviceps*, *F. gynocrates* and *F. puberula* with data from other studies on *Polyergus* and *Formica sanguinea* group slave-makers. Several studies of slave-raiding behavior have been conducted on *P. breviceps* (Topoff *et al.* 1985a; Topoff *et al.* 1985b; Wheeler 1916), *P. lucidus* (Coolkwait & Topoff 1984; Talbot 1967), *P. rufescens* (reviewed by Mori *et al.* 2001), and *P. samurai* (Hasegawa & Yamaguchi 1994; Hasegawa & Yamaguchi 1995). Because slave-makers in different populations often enslave different hosts, collecting data from more populations is critical to gaining a better understanding of the life history of these ants. Indeed, our current knowledge of slave-raiding behavior of *P. breviceps* comes almost exclusively from two populations in Arizona, USA (Topoff *et al.* 1985a; Topoff *et al.* 1985b).

In contrast to *Polyergus*, only a few studies have been conducted on *Formica sanguinea* group slave-makers (Czechowski & Rotkiewicz 1997; Mori *et al.* 2000; Topoff & Zimmerli 1991). Furthermore, to our knowledge, no studies have been

conducted on *F. gynocrates* or *F. puberula*. Because facultative slave-makers might represent a crucial link in the evolution of obligate slave-making behavior (Mori *et al.* 2001), the collection of data on more facultative slave-makers is critical.

Our second objective was to compare slave-raiding behavior of the three slave-makers in our study system to ascertain differences between obligate and facultative slave-makers. Although *Polyergus* often occur in sympatry with *Formica sanguinea* group slave-makers, to our knowledge, no comparative studies of slave-raiding behavior in populations where they co-occur exist. We might expect obligate slave-makers to raid more often than facultative slave-makers because they are completely dependent on slaves for survival. However, a recent field study of the Eurasian facultative slave-maker *F. sanguinea* revealed that slave-raiding can be quite frequent, though slave-raiding frequencies were not compared with obligate slave-makers from the same population (Mori *et al.* 2000). Our system therefore provides a unique opportunity to compare raiding frequencies and behavior of obligate and facultative slave-makers that enslave the same hosts.

METHODS

We studied slave-raiding behavior of three slave-makers, *Polyergus breviceps*, *Formica gynocrates*, and *Formica puberula* on two host species, *F. subsericea* and *F.* near *argentea* in the foothills of the Rocky Mountains, at an elevation of approximately 2000 m, 36 km northwest of Fort Collins, Colorado USA. *P. breviceps* can enslave both hosts, but individual colonies specialize on only one host. *F. gynocrates* enslaves *F.* near *argentea* while *F. puberula* enslaves *F. subsericea*. The study site included open meadows and sparsely spaced ponderosa pine, spruce and fir trees. We found colonies of slave-makers and hosts nesting

under rocks and stones that were abundant on southern and south-eastern exposed slopes.

Behavioral observations

Our study was conducted over two raiding seasons (July-August, 2002, and 2003). We monitored eight *P. breviceps* colonies, two *F. gynocrates* colonies, and two *F. puberula* colonies every day during the slave raiding season from 1300 hours (slave raids are conducted in the afternoon) until slave raids ended in the evening, usually before 2000 hours. We recorded all slave raids for each monitored colony and the location of host colonies using global positioning systems (GPS). We generated maps with Arcview software.

Data Analysis

We made comparisons among *P. breviceps* colonies for the number of raids conducted by a colony, the total number of raids conducted against the same host colony, failure rates (failed raids were those that did not result in brood capture), and average raid distance. We used general linear models (GLM) with colony, year and a colony*year interaction included in the model. All response variables were log transformed to normalize the data, except for the number of raids conducted by a colony, which was normally distributed. The interaction term and the year term were eliminated from the model if not significant (P= 0.10). Unfortunately, small sample sizes for *F. gynocrates* and *F. puberula* limited our ability to make statistical comparisons among the three slave-makers for the number of raids conducted by a colony, the total number of raids conducted against the same colony, failure rates, and average raid distance.

We used correlation analysis to test whether raiding distance increased for colonies as the raiding season progressed and whether the number of raids was related to raid distance for each colony during each year. To determine whether there was a general trend for these correlations to be positive we compared our observations with a binomial probability for the appropriate sample size. We also looked to see whether the average raid distance was positively correlated with the number of raids conducted by a colony.

RESULTS

Raid Descriptions

We observed a total of 175 slave raids against 143 different colonies over two field seasons (74 in 2002 and 101 in 2003). Raids were either simple (a raiding column attacked one host colony), continuous (a column attacked one colony then continued in same direction to another colony), simultaneous (two columns attacked two different colonies), or repeated (raiders returned repeatedly to same colony on the same day (Hasegawa & Yamaguchi 1994). Although the first raids occurred on nearly the same date in 2002 and 2003 (July 1st and 2nd respectively), the duration of the raiding season was longer in 2003, spanning into mid August (Figure 2.1a & b). A maximum of six of the 11 slave-maker colonies was active on a given day during each year. The peak periods of raiding activity occurred at nearly the same time each year, but the peak period was maintained for a longer time in 2003 (Figure 2.1a & b). Table 1 summarizes data on the date of first and last raids for each colony during each year, and shows that active raiding periods for the three slave-makers clearly overlap. Raids occurred on all days during the active period unless it rained; raids also occurred on overcast days when it did not rain.

P. breviceps raids were characteristic of *Polyergus* raids described by others (reviewed by Mori *et al.* 2001). Activity of *P. breviceps* workers commenced in the late afternoon as individuals began circling around the nest area, and raids began when scouts returned after locating a host colony. Raiders left the nest en masse, forming a dense raiding column that moved toward the host nest. When raiders arrived at the target colony they immediately searched for entrances to the nest and penetrated the host colony when an entry point was found. Raiders emerged within minutes and carried pupae or large larvae back to the home nest via the same route.

Formica puberula and *F. gynocrates* raids were similar to those described for closely related *F. sanguinea* (reviewed by Mori *et al.* 2001). The beginning of a slave raid was less conspicuous than that for *P. breviceps*, as raiders left in platoons rather than en masse. Thus, the buildup of raiders at the target host nest was more gradual. Raiders often spent considerable time digging near the host colony before finally penetrating and stealing brood. Raiders stole mostly large larvae and pupae, but were also seen carrying adult ants in the pupal position and, on one occasion, alate females.

Comparison of raid characteristics

Data on the number of raids conducted by each slave-maker colony are summarized in Table 2.2. Although we found no intercolonial differences among *P. breviceps* colonies for the number of different colonies raided (GLM, $p= 0.135$), percentage of raids that were performed on the same colony (GLM, $p= 0.164$), or the rate of failure (GLM, $p= 0.952$), colonies clearly differed in the total number of raids (GLM, $p= 0.019$).

Lack of power because of low sample sizes precluded statistical comparisons between slave-maker species. Nonetheless, it is clear that raiding frequencies for *F. gynocrates* and *F. puberula* are similar to those for *P. breviceps*. In fact, Pb 2 was the only *P. breviceps* colony that raided more times over the two years than Fp 2 or Fg 5 (36 times compared with 28 and 26 respectively). Four colonies (Pb 4, Pb 12, Fp 1, and Fg 12) did not raid successfully in one of the two years, even though slave-makers were still present in the nest.

Raid distances

Data on mean raid distances for each colony are presented in Table 2.3. Intercolonial comparisons among *P. breviceps* colonies revealed strong differences in average raid distance, with means ranging from 4 m to 19.1 m (GLM, $p= 0.006$). Moreover, average raid distance was positively correlated with the number of raids conducted by a colony though this was not statistically significant probably because of low power ($r=0.60$, $p= 0.155$). Although small sample sizes precluded comparisons among slave-maker species, one *F. puberula* colony (Fp 2) averaged the longest raid distance for both years.

We also found evidence that raid distance increased as the raiding season progressed. When we considered all data (all three slave-makers over both years) 12 of 16 correlation coefficients for raid distance vs. raid number were positive, which corresponds to a cumulative binomial probability of 0.038 (Table 2.4). When we considered *P. breviceps* and the *Formica* slave-makers separately, cumulative binomial probabilities were 0.033 and 0.813 respectively, suggesting that this pattern might be unique to *P. breviceps*. However, because data were limited to only three *Formica* slave-maker colonies, we cannot make a reliable determination.

DISCUSSION

Our findings corroborate earlier work suggesting that facultative *Formica* slave-makers can raid as frequently as obligatory slave-makers such as *P. breviceps* (Mori *et al.* 2000). Indeed, colonies Fp2 and Fg 5 raided more frequently over two seasons than all but one *P. breviceps* colony (Pb 2). Moreover, we did not find colonies of either *Formica* slave-maker without slaves, even though all colonies did not raid during both seasons. This, coupled with a relatively high raiding frequency, suggests that *F. puberula* and *F. gynocrates* may, in fact, be more similar to obligate slave-makers such as *P. breviceps* than previously assumed. Moreover, the impact of facultative slave-makers on their hosts is likely to be as high as that for obligate slave-makers.

In general, our behavioral observations of *P. breviceps* raids are similar to descriptions for other *Polyergus* (Coolkwait & Topoff 1984; Hasegawa & Yamaguchi 1994; Hasegawa & Yamaguchi 1995; Mori *et al.* 2001; Topoff *et al.* 1985a; Topoff *et al.* 1985b). *Polyergus breviceps* apparently raids less often than other *Polyergus* species, as our observations are on par with other studies of *P. breviceps* (Topoff *et al.* 1985a; Topoff *et al.* 1985b), but far below raiding frequencies reported for *P. lucidus* (50 raids for one colony; Coolkwait & Topoff 1984), and *P. samurai* (32-63 raids; Hasegawa & Yamaguchi 1995). The reason for this is unclear, but could be related to differences in host colony size. If *P. breviceps* host colonies are larger and raiders retrieve more pupae from a single nest, then fewer raids would be necessary for a colony to maintain an adequate slave pool. A comparison between *P. breviceps* and *P. lucidus* of the number of pupae retrieved per raid suggests that this

is the case as *P. breviceps* typically retrieved far more pupae in a single raid than *P. lucidus* (Coolkwait & Topoff 1984; Topoff *et al.* 1985a; Topoff *et al.* 1985b).

We found strong differences among *P. breviceps* colonies for average distance to target host colonies. Hasegawa and Yamaguchi (1995) also reported intercolonial differences for average raid distance in *P. samurai.* Average distance traveled to target nests over the two seasons in our study was 12.3 m., which is similar to that found for *P. samurai* (11.4 m Hasegawa & Yamaguchi 1995) but much lower than averages of 34 m and 49 m found for *P. breviceps* in two other locations (Topoff *et al.* 1985a; Topoff *et al.* 1985b).

It is unclear what factors underlie differences in average raid distances. Hasegawa and Yamaguchi (1995) suggested that differences in average distance to target nests for *P. samurai* colonies was negatively related with host density and positively related with slave-maker colony size. Although we have no data on host nest density or colony size, we did find a relatively high correlation between average raid distance and the number of raids conducted by a colony ($r=0.60$), though the result was not statistically significant ($\alpha= 0.05$). We could expect a positive correlation between raid distance and raid number because we also found that slave-maker colonies increased raiding distance as the raiding season progressed. Thus, colonies that raided only a few times were likely to raid closer to their home nest, while colonies that raided more often began near their home nest but moved outward as the season progressed, probably when local resources were depleted. More studies are needed to further elucidate the relationships among colony size, average raid distance, and host nest density. Although few studies have been conducted on slave-raiding behavior of *Formica sanguinea* group slave-makers, our observations of *F. puberula* and *F. gynocrates* are similar to other reports in the literature

(Czechowski & Rotkiewicz 1997; Mori et al. 2000; Topoff & Zimmerli 1991). Raids were less organized than those for *P. breviceps* as raiders gradually left the home nest rather than en masse. Moreover, raids were characterized by digging and slow entry into the target host nest. On several occasions we observed raiders carrying reproductive pupae and even alates on one occasion. This is probably common during *Formica* slave-maker raids, as Mori et al. (2000) also observed such predatory behavior in *Formica sanguinea*.

One *F. gynocrates* colony and one *F. puberula* colony did not raid in one of the two years of study. Colony Fg 12 raided only one time during 2002 and did not raid successfully in 2003, though one attempt failed. Colony Fp 1 did not raid in 2002 but did raid five times in 2003. Since these are facultative slave-makers, this result was not surprising, but it is unclear what factors determine how often a colony raids. We do not know whether these are large, old colonies that no longer need slaves, or young colonies that have not started slave-raiding.

To our knowledge there are no published data with which we can compare our data on average raid distances for *F. puberula* and *F. gynocrates.* One *F. puberula* colony conducted longer raids than any other colony of any species at our site, but our sample sizes are too small to make generalizations. There was also a suggestion that *F. puberula* and *F. gynocrates* do not go on longer raids as the raiding season progresses, but again, this might be an artifact of low sample sizes. In fact, the two strongest positive correlations between raid distance and raid number were for colonies Fp 1 and Fg 5 (2003), suggesting that, at least in some years, raid distance increases as the season progresses.

We observed one major difference in the slave-raiding behavior of *F. puberula* and *F. gynocrates* when compared to *F. sanguinea.* Mori et al. (2000)

reported that slaves routinely participated in *F. sanguinea* slave-raids, but we did not see slaves participate in *F. puberula* or *F. gynocrates* raids. On one occasion a few slaves followed along a *F. gynocrates* raiding trail, but were not seen carrying brood back to the slave-maker nest. Regardless, slave participation was certainly not common for either *F. gynocrates* or *F. puberula*.

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Colony	Host species	Date of first raid 2002	Date of last raid 2003	Date of first raid 2003	Date of first raid 2003
Pb 2	*F.* near *argentea*	7/1	7/19	7/4	8/6
Pb 3	*F. subsericea*	7/1	7/20	7/20	8/17
Pb 4	*F.* near *argentea*	7/14	7/14	--	--
Pb 6	*F. subsericea*	7/9	7/27	7/13	8/14
Pb 8	*F.* near *argentea*	7/1	7/16	7/3	7/13
Pb 10	*F.* near *argentea*	7/7	7/20	7/2	7/21
Pb 12	*F.* near *argentea*	--	--	7/9	7/16
Fp 1	*F. subsericea*	--	--	7/24	8/6
Fp 2	*F. subsericea*	7/1	7/28	7/12	7/28
Fg 5	*F.* near *argentea*	7/1	7/30	7/14	8/2
Fg 12	*F.* near *argentea*	7/22	7/22	--	--

Table 2.1. Dates of the first and last raids conducted by *P. breviceps*, *F. puberula*, and *F. gynocrates* colonies during 2002 and 2003.

Colony	Host type	Total successful raids 2002	Different colonies raided 2002	Failed raid attempts 2002	Total successful raids 2003	Different colonies raided 2003	Failed raid attempts 2003
Pb 2	*F.* near *argentea*	12	10	2	24	19	8
Pb 3	*F. subsericea*	9	6	4	12	6	10
Pb 4	*F.* near *argentea*	1	1	1	0	0	--
Pb 6	*F. subsericea*	8	5	3	17	16	6
Pb 8	*F.* near *argentea*	6	6	1	6	6	4
Pb 10	*F.* near *argentea*	7	7	1	9	7	3
Pb 12	*F.* near *argentea*	0	0	--	4	3	1
Fp 1	*F. subsericea*	0	0	--	5	5	1
Fp 2	*F. subsericea*	12	12	4	16	8	4
Fg 5	*F.* near *argentea*	18	17	1	8	7	1
Fg 12	*F.* near *argentea*	1	1	1	0	0	1

Table 2.2. Total number of successful raids, number of different colonies raided, and number of failed raid attempts by slave-maker colonies during 2002 and 2003 raiding seasons.

Colony	Host type	Average raid distance 2002 ± s.e. (meters)	Distance range 2002 (meters)	Average raid distance 2003 ± s.e. (meters)	Distance range 2003 (meters)
Pb 2	*F.* near *argentea*	12.1 ± 2.1	3.7—22	12.2 ± 1.2	3.6—21.2
Pb 3	*F. subsericea*	14.7 ± 1.8	8.1—20.1	10.9 ± 0.7	7.8—15.6
Pb 4	*F.* near *argentea*	8.1 **	--	--	--
Pb 6	*F. subsericea*	8.4 ± 1.8	1.0—12.6	7.3 ± 1.7	10.2—38.0
Pb 8	*F.* near *argentea*	15.0 ± 2.3	5.2—22.0	10.2 ± 3.1	3.7—22.0
Pb 10	*F.* near *argentea*	19.1 ± 3.4	5.6—29.1	12.6 ± 2.2	5.4—25.0
Pb 12	*F.* near *argentea*	--	--	4.0 ± 1.2	1.0—6.7
Fp 1	*F. subsericea*	--	--	8.7 ± 1.4	5.9—14.0
Fp 2	*F. subsericea*	24.5 ± 4.1	4.4—35.5	38.7 ± 3.7	5.2—54.9
Fg 5	*F.* near *argentea*	12.9 ± 1.3	8.9—25.6	13.8 ± 2.8	6.2—24.0
Fg 12	*F.* near *argentea*	13.9 **	--	--	--

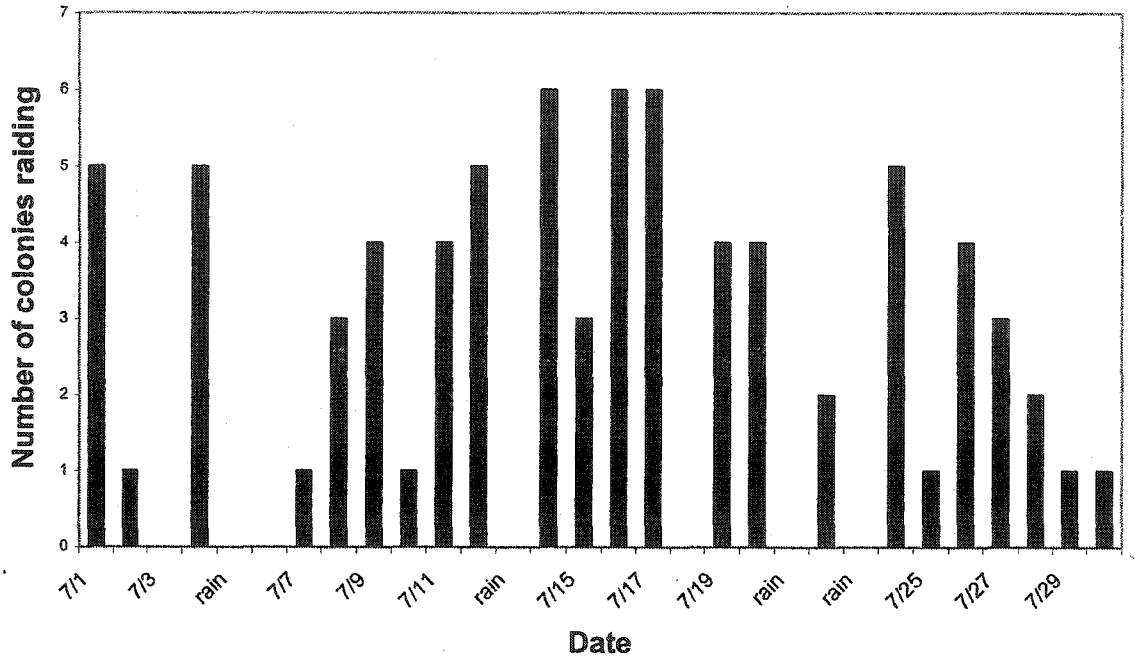
**Colony only conducted one raid

Table 2.3. Average raid distance and distance range for slave-maker colonies in 2002 and 2003.

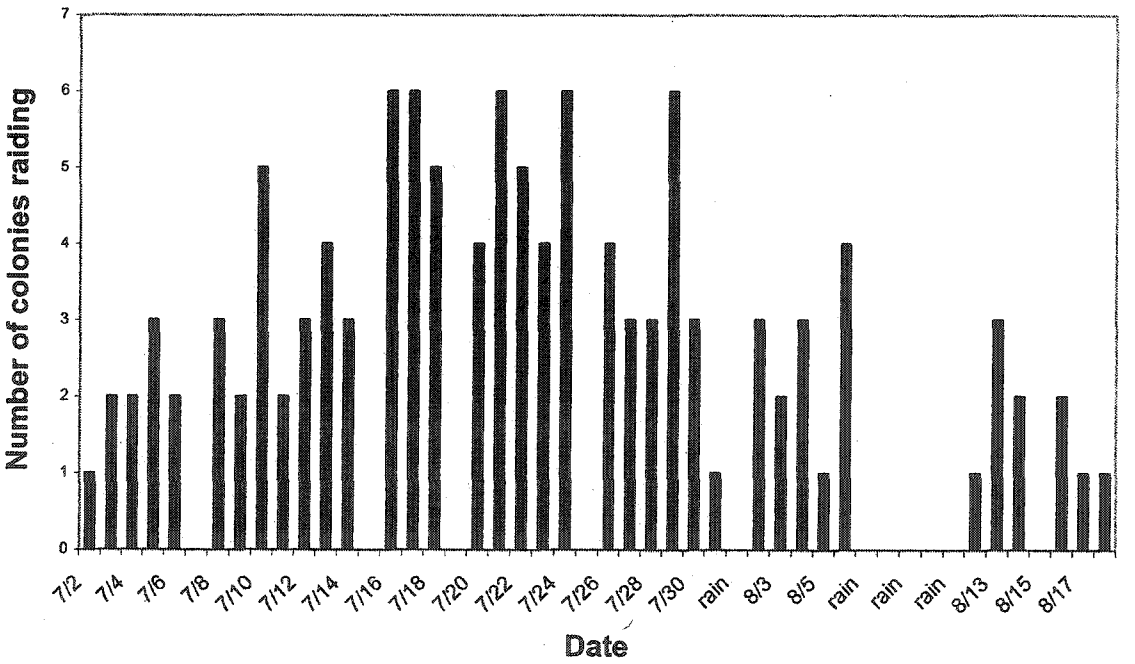
Colony	r—2002 (N)	r—2003 (N)	Binomial probabilities	
Pb2	0.064 (10)	0.615 (24)	All data	0.038
Pb3	0.728 (8)	0.054 (11)	*P. breviceps*	0.033
Pb6	0.704 (8)	0.324 (17)	*Formica*	0.813
Pb8	-0.117 (6)	0.624 (6)		
Pb10	0.668 (6)	0.192 (9)		
Pb12	--	-0.579 (4)		
Fp1	--	0.895 (5)		
Fp2	-0.044 (9)	0.772 (16)		
Fg5	-0.068 (13)	0.960 (7)		

Table 2.4. Correlation coefficients for raid distance and raid number for each slave-maker colony during both years of study. The data were modeled as a binomial assuming correlations can be positive or negative.

A.



B.



Chapter III

Coevolution in a complex system involving three slave-making ants and two hosts

by

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ABSTRACT

Slave-making ants are social parasites that raid neighboring host colonies, steal developing worker brood, and incorporate them into the slave-maker colony as slaves. Recent studies on coevolution between slave-makers and their hosts reveal that the intensity of coevolutionary arms races varies across populations of a particular host-parasite pair. To our knowledge, no studies have investigated coevolution in more complex systems that involve multiple slave-makers and multiple hosts. In this study we report interactions of coevolutionary significance in a complex system involving three slave-makers (*P. breviceps*, *F. puberula*, and *F. gynocrates*) and two hosts (*F. subsericea* and *F.* near *argentea*). Specifically, we had five objectives: (1) To determine whether slave-maker colonies avoid raiding in areas that are used by neighboring slave-maker colonies, (2) to compare estimates of slave-raiding frequency from genetic analyses of slaves with empirical observations, (3) to compare the aggressiveness of the three slave-makers during slave-raids, (4) to compare the aggressiveness of hosts during slave-raids, (5) to determine if slave-raids negatively impact host fitness, and whether host fitness effects differ between slave-maker species. We found that slave-maker colonies avoided raiding areas used by neighboring colonies, probably because fighting when raiding columns intersect is costly. Our estimates of slave-raiding frequency from genetic data on slaves were consistently lower than actual observations; we discuss potential explanations for this pattern. We found clear differences in aggressiveness during slave-raids that are likely attributed to differences among slave-makers rather than hosts. Finally, we found that slave-raids generally negatively impacted host fitness

(except for *P. breviceps* raids against *F. subsericea*), but no patterns of universal parasite prudence or host resistance were evident.

INTRODUCTION

Slave-making ants are social parasites that exploit the labor of workers from closely related host species by incorporating the slave workers into the slave-maker colony. The highly specialized lifestyle of these ants makes them uniquely suited to study a variety of questions ranging from the evolution of sex allocation strategies (D'Ettorre & Heinze 2001) to host-parasite coevolution (Blatrix & Herbers 2003; Davies *et al.* 1989; Foitzik *et al.* 2003; Foitzik & Herbers 2001; Hare & Alloway 2001; Zamora-Munoz *et al.* 2003). The parasitic lifestyle of slave-making ants combines parasitic colony founding by queens with slave-raiding by workers. Slave-making queens are unable to independently initiate a new colony and must instead usurp a free-living host colony by killing the resident queen(s) and gaining acceptance from the host workers. Slave-maker queens then produce their own workers, which replenish the slave supply each year by stealing larvae and pupae from neighboring host colonies. Slave-making ants represent varying degrees of specialization, from obligate slave-makers being entirely dependent on slaves to facultative slave-makers which initiate colonies dependently, but can mature to become independent. Despite the apparent complexity of this kind of host-parasite interaction, slave-making behavior has evolved independently at least 10 times in the subfamilies, Myrmicinae and Formicinae, with particularly high incidence in the tribes Formicoxenini and Formicini.

Recent interest in applying host-parasite coevolutionary paradigms to slave-making systems has provided valuable insights into their dynamic nature (Blatrix & Herbers 2003; Foitzik *et al.* 2003; Foitzik & Herbers 2001; Hare & Alloway 2001; Zamora-Munoz *et al.* 2003). These studies provide clear evidence that slave-makers and their hosts engage in coevolutionary arms races, but the intensity of the arms

race varies across populations of a particular host-parasite pair. Moreover, the presence of additional host species seems to dilute the impact of slave-makers on their hosts (Blatrix & Herbers 2003; Foitzik *et al.* 2003), and could therefore disrupt a potentially tight coevolutionary arms race for a particular host-parasite pair (Thompson 1999). Futhermore, we can imagine pairwise interactions would be further destabilized by the presence of additional parasites, particularly if optimal host defenses are different for each parasite. However, to our knowledge, no studies on coevolution between slave-making ants and their hosts have considered more complex systems that involve multiple parasites enslaving multiple hosts.

Our interest in this study was to identify interactions that have coevolutionary significance in a complex system involving three slave-makers and two hosts. Formicine slave-makers often occur in sympatry and they enslave multiple host species from the genus *Formica*. In our study site, two host species, *F. subsericea*, and *F.* near *argentea*, are parasitized by the highly specialized, obligate slave-maker *Polyergus breviceps*, and two *Formica sanguinea* group slave-makers, *F. puberula* and *F. gynocrates*. The *Formica sanguinea* group consists of 12 species, which range from facultative to obligate slave-makers (Hölldobler & Wilson 1990; Savolainen & Deslippe 1996; Savolainen & Deslippe 2001); thus we expected a range of interactions within the parasite-host community.

Our study had five objectives. First, we were interested in interactions among slavemakers themselves. Slave-raiding, like foraging, takes place away from a colony's nest. Thus, interactions with other ants, both conspecific and heterospecific, are inevitable and potentially drive patterns of space-use by colonies. For slave-maker colonies, space-use might reflect competition for host colonies or the cost of fighting if raiding columns meet. Foraging columns of the army ant (which

are roughly analogous to raiding columns of slave-makers), *Eciton burchelli*, rarely cross, apparently because colonies reject areas that have been recently used by another colony (Hölldobler & Wilson 1990). Slave-maker colonies concentrate raids in certain compass directions while avoiding other areas (Coolkwait & Topoff 1984; Topoff *et al.* 1985a), but no studies have considered how this relates to the raiding of neighboring slave-maker colonies.

Second, we followed the methods of Foitzik and Herbers (2001) to estimate raiding frequency with genetic data on slave relatedness. Because slave-raids in our system can be easily observed, we were able to compare estimates of raiding frequency from genetic analyses and from direct observations of slave raids.

Third, we observed interactions during slave-raids seeking differences in aggression that might indicate different strategies of manipulation and resistance. Studies have suggested that more specialized slave-makers have evolved to minimize their impact on hosts (Hare & Alloway 2001). Indeed, the facultative slave-maker, *F. sanguinea* was more aggressive toward its host *F. cuniculara* in laboratory aggression tests than the obligate *P. rufescens* was toward the same opponent (Grasso *et al.* 1992). *Polyergus* raiders release "propaganda" chemicals that confuse host workers and reduce aggression during slave raids (reviewed by Mori *et al.* 2001). Similar chemicals have been found for some *F. sanguinea* group slave-makers (*F. subintegra* (Savolainen & Deslippe 1996) and *F. pergandei*; (Regnier & Wilson 1971), but are unlikely in others (*F. subnuda*, (Savolainen & Deslippe 1996). Because *P. breviceps* is a highly specialized obligate slave-maker, we expected that it would be less aggressive toward its hosts than either of the *Formica* slave-makers.

Fourth, we looked for differences in aggression levels between the two host species; such variation has been documented in other systems, and may be linked to social structure. Thus *F. subsericea,* which is monogynous, may be more aggressive against raiding parties than *F.* near *argentea*, which is polygynous, because monogynous colonies typically show higher intercolonial aggression than polygynous colonies (Breed & Bennett 1987; Hölldobler & Wilson 1977). Indeed, monogynous colonies of *L. acervorum* were better than conspecific polygynous colonies at defending their nests during attacks by the slave-maker, *Harpagoxenus sublaveis* (Foitzik *et al.* 2003).

Fifth, in order to asses the impact of slave-raids on host fitness, we compared the survival of colonies that were raided to those that were not. In some populations, *L. longispinosous* colonies do not survive *P. americanus* raids (Foitzik & Herbers 2001), though in other populations colonies survive at low frequency (Blatrix & Herbers 2003). Raided host colonies of Formicine slave-makers, however, can probably survive repeated raids (D'Ettorre & Heinze 2001). We were interested in whether survival rates differed between host species because of differences in counter-attack aggression. We also predicted that host colony survival varies with slave-maker species identity. In particular, we predicted that colonies raided by *P. breviceps* would have higher survival than those raided by the *Formica* slave-makers, because *P. breviceps* should have evolved mechanisms to reduce impacts on hosts.

METHODS

Study site

Our study population in the foothills of the Rocky Mountains, at an elevation of approximately 2000 m, is 36 km northwest of Fort Collins, Colorado USA. The system here is comprised of three slave-makers, *P. breviceps*, *F. gynocrates*, and *F. puberula*, and two host species, *F. subsericea* and *F.* near *argentea*,. The study site included open meadows and sparsely spaced ponderosa pine, spruce and fir trees. We found colonies of slave-makers and hosts nesting under rocks that were abundant on southern and south-eastern exposed slopes. We used survey equipment and global positioning systems (GPS) to generate Universal Transverse Mercator (UTM) coordinates of all host and slave-maker colonies we could find prior to our study, which were then mapped with Arcview software. We obtained UTM coordinates of any additional colonies that were found in 2002 and 2003.

Spatial pattern of slave raids

We monitored seven *P. breviceps* colonies, two *F. gynocrates* colonies, and two *F. puberula* colonies every day during the 2002 and 2003 slave raiding seasons from 1300 hours until slave raids ended in the evening, usually before 2000 hours. We recorded all slave raids for each monitored colony and the locations of raided host colonies were mapped with Arcview software. We used the minimum convex polygon method, which is traditionally used to determine the home range of mobile animals, to define the raiding space for each colony. Minimum convex polygons (MCPs) were constructed by connecting the outermost locations of slave-raids for each colony. We excluded raids that were at a distance from the slave-maker nest outside the third quartile of data. Thus, MCPs were constructed using 75% of slave

raids for each colony. Researchers commonly exclude some data points from home range analysis, but there is no generally accepted method for determining which points to exclude (Samuel & Fuller 1994). We chose to use 75% of raids to minimize the influence of rare long-distance raids on the shape of the MCP. The MCP method does not consider the underlying distribution of points within the polygon. Consequently, rare, distant raids would greatly increase the size of polygons, even though most points would be found within a smaller area. Because we hypothesized that space-use would be influenced by interactions with other colonies, we limited the size of polygons to distances where the majority of raids were concentrated for each colony.

Genetic analysis

In June 2002, we collected slaves from eight *P. breviceps* colonies, three *F. gynocrates* colonies, and three *F. puberula* colonies. In May 2003 we collected slaves from the slave-maker colonies that were active the previous summer (seven *P. breviceps* colonies, two *F. gynocrates* colonies, and one *F. puberula* colony). Ants were transported to the laboratory and immediately frozen at –70° C for genetic analysis. We used a salt-extraction protocol to extract DNA from slaves. We removed the abdomen from each ant before placing the remaining parts in 60 µl of Puregene cell lysis solution and grinding with a pestel. These tubes were then incubated at 65° C for 30-60 minutes. Following incubation, we added 20 µl of ammonium acetate to precipitate proteins. Tubes were placed in the freezer for at least 10 minutes, and then centrifuged at high speed for 6 minutes. We pipetted the supernatant into 65 µl 100% isopropanol and cooled the solution in the freezer for at least 10 minutes. We then centrifuged the tubes at high speed for 6 minutes, poured

off the isopropanol and stored tubes inverted for 24 hours to dry the DNA pellet. We resuspended the DNA pellet with 100 µl 1X TE buffer.

We genotyped up to 12 slaves from each colony using microsatellites isolated from other *Formica.* For *F. subsericea* we used two primers: FI21, originally developed for *F. paralugubrus* (Chapuisat 1996) and Fe 49, originally developed for *F. exsecta* (Gyllenstrand *et al.* 2002). For *F.* near *argentea* we used three primers: FI12 and FI29, both developed for *F. paralugubrus* (Chapuisat 1996) and Fe 49. We used PCR to amplify microsatellites by combining 0.5 µL DNA with 1.2 µL 10 X buffer (Promega), 25 mM $MgCl_2$, 4 mM dNTPs, 25 µM of each primer, and 5 units of Promega Taq DNA polymerase to form 12 µL reaction mixtures. All PCR programs consisted of an initial denaturing step of 95° C for 5 min. followed by 36 cycles of 95° C for 30 s, appropriate annealing temperature for 30 s, 72° C for 30 s, followed by a 5 min. extension step at 72° C. Annealing temperatures for the primers were as follows: FI12, 54° C; FI21, 50° C; FI 29, 50° C; Fe49 50° C. We separated samples on 6% polyacrylamide gels and visualized alleles by silver staining gels.

Estimation of raiding frequency

Genetic sampling of host colonies in the spring of 2002 showed that free-living *F. subsericea* colonies were monogynous and monandrous with a population-wide average relatedness of 0.72. *F.* near *argentea* colonies were polygynous with average relatedness of 0.39, and colonies were sometimes polydomous (see chapter 4). We used the program Relatedness v.5.0.8 (Queller & Goodnight 1989) to calculate relatedness of slaves in slave-maker nests (collected in Spring of 2003) using population allele frequency estimates from free-living host colonies in 2002. Overall, we found four alleles that were present in the slave population but not in the

source population. We treated these alleles as having a frequency of 0.0 for relatedness calculations; that these alleles were not sampled in the source population implies their frequency was quite low or that their natal colony had gone extinct. We compared the relatedness of slaves in slave-maker nests (collected in the spring of 2003) to the average relatedness of workers in free-living host colonies (collected in the spring of 2002) to estimate the number of nests represented by the slaves (Foitzik & Herbers 2001). These estimates assume that slaves live in slave-maker colonies for about one year and that raided colonies are represented equally in the slave pool.

Aggression

To compare aggressiveness during slave raids we observed interactions between the slave-making ants and hosts during randomly selected slave raids. We began recording aggressive interactions when the first host worker appeared outside the nest. From this point, we counted the number of aggressive interactions every five minutes for up to one hour (20 total observations or until the raid ended). We considered an encounter to be aggressive when ants were clasped together and clearly fighting. Encounters were rarely ambiguous; ants either interacted in a clearly aggressive manner, or avoided contact altogether.

We observed no aggressive interactions in a large number of raids, and consequently, could not normalize the data. Therefore, we split the analysis into two parts: (1) we used Fisher's exact tests to look for differences in the likelihood of there being at least one aggressive encounter during a raid, and (2) for those raids with aggression, we used mixed model ANOVAs (SAS, proc mixed) to compare the mean number of aggressive interactions per observation (log transformed). We performed

separate Fisher's exact tests to compare the likelihood of aggression between two observers, for *P. breviceps* on different hosts, and the three slave-makers. Our ANOVA model for *P. breviceps* on two different hosts included host species and examiner as fixed effects and colony nested within host species as a random effect. The model for comparison of the three slave-makers included examiner identity, slave-maker species, and a species by examiner interaction as fixed effects and colony nested within species as a random effect. The interaction term was eliminated from the model if it was not significant ($\alpha = 0.10$). We made *a priori* comparisons with the Lsmeans procedure in proc mixed.

Survival of raided colonies

We used data collected in July of 2003 and June of 2004 to compare survival of raided colonies and unraided colonies and to measure the relative impact of the different slave-makers on survival of host colonies. On July 11, 2003 we randomly selected 10 *F. subsericea* nests and 12 *F.* near *argentea* nests that had been previously marked but not raided during the 2002 raiding season to serve as controls. We excluded raided nests that could not be collected because they were under large boulders or because their exact location was uncertain. Using this procedure we selected 28 raided nests in 2003 and and 35 raided nests in 2004 to include in the analysis (summarized in Table 3.1). Unfortunately, we were unable to collect more control colonies in 2004 because most colonies at our site had been raided or collected the previous year. We used Fisher's exact tests to compare the likelihood of survival of raided and unraided colonies, and to test survival of colonies raided by different slave-makers. Small sample sizes limited statistical power so we report one-sided p-values that reflect probabilities associated with expectations from

our *a priori* hypotheses about survival. We pooled data over the two years to compare survivorship of colonies raided by different slave-makers, but we did not combine data for comparisons with controls since we did not collect control colonies in 2004.

RESULTS

Analysis of slave collections revealed that *P. breviceps* enslaved both host species at our site, but individual colonies specialized on only one host. *F. puberula* and *F. gynocrates* specialized on different hosts: *F. puberula* enslaved *F. subsericea*, and *F. gynocrates* enslaved *F.* near *argentea* (Table 3.2). In 2002, one *F. gynocrates* colony (Fg 5) also had a few *F. obscuriventris* slaves of the *Formica rufa* group, though we never observed slave-raids against colonies of this species.

Spatial pattern of slave raids

MCPs constructed from our raiding data reveal minimal overlap of raiding space among slave-maker colonies (Figure 3.1). Moreover, of the 34 raids that were excluded from MCPs because they exceeded the 75% distance criterion only five occurred within the MCP of another colony. It is unlikely that this was caused by overdispersion of slave-maker colonies, because many were close enough that raiding spaces could have overlapped. The only substantial area of overlap occurred between colonies P6 and P8; even so, the raiding activities of these colonies were for the most part temporally segregated. Over two years there were 30 days that included raids by these colonies, but both colonies raided on the same day only twice. MCPs range in size from 10 m^2 to 744 m^2 (Table 3.3), and there was a strong

correlation between the size of the MCP and the number of raids conducted by a colony over the two years of the experiment (ρ= 0.77, p= 0.016; Figure 3.2).

Estimation of slave-raiding frequency

The microsatellite markers that we used to genotype slaves were polymorphic, and therefore useful for calculating slave relatedness. Data on the number of alleles at each locus in both slave and source populations and frequencies of the most common allele at each locus are summarized in Table 3.4. In several cases, we found alleles among slaves that were not found in nearby free-living host colonies. These alleles either represented extremely rare variants or were signatures of previous lethal raids. Data on slave relatedness and estimations of the average number of raids conducted by each species are presented in Table 3.5. Estimation of the number of raiding events is similar over the two years for each species, with the exception of *F. puberula,* but the estimate from slaves collected in 2003 is from only one colony. Our data suggest that *P. breviceps* colonies that enslave *F. subsericea* raid more often than those enslaving *F.* near *argentea*, but differences could arise because few nests included *F. subsericea* slaves.

We are confident that we recorded every slave raid conducted by our focal nests throughout the summer of 2002, which allowed us to compare our estimates of slave-raiding events based on relatedness calculations to actual counts of the number of different colonies raided by slave-makers. In all cases, using genetic relatedness of slaves to calculate raiding frequency led to an underestimate of the true raiding frequency (Table 3.5). The discrepancy is particularly evident for *F. puberula* and *F. gynocrates*, with estimates from genetic data substantially lower than the actual number of raids (Table 3.5).

Aggression

We recorded at least one aggressive encounter in 28 of 53 raids. The identity of the examiner did not influence the likelihood of recording aggression, so we pooled data over observers to increase power (Fisher's exact, P= 0.300). Moreover, we combined all of our *P. breviceps* data, because there was no difference in the likelihood of aggression between colonies that raided different host species (Fisher's exact test, P= 0.718). To compare slave-makers we first tested whether the likelihood of aggression was the same when all three slave-makers were considered together. This test indicated differences among the slave-makers (Fisher's exact test, P= 0.056), so we performed more tests to determine which specific comparisons were different. There were no differences when we compared *F. puberula* and *F. gynocrates*, (Fisher's exact test, P= 0.378; Figure 3.3a), or when we compared *F. gynocrates* and *P. breviceps* (Fisher's exact test, P= 0.326; Figure 3.3a). However, *F. puberula* was more likely to be aggressive than *P. breviceps* (Fisher's exact test, P= 0.035; Figure 3.3a).

For raids that involved aggressive interactions we examined whether the species of slave-maker influenced aggression intensity. We again collapsed *P. breviceps* data over host species, because there was no difference in the number of aggressive encounters per observation for each host species (mixed model ANOVA, P= 0.212 for host species). The amount of aggression differed between slave-maker species, and examiners differered in the aggression observed (mixed model ANOVA, P= 0.003 for slave-maker species; P= 0.001 for examiner). Analysis of least square means (*a priori* comparisons) revealed specific differences among slave-makers, with no difference between *F. gynocrates* and *P. breviceps*,

(Lsmeans, *P*= 0.588; Figure 3.3b), but greater aggression by *F. puberula* than *F. gynocrates* (*P* = 0.003; Figure 3.3b) and *P. breviceps* (*P*= 0.001; Figure 3.3b).

Host Colony Survival

We found no difference in survival between *F.* near *argentea* colonies that were raided by *P. breviceps* and by *F. gynocrates* (Fisher's exact test, one-sided *P*= 0.802; Table 3.6). Although 83% of controls survived compared with 50% and 40% for colonies raided by *P. breviceps* and *F. gynocrates* respectively, differences were not statistically significant in 2003. To increase power, we combined data across all raided colonies, and found their survivorship of raided colonies was only 54% of that for control colonies (Fisher's exact test, one-sided P= 0.058; Table 3.6).

There was a suggestion that *F. puberula* raids on *F. subsericea* were more destructive than *P. breviceps* raids, yielding survivorships of 38% versus 68% respectively (Fisher's exact test, one-sided P= 0.094; Table 3.6). Unfortunately, small sample sizes in 2002 limited statistical power, and we detected no differences between raided and unraided colonies for either slave-maker (Fisher's exact tests, one-sided P= 0.846 for colonies raided by *P. breviceps* and one-sided P= 0.484 for colonies raided by *F. puberula*). However, the low survivorship for *F. subsericea* colonies raided by *F. puberula* when data from both years are combined suggests that these colonies probably suffered reduced survivorship compared to controls.

DISCUSSION

Our study provides new insight into the complexities of systems that involve multiple parasites interacting with multiple hosts. We identified a number of

interactions among parasites and hosts in our study population that have important evolutionary implications, and should help guide future studies on coevolution between formicine slave-makers and their hosts.

Other studies have suggested that slave-maker colonies raid more commonly in certain compass directions (Coolkwait & Topoff 1984; Topoff *et al.* 1985a), but no studies have considered whether this might be related to the raiding activities of neighboring slave-maker colonies. In fact, our results indicate that slave-making colonies confine raiding activities to exclusive areas that overlap minimally with areas used by neighboring colonies; furthermore, the ants made no distinctions between conspecific and heterospecific neighbors. The size of MCPs was positively correlated with the number of raids conducted by a colony, suggesting that colonies that raid more often travel further from their home nest to find host colonies, possibly because local resources become depleted.

Why colonies avoid raiding in areas used by other colonies is unclear. Such a distribution suggests that colonies might defend raiding territories, which would be particularly interesting because territoriality is implicated in the evolution of slave-making behavior (Alloway 1980; Pollock & Rissing 1989; Stuart & Alloway 1982; Stuart & Alloway 1983). However, it is difficult to imagine how such territories could be defended or maintained, at least for *P. breviceps*. We have observed *Formica* slave-makers working outside the nest, which suggests that they might also forage. If so, territories could easily be established and maintained by foraging workers. Yet, *P. breviceps* workers do not forage and do not leave their nest unless they are slave-raiding or scouting for host nests. Consequently, it is not clear how raiding territories would be established and maintained.

Another possibility is that colonies avoid areas used by other colonies because of costs of passing through another raiding column. In two years of observation, we observed raiding columns cross twice. One encounter involved two *P. breviceps* colonies; the other involved a *P. breviceps* colony and a *F. puberula* colony. On both occasions, slave-makers fought for a prolonged period of time, and in the latter case the *P. breviceps* stole some brood items from the *F. puberula* raiders. In both cases, individuals from each colony were killed. Thus, colonies might simply avoid areas that are contaminated by trail pheromones from another colony in order to decrease the likelihood of intersecting another raiding column. A similar mechanism has been proposed for the army ant, *Eciton burchelli*, which forage in large columns that overlap less than expected if colonies moved randomly (Hölldobler & Wilson 1990).

The concentration of raids by slave-makers in certain areas might also explain why our estimates of raiding frequency were consistently below averages calculated from empirical observations. Previous work showed that this population of *F.* near *argentea* is spatially structured, as neighboring nests are sometimes polydomous units of a single colony, or they can belong to different colonies that are related (see chapter 4). Such spatial structuring in host populations coupled with limited space-use by slave-maker colonies would lead to underestimates of raiding frequency. Slaves retrieved from neighboring nests are likely to be related (either because of polydomy or limited queen dispersal), thus inflating the overall relatedness of the slave pool. We do not have comparable data on the colonial structure of *F. subsericea* colonies, but polydomy and population viscosity created by limited queen dispersal are generally not associated with monogyny. Nevertheless,

these traits are common in other *Formica* and we cannot rule out the possibility that they apply to *F. subsericea*.

Another factor contributing to the discrepancy between our estimates of slave-raiding frequency and actual observations was that raided colonies were not represented equally in the slave pool. Indeed, Topoff et al. (1985a) and Topoff et al. (1985b) counted the number of larvae and pupae retrieved during *P. breviceps* slave raids and the range was considerable (e.g. ranging from 4-1610 for one colony). Skew of this magnitude would inflate relatedness estimates, thus leading to underestimates of raiding frequency.

All of our estimates of raiding frequency were low, but particularly low estimates obtained for the *Formica* slave-makers are puzzling. One potential explanation for this could be that raids by *Formica* slave-makers are more spatially clustered than those by *P. breviceps*. However, we have no evidence that the raiding space used by *Formica* colonies is smaller. In fact, the largest MCP was for a *F. puberula* colony (Table 3.3). Another possibility is that slaves live for more than one year, and *Formica* colonies raid the same colonies year after year. Unfortunately, because we collected colonies in 2003 that had been raided in 2002, we have no data with which to test this possibility.

We predicted that because *P. breviceps* is completely dependent on its host species, there would be less aggression during *P. breviceps* raids than raids by *F. puberula* and *F. gynocrates*. As predicted, there was less aggression during *P. breviceps* slave-raids than during raids by *F. puberula* or *F. gynocrates*; differences between *P. breviceps* and *F. gynocrates* were not statistically distinguishable, but there was a clear trend for higher aggression in *F. gynocrates* (Figures 3.3a & 3.3b). Determining whether differences in aggression were because of properties of slave-

makers or hosts is complicated by the fact that *F. puberula* and *F. gynocrates* were host specific. Consequently, we lack data on some potential parasite-host pairings (e.g. *F. puberula* vs. *F.* near *argentea* and *F. gynocrates* vs. *F. subsericea*). Nevertheless, our data suggest that levels of aggression were more likely determined by slave-maker identity than the identity of the hosts. If hosts were responsible for the differences we observed, then we should have seen a pattern where one host was more aggressive whether paired against *P. breviceps* or one of the *Formica* slave-makers. In fact, we found no difference in aggression during *P. breviceps* raids against different hosts. Thus, while *F. puberula* raids against *F. subsericea* had the highest aggression levels of any pairing, we did not find elevated aggression during *P. breviceps* raids against *F. subsericea* when compared with raids against *F.* near *argentea*. Hence, we have little evidence that hosts differed in aggressiveness, despite differences in social structure that would predict otherwise.

We cannot, however, rule out the possibility that aggressiveness of hosts is facultative and depends on the identity of the attacking slave-maker. Hosts may respond non-aggressively to some slave-making species (*P. breviceps*, in this example), while acting more aggressively toward other slave-makers (the *Formica* slave-makers, in this example). Hosts may be relatively non-aggressive during *P. breviceps* raids because raiders release propaganda pheromones; if *F. puberula* and *F. gynocrates* do not use such pheremones or they are less effective than those used *P. breviceps*, then hosts could respond more aggressively. If this is true, then our results are consistent with the prediction that monogynous hosts are more aggressive, because *F. puberula* raids against *F. subsericea* had the highest aggression levels. Again, however, we would need data from all possible pairings (e.g. *F. puberula* vs. *F.* near *argentea* and *F. gynocrates* vs. *F. subsericea*), to

determine with certainty whether differences in aggression were related to differences among slave-makers or hosts.

A facultative aggressive response could also be driven by differences in the costs and benefits of fighting different slave-makers. Morphological specializations such as saber shaped mandibles make *P. breviceps* particularly fierce fighting opponents. Consequently, the costs of fighting *P. breviceps* might far outweigh potential benefits. In contrast, although *F. gynocrates* and *F. puberula* workers are larger than their hosts, they do not have obvious morphological specializations for fighting. Thus, the potential for the benefits to outweigh the costs of fighting might drive more aggressive interactions between hosts and *Formica* slave-makers.

Observational data lend support to this hypothesis because while we never observed a host colony thwart a *P. breviceps* raid attempt by fighting, we did observe one *F. subsericea* colony and one *F.* near *argentea* colony mount an aggressive defense that prevented *Formica* slave-makers from stealing brood. Moreover, widespread fighting during *F. gynocrates* and *F. puberula* raids might have reduced the amount of brood loss even if the raid attempt is not stopped altogether.

Although we predicted that the impact of *P. breviceps* raids on host fitness would be less than that for *F. puberula* and *F. gynocrates*, our support for this hypothesis is equivocal. There was a suggestion that *F. subsericea* colonies raided by *P. breviceps* were more likely to survive than those raided by *F. puberula*, but we found no differences for *F.* near *argentea* colonies raided by *P. breviceps* or *F. gynocrates*. Moreover, survivorship of both colony types was substantially reduced compared to controls. Thus, the impact of slave-raiding on host fitness seems to depend specifically on which host and which slave-maker are involved in the interaction; we found no evidence of parasites that were universally more prudent.

We were surprised that *F. subsericea* colonies raided by *P. breviceps* in 2002 did not have reduced survivorship compared to controls. This could be an artifact of small sample sizes because the comparison with controls included only five raided colonies. However, *F. subsericea* colonies that were raided by *P. breviceps* in 2003 also had relatively high survivorship (64%), suggesting that this was not a statistical artifact. We did not, however, identify an obvious defense mechanisms employed by *F. subsericea* colonies that can account for their relatively high survivorship. Moreover, survivorship is only one component of host fitness; slave-raids may reduce host fitness through other mechanisms. For example, colonies undoubtedly lose a substantial amount of worker larvae and pupae during slave-raids, which could mean that they must compensate by producing more workers the following spring when they otherwise might produce female sexuals.

Mortality of raided *F.* near *argentea* colonies was much higher than controls, suggesting that selection pressure for the evolution of defensive strategies should be strong. Again, we did not identify any obvious defensive strategies, but the polydomous colonial structure of these ants could serve as one line of defense. Polydomy could have the effect of "spreading the risk" for a particular colony, because the loss of a nest due to slave-raiding or colony-takeover by a slave-maker queen does not necessarily result in the loss of the entire colony. In fact, we only measured nest survival, and it is quite possible that other nests that belong to the same colony do survive, even if a slave-raid destroys one nest.

Taken together, our data on aggressiveness during slave-raids and host colony survival reveal complex patterns of coevolution in our study system. Slave-makers clearly used different behavioral strategies during slave-raids, which means that optimal defensive strategies are probably not universal. This probably explains

why we did not find evidence of parasites that were universally more prudent or hosts that were universally more resistant. Instead, the outcome of interactions depended specifically on which host species and which parasite species were involved in the interaction. Indeed, the evolution of parasite manipulation strategies or host defenses is complicated when multiple parasites interact with multiple hosts. Future studies aimed at untangling the coevolutionary complexities of such systems are essential, particularly for formicine slave-making systems, because populations commonly include multiple parasites enslaving the same hosts.

One final important result from our study was that all slave-maker colonies specialized on only one of the two *Formica fusca* group hosts at our site. Host specificity was at the species level for the *Formica* slave-makers, while it was at the colony level for *P.* breviceps. One *F. gynocrates* colony did have a few *F. obscuriventris* (*rufa* group) slaves in 2002, but we did not find slave-maker colonies with slave-pools that included both primary hosts (*F. subsericea* and *F.* near *argentea*). To our knowledge, this is the first report of host specificity for *Formica sanguinea* group slave-makers; they are generally not considered to be host specific, as they often enslave multiple species simultaneously (Goodloe *et al.* 1987; Mori *et al.* 2001). Further work is needed to determine if host specificity is more common in this group than originally assumed and whether this might play a role in speciation.

Colony level host specificity has been found in other slave-makers including *P. lucidus* (Goodloe & Sanwald 1985; Goodloe *et al.* 1987), *P. rufescens* (Mori *et al.* 2001), and *Chalepoxenus muellerianus* (Schumann & Buschinger 1995), but this is the first report for *P. breviceps*. Host specificity in *P. lucidus, P. rufescens* and *C. muellerianus* is found both at the colony founding stage (queens almost exclusively usurp colonies of the same species present in their natal nest), and in slave-raiding

(raids are exclusively conducted against the species present in the natal nest). This system of maternally transmitted host specificity strongly resembles the process of host-race formation in socially parasitic cuckoos (Gibbs *et al.* 2000; Marchetti *et al.* 1998), and should be the focus of future studies.

Our study is the first, to our knowledge, to examine interactions in a complex system of multiple slave-makers and multiple hosts occurring in sympatry. We identified a series of interactions with important evolutionary implications, including those among slave-makers themselves, differences in the aggressiveness of slave-makers, and differences in the survival of hosts raided by different slave-makers. Such complex systems should provide fertile ground for future research, especially for formicine slave-makers, which often have overlapping ranges and co-occur in the same populations.

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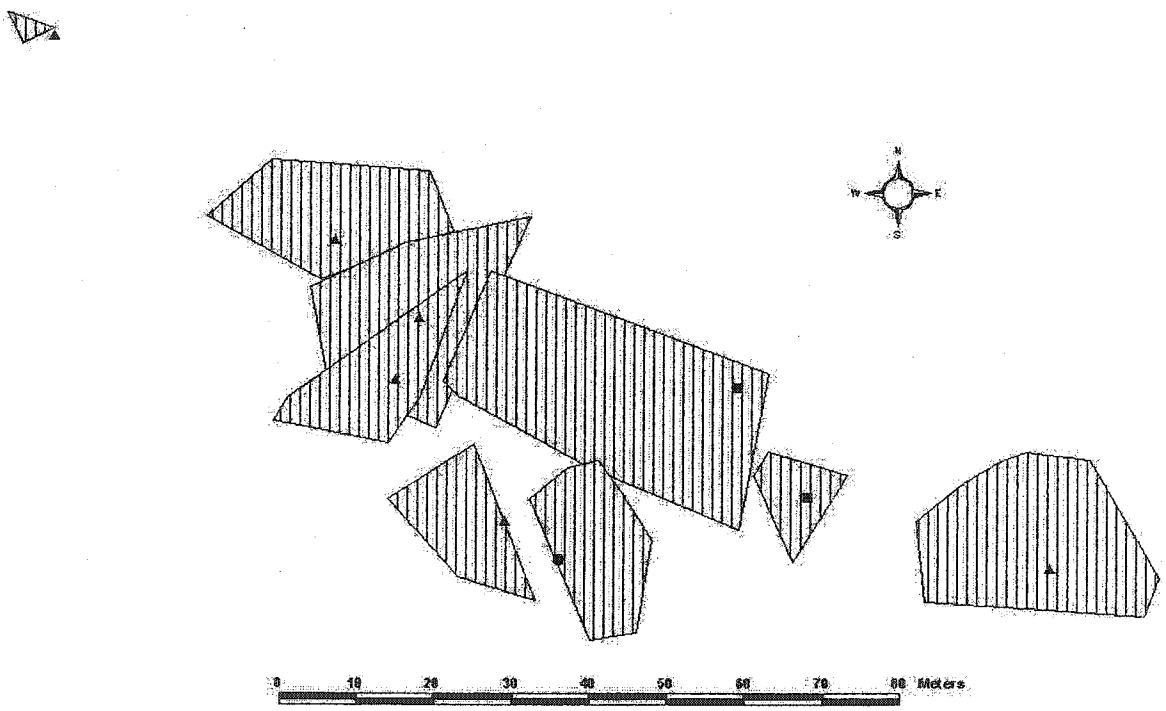


Figure 3.1. Minimum convex polygons formed using data on the location of slave-raids for *F. puberula colonies* (■), *F. gynocrates* (●) and *P. breviceps* (▲). One *F. gynocrates* colony is excluded from the map because it only raided one time.

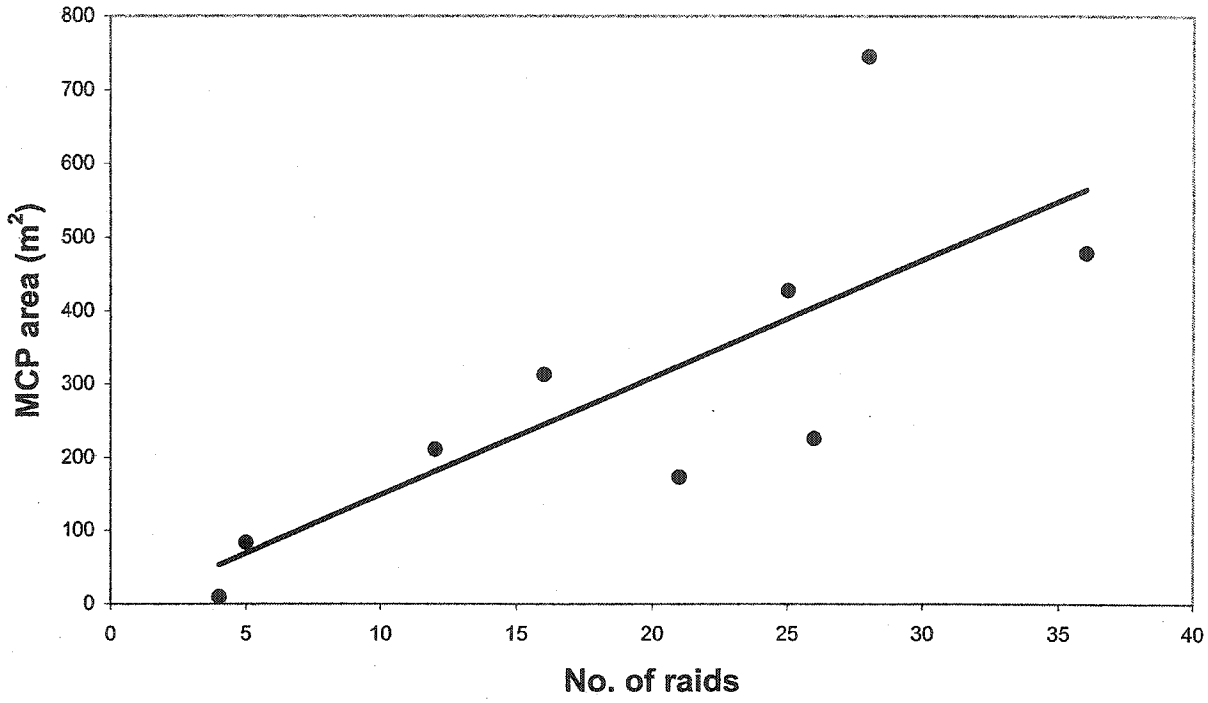
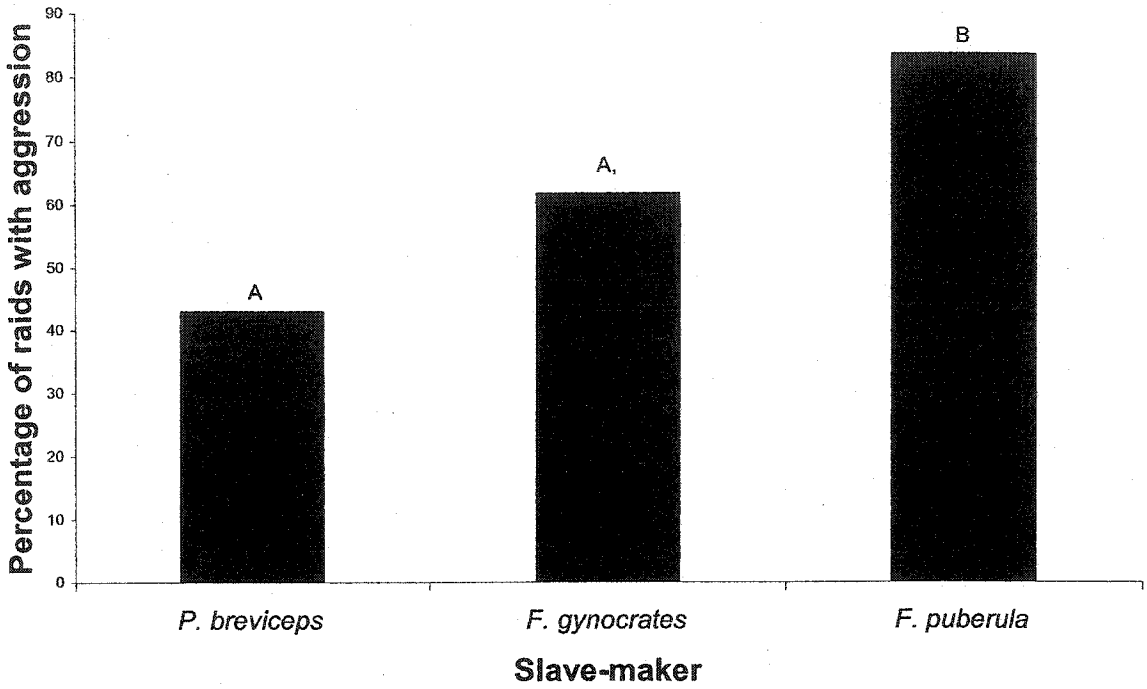
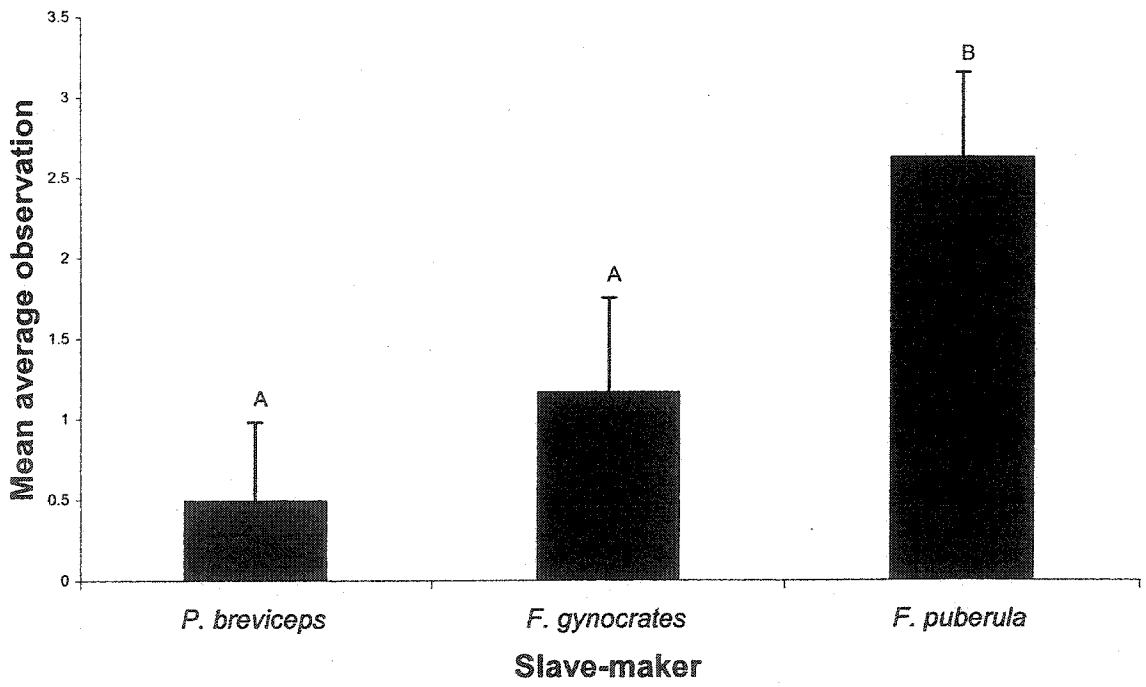


Figure 3.2. There was a strong correlation between the size of the MCP constructed for a colony and the number of raids conducted by the colony over two raiding seasons (ρ= 0.77, p= 0.016).





Host species/slave-maker	No. colonies collected 2003	No. colonies collected 2004
F. subsericea/P.breviceps	6	14
F. subsericea/F. puberula	7	6
F. near *argentea/P. breviceps*	10	15
F. near *argentea/F. gynocrates*	5	2

Table 3.1. Summary of number of raided colonies collected for each host-parasite pair in 2003 and 2004.

Colony	N Slaves sampled 2002	Identity of slaves sampled 2002	N slaves sampled 2003	Identity of slaves sampled 2003
Pb 2	12	*F.* near *argentea*	12	*F.* near *argentea*
Pb 3	11	*F. subsericea*	12	*F. subsericea*
Pb 4	12	*F.* near *argentea*	9	*F.* near *argentea*
Pb 6	12	*F. subsericea*	12	*F. subsericea*
Pb 8	12	*F.* near *argentea*	12	*F.* near *argentea*
Pb 10	12	*F.* near *argentea*	12	*F.* near *argentea*
Pb 11	12	*F.* near *argentea*	--	--
Pb 12	12	*F.* near *argentea*	12	*F.* near *argentea*
Fg 5	12	*F.* near *argentea*	12	*F.* near *argentea*
Fg 12	12	*F.* near *argentea*	12	*F.* near *argentea*
Fg 15	12	*F.* near *argentea*	--	*F.* near *argentea*
Fp 2	12	*F. subsericea*	11	*F. subsericea*
Fp 3	11	*F. subsericea*	--	--
Fp 16	12	*F. subsericea*	--	--

Table 3.2. Number and identity of slaves collected in 2002 and 2003 from nests of three slave-makers *P. breviceps* (Pb), *F. gynocrates* (Fg), and *F. puberula* (Fp).

Colony	Slave species	MCP area (m2)
Pb 2	*F.* near *argentea*	478.7
Pb 8	*F.* near *argentea*	211.2
Pb 10	*F.* near *argentea*	313.6
Pb 12	*F.* near *argentea*	10.0
Fg 5	*F.* near *argentea*	225.6
Pb 3	*F. subsericea*	173.5
Pb 6	*F. subsericea*	427.4
Fp 1	*F. subsericea*	83.5
Fp 2	*F. subsericea*	744.8

Table 3.3. Size of minimum convex polygons (MCPs) formed by connecting the outermost locations of slave-raids conducted by three slave-making ant species, *P. breviceps* (Pb), *F. gynocrates* (Fg), and *F. puberula*).

Host species	Locus	No. alleles in source population	No. alleles in slaves 2002	No. alleles in slaves 2003	Freq. of most common allele in source population
F. subsericea	FI 21	5	6*	6*	0.39
	Fe 49	8	8**	8**	0.22
F. nr. *argentea*	FI 12	10	10	9	0.21
	FI 29	7	8*	8*	0.44
	Fe 49	15	15**	13**	0.31

*One allele was present in the slave population that was not present in the source population

** Two alleles were present in the slave population that were not present in the source population

Table 3.4. Number of alleles in source populations and slave populations of two host species *F. subsericea* and *F. near argentea.* Workers were collected from free-living host populations in the spring of 2002. Slaves were collected in the spring of 2002 and spring of 2003.

Slave-maker	Host species	No. nests 2002	Avg. r of slaves 2002 ± s.e.	No. nests 2003	Avg. r of slaves 2003 ± s.e.	Predicted no. raids from r (2003)	Actual no. raids
P. breviceps	*F. subsericea*	2	0.115 ± 0.01	2	0.105 ± 0.02	6.9	8.5
P. breviceps	*F.* near *argentea*	6	0.118 ± 0.06	4	0.146 ± 0.06	2.7	4.8
F. gynocrates	*F.* near *argentea*	3	0.119 ± 0.09	2	0.205 ± 0.10	1.9	9
F. puberula	*F. subsericea*	2	0.118 ± 0.01	1	0.492 ± 0.07	1.4	12

Table 3.5. Average relatedness of slaves collected in the spring of 2002 and spring of 2003. Standard errors were obtained by jackknifing over loci. Average relatedness of slaves was compared with average relatedness of free-living host colonies to predict the average number of slave-raids for colonies of three slave-makers. These predictions were consistently lower than the number of raids actually observed.

Host	Comparison	No. colonies survived/total	P-value
F. subsericea	*P.* breviceps vs. F. *puberula*	13/19 vs. 5/13	0.094
	P. breviceps 02 vs. control	4/5 vs. 7/10	0.846
	F. puberula 02 vs. control	4/7 vs. 7/10	0.484
F. near *argentea*	*P. breviceps* vs. *F. gynocrates*	13/25 vs. 3/7	0.802
	P. breviceps 02 vs. control	5/10 vs. 10/12	0.113
	F. gynocrates 02 vs. control	2/5 vs. 10/12	0.117
	Raided colonies 02 vs. control	7/15 vs. 10/12	0.058

Table 3.6. Survival comparisons for *F. subsericea* and *F.* near *argentea* colonies collected in 2003 and 2004. P-values are from comparisons using Fisher's exact tests.

Chapter IV

Social structure of an obligate slave-making ant, (*Polyergus breviceps*) and two sympatric hosts (*Formica subsericea* and *F.* near *argentea*)

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ABSTRACT

Data on the social and colonial structure of slave-making ant colonies and their hosts are limited, despite important implications for understanding the evolution of slave-making behavior and for testing models of sex allocation. Evolutionary hypotheses regarding the origin of slave-making behavior implicate polygyny and polydomy (colonies occupy multiple nests) as being important precursors for the evolution of parasitic colony founding and slave-raiding. However, support for these hypotheses is equivocal because most slave-makers are themselves monogynous and monodomous, while host social/colonial structure is more variable. We used microsatellites to determine the social structure of the obligate slave-maker *Polyergus breviceps*, and two sympatric hosts, *Formica subsericea* and *F. argentea*. The microsatellites we used for *P. breviceps* are the first to be isolated from this genus, and we include primer sequences. We show that the seven *P. breviceps* colonies we included in our analysis were all monogynous and monoandrous. *F. subsericea* colonies were also monogynous and monoandrous, while *F. argentea* colonies were polygynous and sometimes polydomous. We discuss the importance of these findings for understanding the evolution of slave-raiding behavior. Moreover, we discuss the suitability of *P. breviceps* for testing models of sex allocation, as slave-making ants have been considered a critical model system with which to test sex allocation theory in ants.

INTRODUCTION

The unique life history of slave-making ants has captivated evolutionary biologists since Darwin's description of slave-raiding in *On the Origin of Species by Means of Natural Selection* (1859). Slave-making ants represent a form of social parasitism in which the slave-making species exploits the labor of workers from another closely related species by incorporating slave workers into the slave-maker colony. Slave-making has evolved independently in two subfamilies of ants, Myrmicinae and Formicinae, and is particularly common in the tribes formicoxeninii and formicinii.

The parasitic lifestyle of slave-making ants involves two components: parasitic colony founding by queens and slave-raiding by workers. A slave-maker queen is unable to independently start a new colony, and must instead penetrate a free-living host colony, kill the resident queen(s), and gain acceptance from the workers. The new queen then produces her own workers, which replenish the slave supply by stealing larvae and pupae from neighboring host colonies. Highly specialized obligate slave-making ants are unable to survive in the absence of hosts, and must replenish their slave supply each year. In contrast, facultative slave-makers are less specialized and can mature to be independent following dependent colony initiation.

Hypotheses concerning the evolution of slave-making behavior must account for parasitic colony founding behavior of queens and slave-raiding behavior of workers. Buschinger (1970) argued that slave-making behavior may have evolved from ancestors who were polygynous (colonies have multiple queens) and polydomous (colonies occupy multiple nest sites). In ants, polygyny commonly results from the adoption of queens into an already established colony. Colonies

that routinely adopted new queens could presumably have been exploited by parasitic queens, and slave-raiding could have evolved from frequent brood transport between polydomous units of a single colony.

Because it is unclear how peaceful brood transport among nests of a single colony could evolve into slave-raiding behavior, Alloway (1980) and Stuart and Alloway (1982) expanded this hypothesis to include territoriality in addition to polygyny and polydomy. Slave-raiding behavior could have evolved from frequent territorial battles among polygynous and polydomous ants that included the robbing of brood from the weaker colony.

Finally, Pollock and Rissing (1989) proposed that intraspecific territoriality among neighboring colonies could have induced selection for brood raiding, but that the evolution of brood raiding specialists (slave-making behavior) is only possible for species with polydomous colonial structure. Nests of polydomous species, they argued, are replaceable because colonies frequently emigrate, establishing new nests spontaneously. For an evolving slave-maker, host nests are a renewable resource, thereby allowing for specialization in brood raiding. Furthermore, queen movement between polydomous nest units could result in queens mistakenly entering a foreign nest, thus setting the stage for parasitic colony founding.

These three hypotheses are not mutually exclusive, and all emphasize the importance of polygynous social structure and polydomous colonial structure as preconditions for the evolution of slave-making behavior. Thus, we expect to find this type of social and colonial structure commonly among slave-making ant species and among their host species. Though data on the social and colonial structure of slave-maker colonies and their hosts are limited, support for these hypotheses is equivocal. Polygyny and polydomy are common in both the formicini and

formicoxenini. Hosts of the obligate slave-maker, *Protomagnathus americanus* are polygynous and polydomous (Foitzik & Herbers 2001a; Herbers & Stuart 1998), but most hosts of *Challepoxenus* and *Epimyrma* are monogynous and monodomous (D'Ettorre & Heinze 2001). Many hosts of the formicine slave-makers are polygynous and polydomous (Pamilo 1982), but others are monogynous and presumably monodomous *(F. subanescens, F. argentea,* and *F. hewitti,* Bennett 1987*)*. Most slave-makers are themselves monogynous, with a few exceptions particularly in the formicines (Buschinger 1990; D'Ettorre & Heinze 2001).

Our interest in this study was to elucidate the social and colonial structure of the obligate formicine slave-maker, *Polyergus breviceps*, and two sympatric hosts, *F. subsericea* and *F.* near *argentea*. As a consequence of haplodiploid sex determination, the social structure of ant colonies can be inferred by inspecting individual genotypes and estimating relatedness among nestmates. For example, workers from a monogynous colony with a singly-mated queen are related by 0.75, because they share one allele from their haploid father and half of their mother's alleles. Relatedness in polygynous colonies is lower and depends on the number of queens and their relatedness to one another. Data on the genetic structure of colonies also can be used to infer whether neighboring nests belong to the same colony (i.e. whether the colony is polydomous) because workers from neighboring polydomous nests are related and the distribution of genotypes among polydomous nests should be identical (Pederson & Boomsma 1999a).

We also use our data on the social structure of *P. breviceps* to determine whether this species might be useful in future studies of sex allocation. In ants, conflict over sex allocation occurs between queens, who favor an even sex ratio, and workers, who favor a sex ratio of 3:1 females. Workers are generally assumed to

win this conflict because their role in brood care affords them opportunities to manipulate sex ratios (reviewed by Bourke & Franks 1995; and Crozier & Pamilo 1996). Trivers and Hare (1976) argued that slave-making ants provide a critical test for sex allocation theory because slaves would gain no fitness benefit by manipulating sex allocation of slave-maker brood. Consequently, slave-making ants could represent a rare case when sex allocation ratios are near the value favored by the queens.

This hypothesis is poorly supported by empirical work, probably because of other genetic factors that are known to influence sex allocation in ant colonies (Herbers & Stuart 1998). For example, factors such as polygyny (Savolainen & Seppa 1996) local mate competition (Buschinger 1989) and local resource competition (Mori *et al.* 1994a; Terayama *et al.* 1993; Topoff *et al.* 1988) probably influence sex allocation ratios for some slave-makers. Moreover, worker production of males can increase male investment, as occurs in *P. americanus* colonies (Foitzik & Herbers 2001b). Clearly, it is important to understand fully the social structure of slave-making ant colonies before assuming that they will be suitable for testing sex allocation models. Although we do not explicitly test models of sex allocation in our study, we do present data on queen numbers and mating frequency of *P. breviceps* queens. These data are relevant to future studies on sex allocation in *P. breviceps* colonies. Moreover, we report primer sequences for microsatellites that we isolated from *P. breviceps*, which are the first for this genus.

METHODS

Study System

We studied a population of the slave-making ant *P. breviceps* and two host species *(F. subsericea* and *F.* near *argentea)* in the foothills of the Rocky Mountains (elev. 2000 m), 36 km northwest of Fort Collins, Colorado USA. Despite the presence of two potential host species, individual *P. breviceps* colonies specialize on only a single host species (see chapter 2). The field site was characterized by open meadows and sparsely spaced ponderosa pine, spruce, and fir trees. We found colonies of the slave-maker and the host species on hillsides nesting under rocks and stones. In May 2002, we collected at least 15 *P. breviceps* workers from each of eight colonies and 9-12 workers from free-living host colonies of *F. subsericea* and *F. argentea* (17 and 18 colonies respectively). We brought workers to the laboratory and immediately froze them at –70° C.

Isolation of Microsatellites

We built a microsatellite-enriched library for *P. breviceps* using biotin-labeled microsatellite oligoprobes [$(TC)_{10}$ and $(TG)_{10}$] and streptavidin-coated microbeads. We used the Quiagen Dneasy tissue kit to extract total genomic DNA from *P. breviceps* workers. We transferred recombinant bacterial colonies onto charged nylon membranes (Hybond N+, Amershand Pharmacia) and screened them with dioxigenine labeled oligoprobes. Fifty-five (18.5%) of the colonies produced a positive screen. Despite the lack of a size selecting step, inserts were of an appropriate size (300-700 bp). We sequenced 27 clones, of which 25 contained a microsatellite locus. We used the computer program Primer3 to design PCR primers for 15 of these loci.

DNA Extraction

We used a salt-extraction protocol to extract DNA from *P. breviceps*, *F. subsericea* and *F.* near *argentea* workers. We removed the abdomen from each ant before placing the remaining parts in 60 µl of Puregene cell lysis solution and grinding with a pestel. These tubes were then incubated at 65° C for 30-60 minutes. Following incubation, we added 20 µl of ammonium acetate to precipitate proteins. Tubes were placed in the freezer for at least 10 minutes, and then centrifuged at high speed for 6 minutes. We pipetted the supernatant into 65 µl 100% isopropanol and cooled the solution in the freezer for at least 10 minutes. We then centrifuged the tubes at high speed for 6 minutes, poured off the isopropanol and inverted tubes for 24 hours to dry the DNA pellet. We resuspended the DNA pellet with 100 µl 1X TE buffer.

Microsatellite analysis

Of the 15 microsatellites that were isolated from *P. breviceps* workers, eight amplified products of appropriate size and four of these (pol1, pol3, pol4 and pol10) proved to be polymorphic in our population. PCR cocktails consisted of 0.7 µL DNA, 2 µL 10X buffer (Promega), 25 mM MgCl2, 4 mM dNTPs, 25 µM of each primer, 5 units of Promega *Taq* DNA polyermase, for a total reaction volume of 20 µL. All PCR temperature programs included an initial denaturing step at 95° C for 5 min, followed by 36 cycles with 30 s at 95° C, 30 s at the appropriate annealing temperature (see table 1), 30 s at 72° C, followed by a 5 min. extension step at 72° C.

We found polymorphic microsatellites for both host species using published *Formica* primers. For *F. subsericea* we used two primers: FI21, originally developed for *F. paralugibrus* (Chapuisat 1996) and Fe 49, originally developed for *F. exsecta* (Gyllenstrand *et al.* 2002). For F. *argentea* we used three primers: FI12 and FI29, both developed for *F. paralugibrus* (Chapuisat 1996) and Fe 49. We used PCR to amplify microsatellites by combining 0.5 µL DNA with 1.2 µL 10 X buffer (Promega), 25 mM $MgCl_2$, 4 mM dNTPs, 25 µM of each primer, and 5 units of Promega *Taq* DNA polymerase to form 12 µL reaction mixtures. All PCR programs consisted of an initial denaturing step of 95° C for 5 min. followed by 36 cycles of 95° C for 30 s, appropriate annealing temperature for 30 s, 72° C for 30 s, followed by a 5 min. extension step at 72° C. Annealing temperatures for the primers were as follows: FI12, 54° C; FI21, 50° C; FI 29, 50° C; Fe49 50° C.

We separated samples on 6% polyacrylamide gels and visualized alleles by silver staining gels. We genotyped 9-12 individuals from 17 different colonies for *F. subsericea* (2 loci), 12 individuals from 19 different colonies for F. near *argentea* (3 loci), and 15-18 individuals from 7 different *P. breviceps* colonies (4 loci).

Data Analysis

We estimated allele frequencies, colony relatedness, and population relatedness with the program RLAT version 5.0.8 (Queller & Goodnight 1989). We weighted individuals equally, and applied a bias correction for allele frequency calculations that accounts for the family structure of colonies. To obtain unbiased estimates of observed heterozygosity for *P. breviceps*, we developed a simulation exercise whereby one individual was sampled randomly from each colony and heterozygosity was calculated for this "population." We ran the simulation 1000

times and calculated the observed heterozygosity as the mean heterozygosity from all 1000 "populations."

Polydomy

Three pairs of *F.* near *argentea* nests that we genotyped were nearest neighbors, so we used methods of Pederson and Boomsma (1999a) to test whether these nests belonged to the same colony. We first calculated the internest relatedness ($r_{1\leftrightarrow 2}$) of workers from the two nests; a positive value indicated that individuals in these nests were related. A positive value for $r_{1\leftrightarrow 2}$ suggests that nests belong to the same colony, but could also result if there is limited queen dispersal, such that neighboring nests are related but not freely exchanging workers. One way to distinguish these possibilities is to calculate Δr which estimates the difference between $r_{1\leftrightarrow 2}$ and the expected relatedness if these nests belong to the same polydomous colony (simply the average of the two intranest relatedness values weighted by sample size from each; equation eight Pederson & Boomsma, 1999a). If the nests belong to the same colony, Δr should be ≥ 0, while negative values indicate that nests belong to different colonies. We also calculated G_{dist} which measures whether genotypes are distributed homogenously between nests. Large values of G_{dist} suggest that the distribution of genotypes is not homogenous and nests do not belong to the same colony. To generate a distribution of G_{dist} for nests that we assumed were not polydomous, we randomly selected 20 nest pairs and calculated G_{dist} for each pair. We then used a one-sided t-test to test the probability that the individual G_{dist} values for potentially polydomous nests could have come from the distribution generated by the 20 sample pairs.

RESULTS

Suitability of genetic markers

Despite our small sample size (seven colonies from one population), four microsatellites that we isolated from *P. breviceps* proved to be polymorphic (pol 1, pol 3, pol 4, and pol 10), with expected heterozygosities ranging from 0.60 to 0.92 (Table 4.1). Thus, these markers provide sufficient resolution to estimate relatedness structure of colonies. Although we did not use these genetic markers to infer population genetic structure of *P. breviceps*, the relatively high levels of polymorphism for these markers over a small sampling scale suggests that they also will be useful for population level analyses.

The microsatellite markers we used for *F. subsericea* and *F.* near *argentea* were polymorphic and therefore useful in determining the genetic structure of these free-living host colonies. Data on numbers of alleles, and observed and expected heterozygosities for these markers are summarized in Table 4.2.

Genetic structure of P. breviceps colonies

Average intracolony relatedness for *P. breviceps* colonies was 0.72 ± 0.04; suggesting that colonies were monogynous and monandrous. Inspection of individual genotypes across the four loci confirmed this inference, because in all cases we found no deviation from genotypes consistent with monogyny (i.e. a single allele from the father and onw of two alleles from the mother). We purposefully genotyped many individuals from each colony (15-18) to increase our power to detect additional patrilines should paternity skew be high (Pederson & Boomsma

1999b). Therefore, we are confident that colonies were, in fact, monogynous and monandrous.

Genetic structure of host colonies

Average intracolony relatedness values for *F. subsericea* were also high (0.72 ± 0.03), and confidence intervals overlap the expectation of 0.75 for monogynous/monandrous colonies (Table 3). Inspection of individual genotypes from each colony also suggests monogyny/monandry, as all individuals in all colonies had genotypes consistent with offspring of individual single-mated queens.

Average intracolony relatedness for *F.* near *argentea* was much lower (0.39 ± 0.04), suggesting either polygyny or multiple mating. Distinguishing polygyny and polyandry is difficult, particularly if queens are related. However, inspection of individual genotypes suggests that colonies are probably polygynous. Moreover, we have observed multiple queens in some colonies, suggesting that polygyny is likely. The range of relatedness values for individual colonies is quite large, suggesting that levels of polygyny/polyandry are mixed in the population (Figure 4.1). If we assume that colonies are polygynous, we can use the average relatedness value for the population to calculate the average effective queen number (N_e) for colonies in the population (equation 3 from Ross, 1993). This equation requires a value for the relatedness of queens (r_q). Because we did not obtain an estimate for this value, we substituted the population average relatedness value (0.39) into the equation, which gave an estimate for N_e of 2.2. If queens were either mother and daughter (r=0.5) or were unrelated, this estimate can either be too low or too high.

Polydomy

We followed the methods of Pederson and Boomsma (1999) to test whether three *F. argentea* nest pairs that were nearest neighbors belonged to the same colony. Nest pair 16-17 had a $r_{1\leftrightarrow2}$ of –0.05 so we rejected the hypothesis of con-coloniality for these nests. The other two nest pairs (97-98 & 101-102) had positive $r_{1\leftrightarrow2}$ (Table 4.4). Nest pair 101-102 appeared to be polydomous because the Δr was positive and G_{dist} was significantly smaller than the mean from the random sample (mean from sample= 27.13; one-tailed t-test, p= 0.0006; Table 4.4). Unfortunately, our samples from *F. subsericea* colonies did not include any nearest neighbors, so we were unable to test whether neighboring nests belong to the same colony.

DISCUSSION

The results of our study provide equivocal support for hypotheses on the evolution of slave-making behavior that implicate polygyny and polydomy as important preconditions for the evolution of slavery. *F.* near *argentea* colonies were polygynous and sometimes polydomous, though we do not know the frequency of polydomy in the population because we only sampled three nest pairs. In contrast, *F. subsericea* colonies were strictly monogynous and monandrous. Since we did not sample *F. subsericea* nests that were nearest neighbors, we do not know whether colonies were also polydomous. Nevertheless, limited queen dispersal and polydomy are traits generally associated with polygyny and are rare in monogynous species (Chapuisat *et al.* 1997; but see Foitzik & Herbers 2001b). Thus, while the social and colonial structure of *F.* near *argentea* fits well with hypotheses that emphasize the importance of polygyny and polydomy during the evolution of slave-

making behavior, the social structure of *F. subsericea* contradicts predictions from these hypotheses. Indeed, variability in social structure seems to be the rule for *Formica fusca* group species (the group most often enslaved by *Polyergus*), because reports of both polygyny/polydomy and monogyny are common (Bennett 1987; Pamilo 1982). Unfortunately, we lack phylogenetic data and information on social structure of more species that would determine which traits are ancestral in this group. If, in fact, polygyny and polydomy are ancestral characters in the group, then frequent switches to monogyny would imply that although polygyny and polydomy were essential for the evolution of slave-making they are not required for the maintenance of this behavior.

Our finding that *F.* near *argentea* colonies were polygynous and polydomous contrasts with a previous study on other Colorado populations of *F. argentea*, which found that colonies were typically monogynous (Bennett 1987). Ants from these two populations may represent cryptic species because, although they are morphologically similar, ants from our population were darker black (A. Francoeur, personal communication). Moreover, differences in social structure potentially provide a mechanism for reproductive isolation.

The occurrence of two sympatric hosts with differing social structures is interesting, especially considering the fact that *Polyergus* queens must usurp existing host colonies to start a new slave-maker colony. Moreover, *P. breviceps* colonies in our population probably specialize on only one host because in samples of slaves taken from two different years we did not find colonies with mixed slave-pools (see chapter 2). Host specificity is also characteristic of *P. lucidus* (Goodloe & Sanwald 1985; Goodloe *et al.* 1987), *P. rufescens* (Mori *et al.* 1994b) and *Chalepoxenus muellerianus* (Schumann & Buschinger 1995), and occurs at the colony founding

stage (new queens start colonies only in nests of the same host species found in her natal nest), and in slave-raiding (colonies primarily conduct raids on colonies of the host species present in their natal nest). Thus, *P. breviceps* queens probably only parasitize nests of the host present in their natal nest. Behavioral or chemical strategies for colony take-over could be different for polygynous and monogynous host species. Polygynous colonies presumably have less specific systems of nestmate recognition, which could facilitate the entrance and acceptance of parasitic queens. However, *Polyergus* queens must eventually kill the resident queen(s), and this could be more difficult in colonies with multiple queens. Further studies are needed to determine whether host specificity could lead to the development of behavioral and chemical strategies for parasitic colony founding that are specific to host social structure (i.e. monogyny or polygyny).

Microsatellite markers that we isolated from *P. breviceps* proved to be polymorphic and were therefore useful in determining colony social structure. Relatedness estimates and inspection of individual genotypes suggests that the seven *P. breviceps* colonies at our field site are monogynous and monandrous. Most other slave-makers are also monogynous, though there are a few exceptions in the formicines (Buschinger 1990; Foitzik & Herbers 2001b; Pamilo 1982; Savolainen & Seppa 1996). Social organization of *P. breviceps* may vary across populations because an earlier study on this species in Canada indicated that colonies were polygynous or polyandrous (Savolainen & Seppa 1996). However, this variation could also represent interspecific rather than intraspecific differences, because *P. breviceps* in the Rocky Mountains is comprised of at least two distinct species (J. Trager, pers. comm.). If these two populations do, in fact, represent distinct species, they also differ in their social structure.

Slave-making ants have traditionally been considered a crucial model system to test theories of sex allocation in ants (Trivers & Hare 1976). However, as the social structure of many slave-makers is revealed, it is clear that factors such as polygyny/polyandry, worker reproduction, local mate competition and local resource competition make prediction of sex ratios more complicated than originally expected. *P. breviceps* is a potential candidate for sex ratio studies because we have shown that populations can be monogynous and monandrous. However, further work is needed to determine whether workers produce males or if local resource competition or local mate competition are present in the population. Local mate competition is unlikely because we have no evidence for inbreeding in our population (F_{is}= -0.17; Table 3), but other studies on *P. breviceps* and other *Polyergus* suggest that local resource competition is a likely consequence of the mating system and colony founding strategy of queens. At least in some populations, *P. breviceps* queens copulate during slave raids and follow raiding swarms to host colonies (Topoff *et al.* 1988). Thus, newly mated queens may settle near their natal nest and local resource competition could ensue. Newly mated *P. rufescens* also have been observed following raiding swarms, and it is generally assumed that queens do not disperse widely (Mori *et al.* 1994a; Mori *et al.* 2001).

Although behavioral studies are suggestive of local resource competition, there currently are no studies on population structure for any *Polyergus* species. The relatively high levels of polymorphism despite limited geographic sampling for microsatellites we isolated from *P. breviceps* suggests that they will also be useful for analysis of population structure. It should also be acknowledged that most of our understanding of the *P. breviceps* mating system and colony founding strategies comes exclusively from studies of one (or a few) populations in Arizona, USA (Topoff

et al. 1988). Intracolonial genetic structure can vary across populations (our study vs. Savolainen & Seppa 1996), and personal observations suggest that the mating system may also vary across populations. In two raiding seasons, we have never observed queens copulating during slave raids, and, to our knowledge, dealate queens did not particpate in slave raids (pers. obs.). On one occasion a dealate queen was found near another *P. breviceps* colony, but we do not know whether this was her natal colony. Indeed, the mating system of *P. rufescens* is diverse, as females copulate after mating flights, during slave raids, and on the ground close to their nest (Mori *et al.* 2001). Moreover, *P. lucidus* queens often return to another *Polyergus* colony, but sometimes this is not their natal colony (Coolkwait & Topoff 1984), which could mitigate the effects of local resource competition in the population. Clearly, much work is needed before we fully understand the mating system and population structure of *P. breviceps*, and some populations may prove to be valuable for testing predictions from sex allocation theory.

D'Ettorre and Heinze (2001) argued that slaves in slave-maker colonies might still control sex ratios even though they do not receive a fitness benefit. If they are selected to produce a certain sex ratio in free-living colonies, they may simply manipulate the sex ratio in the same manner when enslaved. Populations in which *P. breviceps* enslaves multiple hosts with different social structures could provide an interesting system to test this idea. We would expect that sex ratios for monogynous host species would vary from those produced by polygynous host species in accordance with sex ratio theory (Trivers & Hare 1976). If slaves in slave-maker colonies are controlling sex ratios, then *P. breviceps* colonies with different hosts should produce different sex ratios. Moreover, these sex ratios should reflect sex ratios from the respective free-living host populations.

Elucidating the social and colonial structure of slave-making ant colonies and their hosts is essential for understanding the evolution of slave-making behavior and for studies of sex allocation. Future work should focus on determining phylogenetic relationships among hosts and understanding their genetic structure. Furthermore, studies of the genetic structure of *P. breviceps* populations should yield considerable insight into whether this species is suitable for testing sex ratio theories. Our isolation of new microsatellites for *P. breviceps* should facilitate such studies in the future.

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Locus	Repeat motif	EMBL accession no.	Primer sequences (5' – 3')	Annealing temp.	PCR product size (bp)	No. of alleles	H_e	H_o
pol 1	$(GA)_{14}$	AJ577750	TGCCCTACGTATCCACAATCT CGTGGATCCTCGCAACTAT	58	173	11	0.91	0.95
pol 3	$(GA)_7(GT)_9$	AJ577751	GCAACTGCGTTACATAGAGATCA GAGTTTATACGCCTCTGTTTCG	58	111	6	0.75	0.93
pol 4	$(GA)_{20}$	AJ577752	AAGCGGCGACACTTCGAG CGACGCAACGACAATCTTAAT	60	144	5	0.72	0.94
pol 10	$(GT)_{16}$	AJ577753	GAGGTGGCGAGACAAGCTAT TTCTCTTCCGGGATTGGTTT	59	122	4	0.63	0.71

Table 4.1. Repeat motif, primer sequences, amplification conditions, and polymorphism of the four microsatellite loci isolated from *Polyergus breviceps* (N=7).

Species	Locus	Number of alleles	Freq. of most common allele
F. subsericea	FI21	5	0.39
	Fe49	8	0.22
F. near *argentea*	FI12	10	0.21
	FI29	7	0.44
	Fe49	15	0.31

Table 4.2. Number of alleles and frequency of the most common allele for each microsatellite locus used to genotype *F. subsericea* and *F.* near *argentea* workers.

Species (N)	R (95% c.i.)	F_{is} (95% c.i.)
P. breviceps (7)	0.72 (0.60, 0.84)	-0.17 (-0.88, 0.04)
F. subsericea (17)	0.72 (0.66, 0.78)	-0.07 (-0.94, 0.80)
F. near *argentea* (18)	0.39 (0.31, 0.47)	-0.04 (-0.13, 0.05)

Table 4.3. Population mean relatedness and inbreeding coefficients for the slave-making ant *P. breviceps*, and two sympatric hosts, *F. subsericea* and *F.* near *argentea*. Confidence intervals were constructed using standard errors that were obtained by jacknifing over colonies.

Nest pair	r1↔2 (s.e.)	Δr	Gdist	Gdist p-value	Same or different colony
97-98	0.23 (0.07)	-0.25	23.16	0.141	Different
101-102	0.27 (0.05)	0.02	3.25	0.001	Same

Table 4.4. Data used to determine whether neighboring *F.* near *argentea* nest pairs belonged to the same colony. Standard errors for internest relatedness values were obtained by jacknifing over loci.

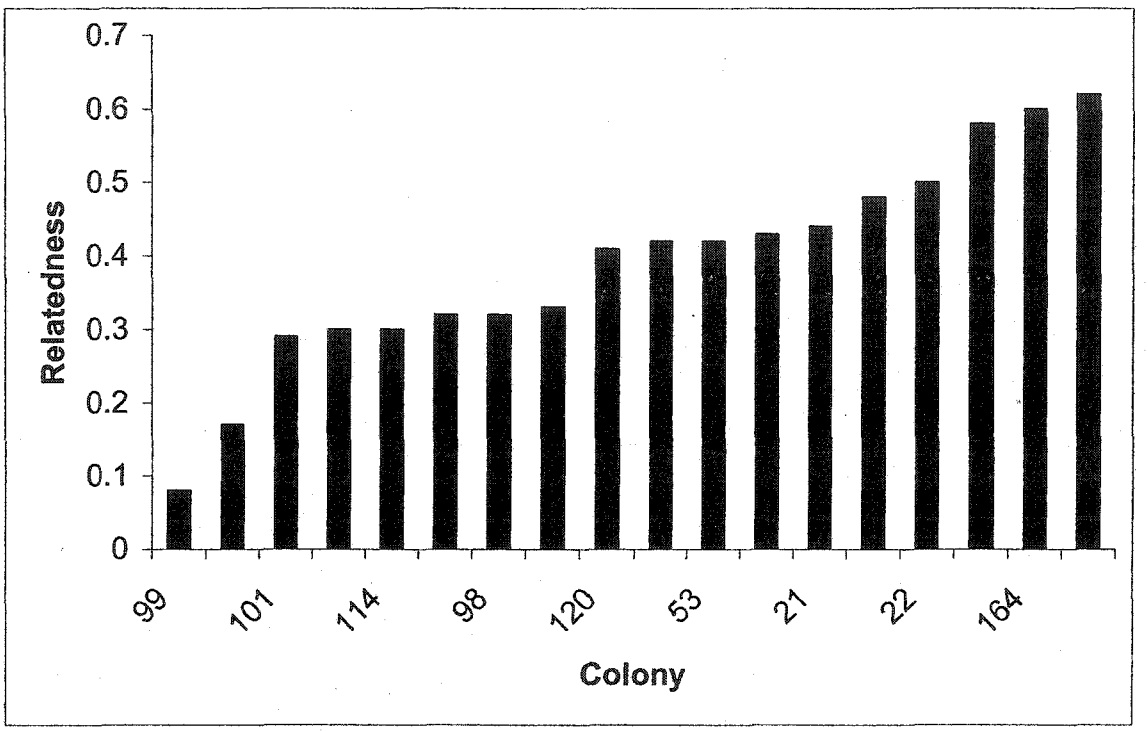

Figure 4.1. Intracolonial relatedness values for F. near *argentea* colonies sampled from Colorado, USA. N=18