Introduction

Parasitism is ubiquitous and most organisms are host to one or more parasites. However, which characteristics facilitate the evolutionary transitions from non-parasitic to a parasitic lifestyle are unclear. I will attempt to answer this question by studying a tractable social parasitic system. **Social parasitism**, the exploitation of a society by other social organisms, is widespread across social insects, birds, spiders and other animals.[1,2,3] The host's fitness may suffer to varying degrees, depending on the resource acquired (e.g. food, webbing, shelter, work through enslavement).

Investigations of social parasitism have focused on providing specific parasitic species accounts, on the phylogenetic relationship of parasites and hosts and on parasites' chemical and behavioral mechanisms used in host suppression.[3,4,5,6,7,8,9] Though this research has highlighted general characteristic correlated with parasitism, it has not determined the behavioral characteristics that may facilitate evolutionary transitions from free-living to parasitic lifestyles. One hypothesized behavioral precursor to parasitism is predation. The **Predation Hypothesis**, originally proposed by Darwin (1859), suggests that predatory raids on other species evolutionarily precede parasitic interactions.[10] Thus, the evolutionary refinement of infiltration strategies may lead to cohabitation rather than thievery. Buschinger (1989) suggested that there must be "widespread traits in the ordinary behavior of non-parasitic ants" that facilitate the establishment of parasitic associations.[11] Surprisingly, few of these "widespread traits" have been identified. I will look for behaviors that accompany predation and that may serve as important catalysts prompting the evolution social parasitism.

The **focal group** for this study is a monophyletic clade of ants in the genus *Megalomyrmex* (Tribe: Solenopsidini), some species of which parasitize distantly related hosts in the ant tribe Attini. While some species are completely dependent on their host for survival, others are facultatively associated with a host and still others are free-living predators. These various associations suggest a possible evolutionary transition from a free-living to parasitic state. A preliminary phylogeny, in fact, (Fig.2) suggests multiple origins of social parasitism across the clade. Using an integrative approach, the Predation Hypothesis will be examined. Phylogenetic analysis will elucidate the origins of social parasitism, while behavioral, ecological, and venom alkaloid data will distinguish which characteristics are necessary for social parasitism to evolve and help explain how social parasitism is maintained.

<u>Principle lines of inquiry:</u>

Question 1: Did social parasitism evolve once in the *Megalomyrmex* genus? If so, the parasites will form a monophyletic group. Alternatively, if social parasitism evolved multiple times, the parasites will form a polyphyletic group.

Question 2: Did social parasitism arise from behaviors related to a primarily predatory lifestyle, following the Predation Hypothesis? Behaviors involved in interspecific interactions, including chemical communication and host suppression, will be investigated.

Study System: Megalomyrmex

Megalomyrmex species are found from southern Mexico to Argentina. The genus currently comprises 32 described species and has been divided into four species groups (Silvestrii, Pusillus, Leoninus, and Modestus) based on taxonomic characters.[12,13] Outside of taxonomic description, most work reporting on *Megalomyrmex* are no more than obscure natural history accounts.[14,15,16] With the exception of alkaloid studies and taxonomic revisions, my recent work is the only work published on *Megalomyrmex* in almost twenty-five years.[12,13,17,18,19,20,21]

The paucity of life history data on *Megalomyrmex* is striking given their fascinating associations and habits. All *Megalomyrmex* species found outside the Silvestrii species group are assumed to be predatory.[12] Some of these species also tend homopterans, consuming honey dew in exchange for protection.

Focal Species: *Agro-predators and social parasites*

Eight *Megalomyrmex* species are classified in the Silvestrii species group and are assumed to be associated with the fungus-growing ants (Tribe Attini).[12] The attine ants have an obligate mutualism with their specific fungal cultivar strain, feeding, protecting, and dispersing it in exchange for nutrients.[3] However, the garden is a large food store, susceptible to consumption by other organisms through usurpation and parasitism. *Megalomyrmex* species may be facultatively or obligately associated with fungus-growing ants; the particular association varies depending on the *Megalomyrmex* ant species.[12,22] For example, *M. silvestrii* colonies are **facultative social parasites** capable of living in or outside their parasitic association.[12,22] *M. wettereri* colonies are **obligate agro-predators**, aggressively attacking attine host ants and usurping their garden, unable to live outside the association.[21] In contrast, *M. mondabora* and *M. symmetochus,* **obligate social parasites**, can only live with their host, sharing the fungal resource. These four species associate with several host species spanning both the basal and derived attine genera (Fig. 1).[21,23,24] For example, *M. wettereri* has been found associating with *Trachymyrmex bugnioni,* a higher attine closely related to the leaf cutting ants, and *Cyphomyrmex longiscapus*, a basal lineage.[23] Also, my laboratory experiments showed that *M. wettereri* can subsist on a variety of fungal garden strains from different attine host species.[21] In addition, they may use their alkaloids as universal repellents, similar to other Solenopsidini species.[22,25] This flexibility in fungal diet and potential nest infiltration strategy, suggests that *M. wettereri* ants are not specialized on their host's fungal garden strain. Therefore, fungal cultivar does not limit host choice and the universal repellents make more than one ant host species susceptible to parasitism.

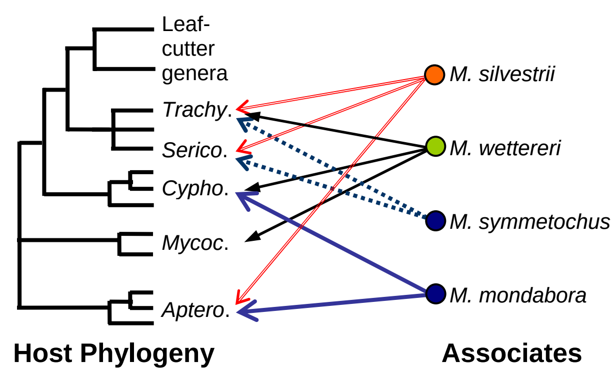

Figure 1: *Megalomyrmex* host association
The social parasitic species associate with a range of hosts spanning their phylogenetic tree.
*Host tree after Schultz and Meier 1995.[24]

The *Megalomyrmex* genus is ideal for studying the evolution of social parasitism for the following reasons: **1)** Taxonomy is clearly defined in this genus.[12,13] Thus, species identification is possible. **2)** *Megalomyrmex* ants exhibit a range of interesting life history characteristics. First, their omnivorous diet ranges from arthropod prey to fungal garden to honey dew. Second, they maintain predatory, parasitic, and mutualistic relationships. Third, some species produce wingless queens, others produce "true" winged queens, while still others produce both, thus impacting gene flow and population structure. **3)** The evolution of social parasitism within the *Megalomyrmex* genus is unusual because the parasites are distantly related to their host (many parasites are sister to their host).[26] Therefore, social parasitism has clearly evolved independent of the host lineage. **4)** *Megalomyrmex* ants produce unusual venom alkaloids that can be synthesized and tested in behavioral bioassays.[17,18] Determining their function will help clarify how social parasites infiltrate their host colonies. By using this tractable parasitic system, I will integrate evolution, behavior, sensory ecology, and chemistry, providing a comprehensive study elucidating the origins of social parasitism within this genus of ants.

PROPOSED RESEARCH

Evolutionary Relationships and Transitions

Question 1: Did social parasitism evolve once in the *Megalomyrmex* genus? If so, the parasites will form a monophyletic group. Alternatively, if social parasitism evolved multiple times, the parasites will form a polyphyletic group.

The genus *Megalomyrmex* has been taxonomically revised; however the phylogenetic relationships between the species in this genus have not been established (but see Fig. 2).[12,13] I will determine which behavioral and ecological characteristics lead to the evolution of social parasitism by examining them in a phylogenetic context. If parasitism evolved once, then the association between parasitism and other traits cannot be determined statistically, however these results would nevertheless be a critical first step in understanding how parasitism evolves. If there are multiple origins of social parasitism within this genus, then I can use the comparative method to test which behavioral and ecological traits are signifi-

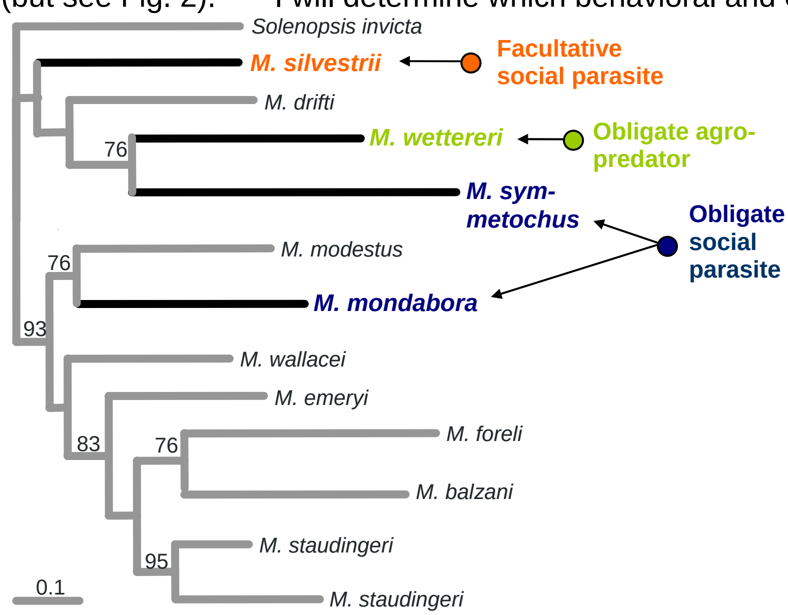

Figure 2: Multiple origins of social parasitism
The social parasites are polyphyletic, suggesting social parasitism has evolved more than once within this genus. *M. mondabora,* an obligate social parasite, is in a well-supported clade with non-parasitic species. *Numbers indicate Bayesian Posterior Probabilities above 75.

cantly associated with the evolution of parasitism in the context of the *Megalomyrmex* phylogeny.[27] In either scenario, the ecological and behavioral conditions prompting the evolution of social parasitism can be compared with other social-parasitic systems to begin understanding the patterns found on a larger scale.

Preliminary evidence

Using 12 species, I sequenced over 1000 base pairs of the mitochondrial cytochrome oxidase I (COI) gene and created a phylogeny using Bayesian methods (Fig. 2). Results reveal that the social parasites are polyphyletic and places *M. drifti*, a non-parasite, within a clade with social parasites. Although preliminary, these results, suggest social parasitism has been lost multiple times or evolved more than once within the *Megalomyrmex* genus. Sequencing additional *Megalomyrmex* species using COI and a nuclear gene will clarify the relationships within *Megalomyrmex*. In addition, a second independently derived phylogeny, using morphological characters, will be constructed and compared.

Proposed Research

Collecting: My collection currently consists of material I have colleted from nine field sites (in Costa Rica, Panama, Brazil, and Peru), mounted specimens on loan, and specimens I have received from other researchers. To improve my current phylogeny and complete the proposed behavioral research, I will carry out three expeditions (Costa Rica, Peru, and Ecuador) to both established and new sites with diverse *Megalomyrmex* species insuring success. I will use the same sampling techniques I have used for the last five years: baiting, nest sampling, and leaf litter sifting using Winkler Extractors to continue my collections.[28]

Outgroup: For adequate outgroup representation, I will use several species from the 12 other genera found within the Solenopsidini tribe for both morphological and molecular analysis. Two of these genera contain social parasites (*Solenopsis* and *Oxyepoecus*) and at least two (*Solenopsis* and *Monomorium*) biosynthesize alkaloid compounds for various competitive and aggressive interactions with other ant species.[25,29,30,31,32] Phylogenetic analysis will determine the most closely related sister taxa to the *Megalomyrmex* genus. Behavioral, morphological, and ecological features of the sister taxa can then be compared with those of *Megalomyrmex* species.

Morphology: I have on loan, from three museums, 28 of the 32 *Megalomyrmex* species. Morphological characters will be scored for all 32 *Megalomyrmex* species and several outgroup species. Five non-nestmate individuals will be photographed using Automontage imaging software and a high powered microscope. Several pictures from different angles will correspond to the morphological characters used to define *Megalomyrmex* species (full length side, head, mouthparts, dorsal view, and sting). The three dimensional photographs will then be analyzed and measured. Although there are external characters that clearly distinguish species (e.g. dental formula, petiole denticle) I will also examine the internal sting apparatus as recommend by Dr. Brandão. During my visit to his lab in October 2004, we discussed which characters will be reliable and the usefulness of the sting morphology.[33] All morphological work will be conducted in collaboration with two well known ant taxonomists – Drs Ted Schultz and Beto Brandão (see Letters of Support).

Molecular: The data will be generated using material stored in ethanol and from dried museum specimens. Sequence data will be generated using previously developed amplifiable primers for two gene regions, nuclear elongation factor-1 alpha (EF1a) and mitochondrial cytochrome oxidase I (COI).[34] Samples will be analyzed on the ABI 3100 automated sequencer in the Mueller lab and at the Human Genome Research Center in Brazil (see Budget Justification). I will examine the data using several phylogenetic methods, including unweighted/weighted parsimony, implied-weight parsimony, joining, maximum-likelihood, and Bayesian analyses.[35,36,37] Phylogenies will be independently estimated using multiple molecular data sets and morphological data sets. Congruence across these data sets will be tested.

Behavioral Processes and Parasitic Mechanisms

Question 2: Did social parasitism arise from behaviors related to a primarily predatory lifestyle, following the Predation Hypothesis? Behaviors involved in interspecific interactions, including chemical communication and host suppression, will be investigated.

Background

The Predation Hypothesis can be examined by studying behavioral transitions leading to social parasitism. It seems likely that the extreme dietary shift in *Megalomyrmex* evolution (from carnivorous to fungivorous) may have resulted from opportunistic foraging. It is possible that *Megalomyrmex* ant species entered attine nests to prey on brood and later shifted to consuming the abundant fungal resource. Identifying behavioral and ecological precursors **(Part A)**, as well as examining how venom alkaloids are used in non-parasitic and parasitic species **(Part B)**, will provide a necessary foundation to compare social parasites with non-parasitic species.

<u>PART A: Behavioral Processes</u>

Accomplished Behavioral Work

Since 1998, I have observed nine *Megalomyrmex* species in the laboratory to determine species-specific and clade-specific behaviors. As mentioned above, the social parasites in *Megalomyrmex* maintain distinctive relationships with their hosts and likely impact them in various ways. For example, I have observed both *M. wettereri* and *M. mondabora* consuming host brood and fungal cultivar. In addition, I have observed *M. symmetochus* workers chewing the wings of their host's female reproductives (unmated queens), rendering them functionally sterile (Fig.3). The consumption of offspring and wing removal of future queens benefits the parasite (wingless queens perform worker tasks) and negatively impacts the ant host and their fungal mutualist (neither the ants nor the fungus disperse). These unfavorable consequences of the parasitic interaction intuitively lead to the question of how the parasite can persist when natural selection is likely favoring resistance by the host ants as well as their cultivar. A co-evolutionary arms race is expected unless the parasites are too rare to exert selection.[38] It is possible that the parasites are not specialized on a sin-

Figure 3: *M. symmetochus* workers remove their host's reproductives wings, forcing them to perform worker tasks rather than dispersing with the garden.

gle host species and use general strategies that can be applied to many host species, just as a predator may hunt different prey using the same strategy. Previous studies suggest that *Megalomyrmex* use venom alkaloids similarly to *Solenopsis* and *Monomorium* species as repellents during competitive interactions or during host nest infiltration.[22,32] It is feasible that predatory behaviors found in *Megalomyrmex* species could shift to parasitic functions over evolutionary time.

Proposed Research

I will conduct extensive observations using a video camera to record *Megalomyrmex* colony behavior both in the field and in captive colonies housed in nest boxes in the laboratory. I will first establish peak activity time and duration by recording colonies in two second frames, every five minutes, over 24 hours. Then I will record each colony for six hours in 1.5 hr bocks on separate days during peak activity. A list of behaviors found in each species will be compiled (i.e., trail laying, alarm postures such as gaster-flagging and gaster-tucking). I will mentor and train undergraduate students to help with analysis as I have done successfully in the past.

Each behavior found in a parasitic species will be compared to similar behaviors seen in non-parasitic species. For example, some species of *Megalomyrmex* have distinct alarm postures. Using my phylogenetic results, ancestral behavioral character states will be proposed by character mapping. By mapping these characters, I will be able infer evolutionary transitions.

<u>PART B: Parasitic Mechanisms</u>

Background

There is a trend among researchers to examine chemical mechanisms used in nest infiltration, specifically how hydrocarbons and other chemicals are used by the parasites.[8,9,39,40,41,42,43] Alkaloids, stored in ant venom glands, likely have many functions. Some Solenopsidini ant species use them as repellents and as trail pheromones while others use them in nest raiding like *Solenopsis fugax*.[19,25,31,32] Elaborating on previous alkaloid work, I will continue to study alkaloids produced by *Megalomyrmex*.[17,18,19,25,32] If alkaloids are used as repellents by predatory *Megalomyrmex,* then perhaps the parasitic species use them in a similar way.

Preliminary Evidence

Behavioral evidence and alkaloid use in host suppression. *Megalomyrmex* species appear to use alkaloids when entering a naive host colony. Through extensive observation I have determined that several host species react immediately when in contact with *Megalomyrmex* ants.[20,21] When a foreign *M. symmetochus* ant is introduced to a host colony (*Trachymyrmex* cf. *zeteki*), host workers immediately attack, and not until they have received several repeated antennal taps by the parasite, does their aggression subside. *Cyphomyrmex longiscapus* ants become alarmed, collecting brood and fungal garden, when *M. wettereri* ants are near. *M. wettereri* uses an alarm posture called gaster-flagging, during which they likely emit volatile repellent pheromones. *Cyphomyrmex cornutus* is initially aggressive towards foreign *M. mondabora* ants, using jerking threats with open mandibles until touched by the *Megalomyrmex* ant. Immediately after contact, the *C. cornutus* ant pulls in her antennae, tucks her head under, and backs away. These responses suggest chemical signaling received by the host.

Alkaloid characterization and synthesis. My collaborator, Dr. Jones, a chemist from Virginia Military Institute, determined the alkaloid compounds found in ten *Megalomyrmex* species that I have collected and five more samples collected by other researchers. In addition, he recently completed the first synthesis of an alkaloid compound biosynthesized by the social parasite, *Megalomyrmex mondabora*. This is an excellent species for field behavioral studies because it parasitizes four different attine hosts and one of these, *Cyphomyrmex cornutus* has accessible, abundant, and observable colonies (40 nests are currently marked at La Selva Biological Station).[20] When I return to La Selva next summer, I will use these colonies for alkaloid bioassays.

Interspecific response to Megalomyrmex alkaloids. I predict that alkaloids are used by non-parasitic *Megalomyrmex* species as repellents during interspecific competition much like the parasitic species use them when infiltrating a host colony. In order to observe how non-parasitic *Megalomyrmex* species might use alkaloids against potential competitors, my undergraduate assistant and I conducted a pilot study. We observed interspecific and intraspecific response to prominent alkaloids characterized from *M. modestus* ants. Responses to those alkaloids were tested in *M. modestus* and *M. wallacei*, all of which are sympatric at La Selva Biological Station in Costa Rica. *M. wallacei* and *M. modestus*, are both non-parasitic *Megalomyrmex* species and possibly compete for food. *M. wettereri*, a fungus growing ant associate, consumes primarily fungal garden and therefore is an unlikely competitor with non-parasitic species. These three species were given a choice between a test solution (9µl of red sugar water and 1µl of ethanol laced with 1µg of the alkaloid 2-butyl-5-heptylpyrrolidine isomer mixture) and a control (9µl of red sugar water and 1µl of ethanol). As expected, *M. modestus* showed no preference for or avoidance of either the control or test solution containing its own alkaloid compounds. In contrast, *M. wallacei* and *M. wettereri* showed a preference for the pure sugar solution and avoided the test solution. These results suggest that this heptylpyrrolidine may repel congeneric ant species, even those that are unlikely competitors. Similar studies will be conducted using host workers and comparable alkaloid solutions isolated from parasitic species to see if host ants have similar responses to interspecific intruders.

Proposed Research

To examine chemical infiltration mechanisms, each *Megalomyrmex* species will be analyzed by Dr. Jones using gas chromatographic-mass spectral analyses. The predominant alkaloids found in the social parasites and their sister species will be synthesized and diluted into solvent (ethanol). These dilutions as well as the venom sac contents will be tested in bioassays to determine what behavioral responses may be elicited from exposure.[19] Video recordings of all trials will be scored blind.

The prominent alkaloid or the entire venom sac content is used as a repellent by *Megalomyrmex* ants when interacting with their host.

Prediction & Test ❶: If prominent alkaloids used by *Megalomyrmex* act as repellents, I predict avoidance behavior will be observed in the following experiment. Two adjoining food chambers will be connected to the host colony chamber. One will contain a food dish with red sugar water mixed with the synthesized alkaloid solution and the other chamber will contain a food dish with sugar water mixed with the solvent and will serve as a control. Individuals drinking from the sugar mixture and walking around the food chamber will be counted separately. Each host colony will be tested once (n=20) and chamber positions will be randomly selected.

Prediction & Test ❷: If alkaloids used by *Megalomyrmex* act as repellents, I predict that when host workers detect alkaloids, they will flee or avoid the alkaloid substance in the following experiment. Using an experimental design similar to Mori et al. (2000), host species will be exposed to alkaloid solutions and their reactions will be scored.[44] One week prior to the start of the experiment, 20 host colonies will be subdivided in to two subcolonies containing five workers and a small garden fragment. After one week, a filter paper punch (4mm diameter) will be saturated (5µl) with the alkaloid solution, solvent, or the entire contents of the venom sac from one individual, and then placed on the host's fungus garden. Reactions by the host workers will be scored [strong avoidance (no contact), submissive response upon contact, paper removal, and aggression (lunge, bite, etc.)].

INTELLECTUAL MERIT & TIMELINE

Because all organisms are susceptible to parasites, there is diverse evolutionary and ecological research on host-parasite interactions. Surprisingly, few studies have identified which characteristics facilitate the evolutionary transition from non-parasitic to a parasitic life-style. Using the *Megalomyrmex* ant genus, I will apply my skills in phylogenetics, field techniques, and behavioral experimentation to elucidate the origins of social parasitism. By gathering behavioral, ecological, and sensory ecology information, and analyzing it in a phylogenetic context, the *Megalomyrmex* system will be comparable with other parasite systems, and will thus broaden our understanding of the evolutionary processes associated with the onset and maintenance of host-parasite associations.

Oct.-Jan'04	Jul–Aug'05	Sep'05–Jul'06	Aug'06–Jan'07
Brazil & Wash.D.C.: Morphological training & data collection **Panama:** Ant collection & behavior	**Costa Rica:** Collection & behavior **Salt Lake City:** Animal Behavior Meeting **Manuscript prep.:** -Alkaloid Description	**Austin:** Molecular & morphological data collection **Peru/Ecuador:** Ant collection & behavior. **Manuscript prep.:** -Behavioral Study -Alkaloid. Function	**Wash. D.C./Virginia:** Morphological completion, IUSSI Meeting **Brazil:** Molecular data completion **Manuscript prep.:** - Alkaloid. Description - Phylogenetics
Funded	$2,188/partially funded	$3,951	$ 8,404