

## PROJECT SUMMARY

### **Objectives and Methods**

Ecological studies of local and regional drivers of community assembly of tropical organisms are often limited to observation of static assemblages because manipulations are impractical or impossible. This proposal takes advantage of an easily manipulated arboreal twig-nesting ant community in a coffee agroecosystem to explore ecological evidence for multiple drivers of community assembly. The project will use environmental niche partitioning in colony arrival and survival as a conceptual framework for addressing assembly processes for these ants. The main objectives are to 1) examine life history traits including colony size, fecundity, dispersal timing, dispersal limitation, and competitive ability, 2) examine changes in relative abundance of ants in years following a large-scale disturbance and correlate observed changes with life history traits of twig-nesting species, 3) examine the relative impact of environmental variation, interactions with twig-nesting ants, and interactions with a canopy dominant ant on colony recruitment, 4) examine the relative impacts of environmental variation, interactions with twig-nesting ants, and interactions with a canopy dominant ant on colony survival. Surveys and manipulations will be conducted in a 45-ha plot where drastic canopy cutting occurred in 2007 and 2009, and in an uncut 6-ha control plot. Two large-scale experiments with nest additions and reciprocal transplants will be used to directly compare the influence of multiple factors (e.g. local and large scale environmental variation, and competition from twig-nesting ants and a canopy dominant) on recruitment and colony survival.

### **Intellectual merit of the proposed study**

The overarching goal of the research is to examine multiple drivers of community assembly in a tropical twig-nesting ant community. Although others have examined coexistence and assembly processes for tropical ants, studies and observations have been largely limited to a few species, and much previous work is not experimental or spatially explicit. Furthermore, previous studies have rarely examined the relative importance of multiple driving factors. The proposed work will evaluate the importance of environmental variation at two distinct spatial scales and will document a suite of important life-history traits for a relatively little known assemblage of diverse twig-nesting ants. The proposed observations, surveys, manipulations, and analyses will mechanistically test the importance of different factors in ant community assembly, and will enable examining the relative importance of each factor. The proposed work will generate a large, long-term, spatially explicit, community-level data set. The data will be the first of its kind for a tropical insect, despite the importance of insects for ecosystem function. Finally understanding community assembly of ants in coffee agroecosystems will contribute to general ecological theory and to coffee management as ants are important predators and have large influences on resources in tropical ecosystems.

### **Broader impacts resulting from the proposed activity**

The proposed activity will result in an increased understanding of community ecology and the relative importance of different factors influencing community assembly. The proposed work will result in numerous high-quality publications. The project will train undergraduate and graduate students both from the University of Toledo, and from Mexico and will form the framework for several undergraduate, MS and PhD theses. The project will also prepare a postdoctoral researcher for a successful professional career with careful mentoring. Students and the postdoc will be chosen from a diverse pool, including internationally, and one aim of the project will be to increase diversity and the representation of women ecologists. The extensive ant material collected will be used to a) enhance museum collections in the US and Mexico, b) teach undergraduates and graduate students in ant taxonomy and identification, c) create high resolution photographs of ants to publish web pages and field guides for the ants of Chiapas. Furthermore, coffee is of extreme importance from a conservation standpoint, and ants are important biological control agents in coffee agroecosystems. Elucidating factors contributing to ant assembly will be important for management decisions and for sustainable agricultural production.

# TESTING MULTIPLE HYPOTHESES OF COMMUNITY ASSEMBLY USING A TROPICAL INSECT: TWIG NESTING ANTS IN A COFFEE AGROECOSYSTEM

## I. RESPONSES TO REVIEWERS

I thank the reviewers for their helpful comments. I respond to their general concerns here.

1. General questions about conceptual framework & structure. I have altered the proposal to examine environmental niche partitioning in colony arrival and survival processes as a general conceptual framework. I have restructured the proposal as suggested by reviewer 3.

2. Improvement in experimental methodologies. For trials examining *competitive hierarchies* of twig-nesting ants (TNAs), reviewers questioned lab trials, statistical methods to examine hierarchies, and whether entire colonies vs. individuals would be fought. I have added field experiments to examine competitive hierarchies among TNAs and added treatments to the recruitment and reciprocal transplant experiments that will allow for examining the influence of existing ant colonies on recruitment and survival. For lab trials, I will statistically examine hierarchies as outlined by LeBrun 2005, as suggested. I will primarily fight entire colonies of species, but may attempt to conduct “gladiator” trials (sensu Yu *et al.* 2004) to fight foundress queens as time and their discovery allow. One reviewer asked about potential *bias in identification* or encounter of species based on sample location. There is not, as all ants will be sampled from coffee plants by removing all dry twigs and opening them to find ants. They are very easy to see. One reviewer again questioned *collecting alates* – I have retained this part of the methodology, but have reframed tests to primarily examine for a) reproductive phenology of TNAs, b) clustering of dispersing queens, and c) dispersal rates and will not examine directly dispersal distances (also hindered because not every plant would be sampled). Explanations of dispersal limitation were modified.

3. Ensure that the transplants will work: Last summer, I conducted a successful pilot experiment. Results are discussed in preliminary data. All transplanted colonies will be arboreal TNAs, not litter ants, as questioned by one reviewer.

4. Perhaps not measuring the right environmental variables? What if food is the important factor? Of course any set of experiments is limited by what is measured. I argue that because TNAs are nest-site limited, that this is likely a strong factor affecting assembly processes, and preliminary evidence indicates that nest abundance and canopy complexity also affect TNAs. It is possible, and even likely, that other factors affect TNAs, however, a) field observations of TNA foraging in the field are relatively difficult (rarely seen at baits), and b) as the proposal includes a large workload, I have chosen to focus on nests and the canopy as two potential factors and hope to examine other factors, including food, in the future.

5. How will the different factors be compared? I have modified experiments so that factors influencing recruitment and colony turnover or survival processes can be directly compared with Multiple Regression Trees (MRT). MRT follows a hierarchical model, selects a series of predictor variables to explain the response variable (e.g. relative abundance of different ant species), and can thus determine the relative importance of different factors. Additional tests of life-history trade-offs and experimental results will be used to examine the relative importance of environmental variability at two spatial scales, and competition from TNAs and *Azteca* canopy dominants.

6. Is sampling 1 coffee plant around each tree enough to detect all ants? I have modified the larger scale surveys to focus on a set of hectares that will be sampled as described before (large-scale) but also in targeted 20 x 20 m plots to improve detection.

7. One reviewer suggested focusing on one or a few ant species, perhaps *Azteca*. First, *Azteca* is not a TNA, thus would not be a good representative for examining mechanisms of coexistence for the TNA community. Second, I argue that the use of a diverse assemblage of ants is a strength and novel component of the proposal.

8. Detailed comments (e.g. tree spacing, timing of experiments) are changed in the text. Other comments (e.g. related to phylogenetic community structure) are no longer relevant due to proposal restructuring.

## II. CONCEPTUAL FRAMEWORK

The enigmas associated with the structure of ecological communities have long fascinated ecologists, from Darwin's tangled bank to the varied and complex questions currently populating the ecological literature about community assembly (e.g. Bell 2001, Hubbell 2001, Leibold *et al.* 2004, Chase 2005, Gotelli & McGill 2006). Several theories outline mechanisms for species co-existence at both local and regional scales (Chesson 2000, Amarasekare *et al.* 2004, Holyoak *et al.* 2006). In the case of ecological dynamics, categorization of mechanisms into those that operate at a local level (e.g. interspecific competition, differing resource requirements, predation) and those that operate at a more regional level (e.g. dispersal, recruitment limitation) remains a useful framing (Ricklefs 1987). It is a framework that has been applied most frequently to sessile organisms like plants (e.g. Hubbell 2001, Foster *et al.* 2004, Tilman 2004, Karst *et al.* 2005). Due to their intrinsic mobility, this community assembly framework has not been commonly applied for terrestrial (or tropical) animals (but see Cottenie & DeMeester 2004, McCauley 2007).

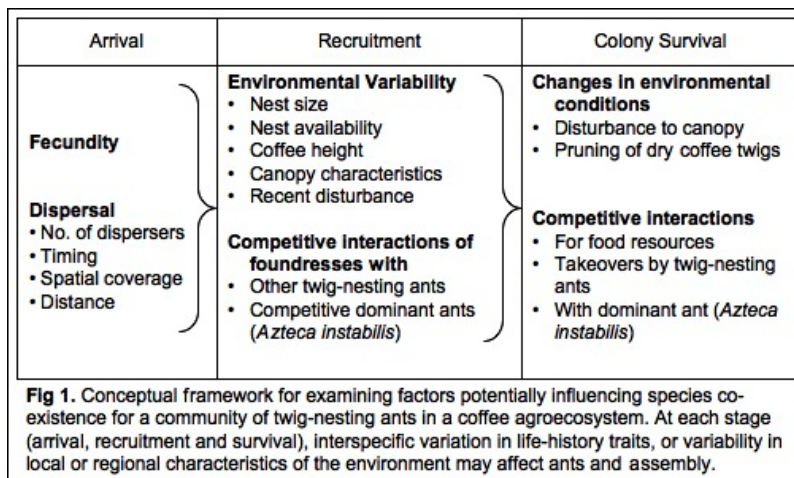
Recently, these local and regional drivers have been synthesized in the metacommunity approach. A metacommunity is a network of local communities linked by dispersal, and the four main metacommunity perspectives emphasize competition-colonization trade-offs (patch dynamics), environmental differences in patch types coupled with differences in species traits (species-sorting), high levels of immigration and emigration from patches (mass-effects), and random walks altering relative abundance of species (neutral) (Leibold *et al.* 2004). Even though differences in local vs. regional processes are implicit in metacommunity formulations, there is acknowledgement of a lack of attention to the role of explicit landscape structure in models used to describe community assembly (Leibold *et al.* 2004). In a spatially explicit model of a metacommunity, several factors can influence community assembly including quality local habitat patches, patch arrangement, the locations and dispersal ability of individuals of species, and species-specific responses to invading competitors (Moquet & Loreau 2003, Cadotte 2006).

Here I take advantage of an effectively sessile animal community (arboreal twig-nesting ants) in a relatively uniform environment (a coffee plantation) to explore the relative importance of multiple factors in community assembly. In many ways, this is an ideal and important study system in which to assess community assembly processes of ants. First, many questions regarding community assembly and other aspects of ecology are difficult to test in more complex natural systems making the somewhat less diverse agroforests ideal (Greenberg *et al.* 2008). Diversity of twig-nesting ants in coffee agroecosystems is high (~40 species), but not so large as to be unmanageable. Second, twig-nesting ants live in discrete nest sites (e.g. twigs) and are easy to manipulate making possible experiments that are difficult with other groups of tropical insects. Third, managed agricultural systems cover approximately 60% of the earth's terrestrial surface (Scialabba & Williamson 2004). Thus understanding ecological processes therein is of the utmost importance. Especially in the context of global environmental change (spurred by changing climates, habitats, and invasive species), understanding ecological processes in managed systems, and systems that vary in ecological complexity (such as coffee agroforests) will provide important data. Coffee systems in particular are extremely important habitats in N. Latin America and elsewhere providing critical habitat for biodiversity (e.g. Philpott *et al.* 2008), and livelihood for millions of people.

Ants make ideal organisms for testing hypotheses about community assembly and species co-existence (e.g. Palmer *et al.* 2000; 2003, Stanton *et al.* 2002, Sanders *et al.* 2003, McGlynn 2006, Dunn *et al.* 2007). Ants are ubiquitous and diverse; in tropical ecosystems ants represent up to 80 % of animal biomass (Hölldobler & Wilson 1990). Understanding spatial distribution and subsequent formation of tropical ant assemblages has long intrigued community ecologists (Room 1975, Majer 1978). At the local level, several factors may contribute to ant community assembly including, but not limited to, 1) preferences or ecophysiological requirements for, availability, or diversity of particular microhabitats (Nestel & Dickschen 1990, Morrison 1998, Kaspari & Weiser 2000, Albrecht & Gotelli 2001, Ribas *et al.* 2003), 2) lack of preferred size or type of nesting site (Leston 1978, Herbers 1989, Torres & Snelling 1997, Armbrrecht *et al.* 2004), 4) food availability and distribution (Kaspari *et al.* 2000, Blüthgen *et al.* 2004), 5) interspecific competition from native or invasive ants (Leston 1978, Savolainen & Vepsäläinen

1988, Holway *et al.* 2002, Parr & Gibb 2010), 6) changes in competitive hierarchy due to environmental conditions (Cerda *et al.* 1997), and 7) impacts from predators, fungal diseases, or parasites (Feener 2000, LeBrun 2005). At regional scales, dispersal limitation may affect ant assembly (Yu *et al.* 2004, Bruna *et al.* 2005). Several studies use null models to detect assembly rules in ant communities (e.g. Gotelli & Ellison 2002, Sanders *et al.* 2003, Dunn *et al.* 2007) and have reviewed empirical evidence for different assembly mechanisms across systems (e.g. Andersen 2008). Although competition is the ‘hallmark’ of ant ecology (Parr & Gibb 2010), several factors likely contribute to assembly processes for ants (Fig. 1) (Palmer *et al.* 2003, Andersen 2008). Among the areas of ant community assembly that have received little attention include a) processes acting at the recruitment stage, b) effects of environmental differences on outcomes of competition, and c) the influence of patchily distributed dominant ants on other ant species and diversity (Andersen 2008). Further, compared with other taxa, little manipulative work has synthetically examined local and regional drivers of community assembly within a single study system (but see Palmer *et al.* 2000). Thus many questions about assembly of diverse tropical ant assemblages have not been addressed.

I will follow environmental niche partitioning in colony arrival and survival processes as a general conceptual framework for



addressing assembly processes for these ants. This idea most closely follows the ‘species-sorting’ perspective of metacommunity dynamics. Environmental niche partitioning hypothesizes that coexistence results from life-history trade-offs among competing species co-occurring in habitats with at least one source of environmental heterogeneity (Yu & Wilson 2001, Debout *et al.* 2009). In obligate plant-ants, several authors have documented life-

history trade-offs in traits such as competitive ability, fecundity, colony size, and dispersal ability (Palmer *et al.* 2000, Yu *et al.* 2004, Debout *et al.* 2009). For twig-nesting ants, existing in frequently disturbed coffee agroecosystems, I will examine differences in life-history traits related to colony size, fecundity and dispersal rates of ants. Then using two large manipulative experiments, I will examine the relative influence of environmental heterogeneity (at two distinct spatial scales) and competition (from other twig ants and a dominant ant species) on colony recruitment and long-term survival of ant colonies.

### III. RESEARCH OBJECTIVES

The proposed research will investigate multiple factors affecting community assembly for twig-nesting ants (TNAs) in coffee agroecosystems. I will use an integrative approach by combining surveys and experiments, and a large ecosystem disturbance to examine assembly processes for a community of twig-nesting ants in a coffee agroecosystem. The overarching objectives of the research are to:

- 1) Examine variation in life history traits among TNAs including colony size, fecundity, dispersal timing, dispersal limitation, and competitive ability
- 2) Examine changes in relative abundance of ants in years following a large-scale disturbance and correlate any observed changes with life history traits of individual species in the TNA community
- 3) Examine the relative impact of environmental variation at the local and regional scale, interactions with TNAs, and interactions with a canopy dominant ant on recruitment processes of twig-nesting ant colonies
- 4) Examine the relative impact of environmental variation at the local and regional scale, interactions

with TNAs, and interactions with a canopy dominant ant on colony survival of twig-nesting ant colonies

This work is exciting and novel because it will develop a spatially explicit, long-term data set on community dynamics for a group of tropical insects. The data generated will be the first of its kind for a *diverse* group of tropical ants. The work takes advantage of the fact that TNAs are easy to study and manipulate, and that they exist in an environment in which I can test predictions about how community assembly varies along habitat gradients. I will simultaneously measure a suite of life-history traits for this ant diverse community, and examine the potential for and particular form (e.g. nonlinear) of trade-offs. Understanding the spatial distribution and community assembly of ants in coffee agroecosystems is important both for informing general ecological theory and for management practices, as ants are important biological control agents and have large influences on food webs in tropical ecosystems.

#### IV. BACKGROUND

**a. Study site and system:** Fieldwork will take place in a coffee agroecosystem in Chiapas, Mexico. *Finca Irlanda* (15°20' N, 90°20' W) is a 300 ha certified organic coffee farm with ~250 shade trees ha<sup>-1</sup>. Trees are spaced approximately between 5-8 m apart and are uniformly distributed in the farm, except along roads where they are clumped (Vandermeer *et al.* 2008). Previous work at Irlanda, funded by four NSF awards (DEB-9981526, DEB-0349388, and GRFs to SMP and G. Livingston), provides detailed background on the arboreal ant community allowing for testing detailed hypothesis about mechanisms driving community assembly. There are >80 arboreal-foraging ant species on the farm (Philpott *et al.* 2006). These can be divided into three general groups: ground-nesting ants that opportunistically forage on baits, competitive dominants that live in carton nests or in tree trunks, and arboreal twig-nesting species that nest in hollow cavities of twigs in trees and coffee plants. I will focus primarily on assembly of the arboreal twig-nesting ant (TNA) community, and interactions between TNAs and one competitive dominant ant, *Azteca instabilis* (hereafter *Azteca*). There are at least 33 species of arboreal TNAs at the study site: *Brachymyrmex* (4 species), *Camponotus* (2), *Cephalotes* (1), *Crematogaster* (8), *Dolichoderus* (1), *Myrmelachista* (2), *Nesomyrmex* (2), *Procryptocerus* (1), *Pseudomyrmex* (11), and *Technomyrmex* (1) (Philpott & Foster 2005, Livingston & Philpott, *in review*). Although community-level studies of ants are made difficult by low taxonomic resolution (Gotelli 2004), species accumulation curves generated from recent data demonstrate that most TNAs at the site have been collected. Most have been identified to species, and queens have been collected from nests for most species. TNAs in coffee agroecosystems are nest-site limited in terms of number (Philpott & Foster 2005) and diversity (Armbrecht *et al.* 2004) of nesting resources. Loss of ant diversity with coffee management intensification is associated with decreases in canopy cover and tree richness (Philpott *et al.* 2008), changes in competitive hierarchies under different microclimates (Perfecto & Vandermeer 1996), and distance to forest fragments resulting from a lack of needed resources or dispersal limitation (Perfecto & Vandermeer 2002).

Six years ago, a 45-ha plot was established at Irlanda within which ~11,000 tagged and mapped trees have been monitored for *Azteca* each year. During 2007 and 2008, owners of the farm conducted what I consider a large experimental manipulation in which >3000 of the trees were removed in the 45-ha plot. The stated goal of the farmers was to reduce the canopy cover from 75% to 50% throughout the affected area of the farm. This change in the farm management provides an opportunity for examining the effect of environmental heterogeneity, specifically a large-scale disturbance, on assembly processes in the ant community. Since the experimental manipulation, a new 6-ha plot has been established in an additional area of the 300-ha farm that has not undergone any manipulation to the shade canopy and remains under 75% shade cover. This 6-ha area thus acts as a control and an area in which the distribution of trees and *Azteca* ants is not significantly different that in the affected area of the farm (Perfecto *et al.*, *in review*).

Although both of these areas have been used for extensive censuses on *Azteca*, scale insects, an entomopathogenic fungus, and coccinellid beetles (e.g. Liere and Perfecto 2008, Vandermeer *et al.* 2008, Jackson *et al.* 2009) no work has comprehensively examined the assembly processes of TNAs that

comprise a large fraction of the arboreal ant community until now. I will survey TNAs within the plot to examine life-history traits, arrival, recruitment, and survival processes along environmental gradients and will experimentally manipulate TNAs to examine the relative impact of multiple factors driving community assembly and species coexistence. Working at this extensive spatial scale will be beneficial in capturing the range of environmental and microclimatic conditions at the site. Important changes in the biotic environment can only be captured at larger spatial scales (e.g. presence and abundance of *Azteca*, habitat quality). Ant dispersal spreads from 10's to 1000's of meters, and thus a larger spatial extent is necessary for capturing the majority of dispersing queens in order to compare the roles of local vs. regional factors in structuring the ant community.

**b. Agroecosystems:** Research in agroecosystems is important for several reasons. Examining mechanisms of community assembly is difficult in highly heterogeneous tropical forests where ant diversity is highest. Alternatively, agroforests, crop systems with trees, are model systems for examining spatial ecology, habitat choice, trophic interactions, diversity-function relationships, and landscape influences on biodiversity (Greenberg *et al.* 2008). In fact, pioneering work on ant ecology was conducted in agroforests (Leston 1978, Majer 1978). Agroforests are homogeneous relative to tropical forests yet habitat characteristics that affect ant assemblages in forests (e.g. canopy cover, tree richness, nest site availability) vary at small scales within agroecosystems. Such variation is due both to inherent differences in agricultural management and due to the increased accessibility of environmental manipulations in agroforests compared with tropical forest systems. Thus work in agroecosystems allows testing how mechanisms of community assembly vary across environmental gradients. From an ant's eye view, physically separated coffee bushes in agroforests represent separate habitats, and as such, ant community dynamics in coffee plants can be modeled as distinct habitats connected by dispersal. Finally, although some argue that studying pristine environments is more valuable, agricultural systems represent a large fraction of tropical landscapes. Understanding community assembly in disturbed ecosystems is thus important from a standpoint that incorporates conservation, global change, ecosystem function, and food production into our ecological lens.

## V. RESEARCH HYPOTHESES

***Life History Traits:*** One primary objective of the research is to determine whether there is interspecific variation in a suite of life history traits of TNAs and whether that variation influences community assembly. Several authors have argued that life-history trade-offs are important in maintaining coexistence of tropical ants. For example, Stanton *et al.* (2002) identified competition-colonization trade-offs between foundress queens for nest-site colonization, and between mature colonies during colony expansion processes onto nearby trees. Further, Yu *et al.* (2004) concluded that of three life history trade-offs discovered among plant-ants in the Amazon that fecundity-dispersal trade-offs best explained relative abundance patterns; fecund ants are more common in patches of high host tree density, better dispersers are more abundant in areas of low host tree density. Dispersal is also important to coexistence patterns and assembly, but few have examined dispersal of tropical ants (Bruna *et al.* 2005). Queen dispersal is usually density dependent (Cole & Wiernasz 2002, Yu *et al.* 2004) and dispersal limitation is implicated as one mechanism limiting ant diversity in both fragmented (Bruna *et al.* 2005) and urban areas (Pacheco & Vasconcelos 2007). Furthermore correlating changes in relative abundance of ants with time since disturbance may allow an examination of trade-offs in life history traits, as those ants becoming more or less abundant may share specific traits. Based on this background, I propose the following hypotheses regarding life history trade-offs for TNAs:

- H1: TNA species will differ in colony size and will exhibit a strong gradient in alate: worker ratio.
- H2: TNA species will differ in timing of dispersal events and relative abundance of dispersing queens.
- H3: TNA species will vary in degree of dispersal limitation.
- H4: TNA species with high fecundity, and high dispersal rates will have higher relative abundance in more recently disturbed habitats.

**Competition:** Competition is one of the foundations or “hallmarks” of ant community ecology (Leston 1978, Savolainen & Vepsäläinen 1988, Holway *et al.* 2002, Parr & Gibb 2010). For TNAs, nest takeovers are common and nest sites are often limiting, thus competition for nest sites is commonly inferred (Brian 1952, Yamaguchi 1992), and in at least one case has been experimentally demonstrated (Palmer *et al.* 2000). In addition, competition between queens or individuals of budding colonies may limit the founding of new colonies (Stanton *et al.* 2002). It is clear that ants in coffee agroecosystems are nest-site limited (Philpott & Foster 2005), but it is relatively unknown what are the specific roles of other TNAs in limiting recruitment of new colonies, or which species have the ability to take over nests from others or hinder recruitment. In addition, competition from *Azteca instabilis*, a canopy dominant may affect recruitment to new nests, or may act as a factor altering competitive hierarchies between TNAs. Although competition for resources other than nest sites may be important in structuring the twig ant community, I will focus on what is an important limiting resource for the twig-nesting community. Specifically, I hypothesize that:

H5: TNAs form a clear dominance hierarchy for nesting sites in controlled environments and TNAs compete for nest sites in the field.

H6: Presence of twig-nesting ant nests hinders colonization by foundress queens and alters survival of mature colonies.

H7: Presence of *Azteca* on coffee plants hinders colonization by foundress queens and alters survival of mature colonies.

H8: Relative abundance of competitively dominant species increases with time since nest establishment or disturbance.

**Environmental variability:** Modern formulations of niche theory for community assembly posit that variations in biophysical characteristics of the environment (including temporal and spatial variation, and changes in species interactions in time and space) create networks of available locations into which species with different requirements can colonize and persist (Chase 2005). For TNAs in this coffee system, there are three primary sources of environmental variation, each that act at a distinct scale: twig-size, nest availability on a coffee plant, and the environment surrounding the nest. In coffee agroecosystems, in particular, where coffee is grown under a canopy of shade trees, reduction of canopy complexity influences ant richness (e.g. Perfecto & Vandermeer 2002, Philpott & Foster 2005, Philpott *et al.* 2006). In addition, some TNA species also inhabit canopy twigs, so canopy disturbance may strongly affect the ant community within coffee twigs (Philpott, *in prep*). If environmental differences are important to TNA assembly, I hypothesize that:

H9: There will be strong differentiation in nest size distributions for TNA species and TNAs will show strong preferences for different sized nests.

H10: Nest availability on coffee plants will affect the colonization and survival of TNAs

H11: TNA relative abundance will vary along gradients of nest availability.

H12: Relative abundance of TNAs will vary along gradients of canopy structure (e.g. canopy cover, tree density, tree diversity).

H13: Colonization and survival of TNA colonies will vary along an environmental gradient of canopy structure.

## VI. PRELIMINARY DATA

**Life-history traits:** Some preliminary data has been collected on basic life-history traits of TNAs such as colony size, number of queens, and worker:alate ratios in both natural nests (Livingston & Philpott, *in review*) and artificial nests (De la Mora & Philpott, *in prep*). Among natural nests colony sizes vary by an order of magnitude from 117 individuals (*Myrmelachista mexicana*) to 7.5 individuals (*Dolichoderus lutosus*); the fraction of colonies comprised of alates also varies greatly from 71% (*M. mexicana*) to 0.8% (*Pseudomyrmex elongatus*). Furthermore, 66% of twigs housing *D. lutosus* only contained queens,



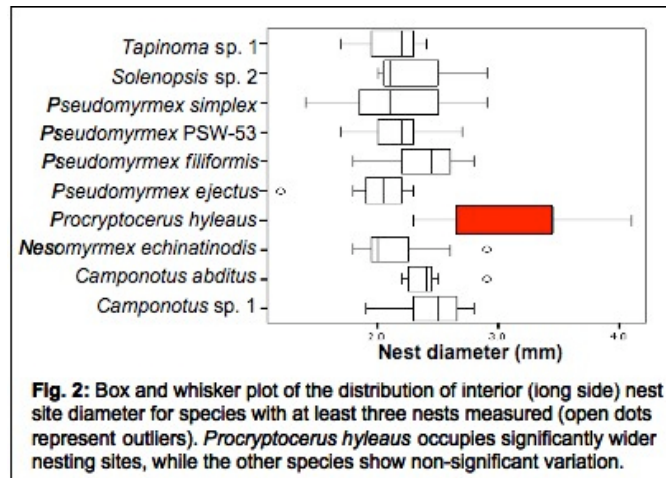
whereas only 2.5% of twigs with *Nesomyrmex echinatinodis* contained lone queens. These data together indicate there may be differences in fecundity, dispersal timing, dispersal limitation, or recruitment capabilities of different species. Furthermore, more species colonize artificial nests in coffee sites with higher levels of canopy complexity (e.g. tree richness, canopy cover, tree density) relative to richness found in artificial nests than in vegetatively simple habitats showing that TNAs are dispersal or recruitment limited at the 45-ha plot scale (Philpott & Foster 2005). These are all interesting patterns that have not yet been tested in a spatially explicit experimental fashion.

Preliminary work has examined competitive hierarchies of TNAs in lab and field settings. In the lab, paired species of ant colonies (including workers, brood, and alates) were dislodged from natural nests, placed into plastic tubs with a bamboo twig, and given 24 hours to colonize. The ‘winner’ was noted as the species with the majority of its individuals within the twig. To date, a total of 193 trials with 30 unique species pairs have been conducted (Livingston & Philpott, *in review*; Philpott, *unpubl. data*), and four tiers of competitive ranks emerge with some common species ranked as competitive dominants (*P. PSW-53*, *M. mexicana*) others ranked in the 3<sup>rd</sup> (*P. simplex*) or 4<sup>th</sup> tiers (*P. ejectus*). To corroborate lab data, one pilot study (Livingston & Philpott, *unpubl. data*) examined competitive interactions between *P. simplex* and *P. ejectus* in the field. We located colonies (i.e. all twigs of one species on one coffee plant) by breaking off twigs at the base, searching for nest entrances, and placing entire twigs in large plastic bags then placed in the sun. After a few minutes, ants will leave the nest, upon which time they can be identified without nest destruction. Colonies of *P. simplex* and *P. ejectus* were then paired at random on new coffee plants, and the occupant of each nest marked with flagging. Nests were checked after 1 month for any takeovers and nest abandonment. No clear hierarchy emerged; *P. simplex* took over 1 *P. ejectus* nest (of 51 total) and *P. ejectus* took over 2 *P. simplex* nests (of 45). Nest retention was high; however, with only 7% of nests abandoned during the month-long experiment showing the feasibility of such transplant experiments.

**Nest size preference:** Twig characteristics may influence TNA recruitment (Byrne 1994) but because TNAs are often nest limited, opportunistic, rather than specialized twig use is also expected (Kaspari 1996, Philpott & Foster 2005). For example, in one agroforest study, TNAs did not show preferences for twig species, temperatures, or moisture conditions (Armbrecht *et al.* 2004). For coffee twigs, principal sources of local variation are nest size and degree of decay (Byrne 1994). Preliminary field data show widespread overlap in nest sizes of several TNA species (Fig. 2, Livingston & Philpott, *in review*).

In laboratory ‘real estate’ experiments, occupants were dislodged from natural nests and allowed to choose nests varying in size. *P. simplex*, *P. ejectus* and *N. echinatinodis* chose small nests (1.5-2.5 mm) and *P. hyleaeus* chose large nests (2.6-4 mm) significantly more often (Chi-squared Test,  $P < 0.01$ ). Thus for most TNAs (except *P. hyleaeus*), nest size selection does not seem to represent an axis of environmental variation among species, largely rejecting  $H9$ .

**Nest abundance:** Another potential source of environmental variation is nest availability at the scale of a coffee plant. In TNA surveys conducted during 2007-2009, I recorded the number of hollow twigs on all coffee plants sampled, and the number of nests occupied by each TNA. For 9 of the 10 most common ant species, the proportion of nests occupied on individual plants declined with increased numbers of nests on that plant ( $P < 0.01$ ). Across the community, however, relative abundance of some species increased on plants with more nests (*Camponotus abditus*,  $P < 0.001$ ), and the relative abundance of some species was

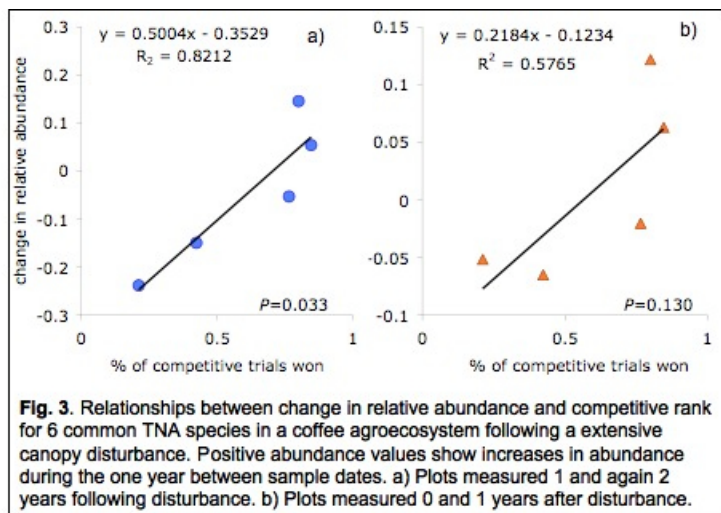




higher on plants with fewer nests (*M. mexicana*, *P. simplex*, *P. elongatus*, *P. filiformis*, *P. PSW-53*;  $P < 0.07$ ). These patterns indicate that something about nest availability may affect TNA coexistence or assembly processes, but this requires further study, especially with experimental manipulations of nest abundance (see proposed work below).

***Distribution and composition of twig-nesting ants in the 45-ha and 6-ha plots:*** TNAs have been sampled at the field site since 1999 and systematically in the 45-ha plot since 2007. Thus there is an excellent understanding of which species occur in the plot. Surveys conducted in uncut areas of the 45-ha plot in 2007 suggest species composition varies greatly between hectares. TNAs were sampled on >500 coffee plants across 27 hectares (Livingston & Philpott, *in review*). Among hectares with >20 colonies, rarefied ant richness did not vary with on average 5.17 species per 20 nests encountered (and significant overlap in 95% CIs among hectares). Species composition also varied with few hectares sharing >50% of species. Of 27 species found, 7 were found in a single hectare, and 4 were found in only two. Thus, TNAs are not uniformly distributed in the plot. Relative abundance patterns of individual TNA species varied qualitatively with larger-scale environmental variation (e.g. tree density, tree richness and fraction of tree individuals in the genus *Inga*). However, more data is needed to quantitatively examine which sources of environmental variation relate to assembly processes such as recruitment and survival.

TNAs have also been sampled in the 45-ha plot to document changes in nest occupation, richness, and relative abundance since the canopy manipulation conducted by farm owners. I sampled ants in July 2008 and 2009 in a series of hectare-sized plots forming a treatment gradient of time since disturbance (uncut = control plots in the 6-ha plot; cut in 2007; cut in 2008). In each treatment area, I randomly chose 2 or 3 hectares, and sampled ants on the coffee bush nearest to each tagged tree. I found 19 TNA species



from 534 nests. Nest availability did not differ with treatment, but more available nest sites were occupied in most recently cut sites (82.9%) than in areas cut a year earlier (70.7%) or in uncut hectares (57.7%). Species accumulation curves demonstrated ant richness was highest in recently cut sites (16 species) and lowest in uncut hectares (11). Species relative abundance drastically differed in the three areas. I re-sampled plots in 2009, calculated changes in relative abundance for each species with time since disturbance, and examined if changes correlated with competitive rank as determined in the lab. I found positive

correlations between competitive rank and increase in relative abundance with time; significant with longer time since disturbance (Fig. 3, Philpott *et al.*, *in prep*). Thus it is clear that disturbance affects occupation, richness, and relative abundance, and that competitive exclusion may be important; however, further variables must be examined before concluding the predominant factor determining assembly.

***Effects of Azteca on recruitment:*** *Azteca* is a canopy dominant that acts as a “keystone” species in several trophic interactions (Perfecto & Vandermeer 2008, Vandermeer *et al.* 2008). Competitive dominants may strongly affect assembly, both in tropical assemblages, and in communities dominated by invasive ants (Sanders *et al.* 2007). Further, it has been hypothesized that dominant ants, when patchily distributed, create gaps into which subordinate species may colonize (Andersen 2008). *Azteca* is distributed in patches (Vandermeer *et al.* 2008). In order to affect twig-nesting ant assembly, *Azteca* must limit colonization and recruitment or alter composition, either by evicting ants or competing for resources to the extent that TNAs abandon nests. I previously examined colonization rates into artificial nests on

plants with and without *Azteca*. Results indicate that *Azteca* limit colonization and alter the relative abundances of several common TNA species colonizing nests in areas with and without *Azteca* (Fig. 4, Philpott, *in revisions*). Thus it is clear that *Azteca* do affect recruitment of TNAs (consistent with *H7*, but relative importance of *Azteca* and other factors affecting recruitment is not clear.

## VII. PROPOSED STUDIES

### A. Life history traits of twig-nesting ants

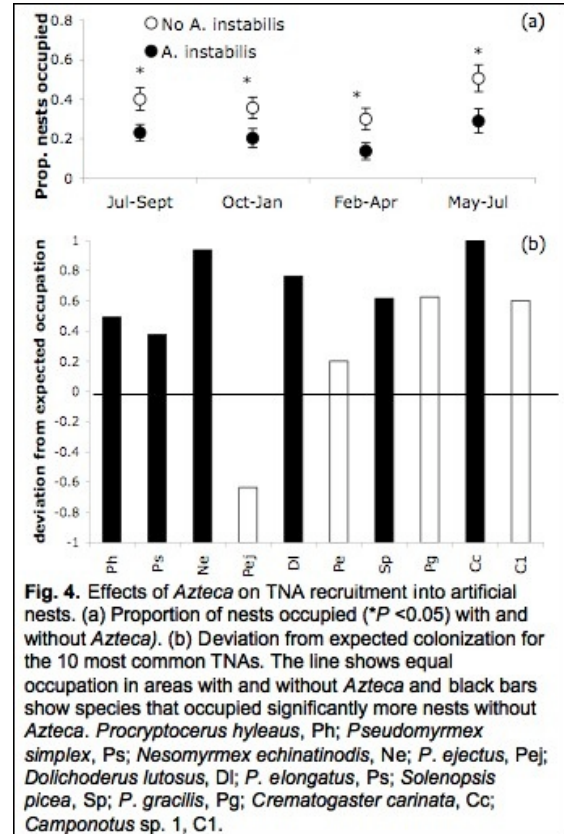
**Colony size and fecundity:** To examine *H1*, adding to preliminary data, I will open 50 colonies of each species (i.e. all nests of a single species on one coffee plant) from the three habitat types and count the number of individuals including queens, males, workers, and brood. These numbers will allow examining the average colony size for the most common ant species, and will be used to create a alate: worker ratio among ant species, and also to assess relationships between the fecundity of ant species and competitive rank, dispersal ability, and colonization rates in nest addition experiments. This will take approximately 250 hours total.

### **Dispersal timing and relative abundance of dispersing**

**queens:** To examine *H2*, I will examine dispersal timing and rates of TNAs. I will collect queens in pan traps oriented throughout the 45- and 6-ha plots. I will set up 204 traps (180 in the 45-ha and 24 in the 6-ha plot) distributed along major trails (to facilitate collection) approximately every 40 m, but placed at least 5 m from trails, throughout the two plots. Traps will consist of 0.5 m radius cones affixed with large cups with alcohol and will be suspended from shade trees ~1.5 m above ground. Trap contents will be collected bi-weekly for a year (5304 samples) and all TNA queens collected will be identified. Based on collection of queens on sticky traps, I estimate I will collect approximately ~10,000 alates, a subset of which will be arboreal TNA queens. Collecting samples will take 2 people ~2 days every other week. Identification will be facilitated by existing collections; most twig-nesting queens are known from nest series collections at the site. Collecting queens weekly will yield an estimate of the reproductive phenology of TNAs (Kaspri *et al.* 2001), and will enable examination of temporal variation in dispersal. I will correlate dispersal frequency data with fecundity measures to examine for a possible fecundity-dispersal trade-offs.

**Dispersal limitation:** To examine whether dispersal is limited at the community level (*H3*), I will examine queen (i.e. seed) limitation, source limitation, and dispersal limitation (sensu Dalling *et al.* 2002). Queen limitation is defined as the proportion of traps not receiving queens of a given species. Source limitation, a measure that shows restraint in queen arrival due to low numbers of queens overall and is defined as the proportion of traps not receiving queens as a function of the proportion of traps that would have received queens if dispersal were random. Thus source limitation relates to low fecundity or low numbers of dispersers in general. Dispersal limitation is a metric showing how queen arrival is limited beyond restraints due to low queen number and is defined as the proportion of traps receiving queens divided by proportion of traps that would receive queens if queens were randomly distributed. Values for dispersal limitation will reflect the extent to which ant species are limited by dispersal numbers and dispersal capability and will be used to examine tradeoffs between other life-history traits.

**Competitive ability:** I will examine the relative competitive ability of different TNAs by constructing



**Fig. 4.** Effects of *Azteca* on TNA recruitment into artificial nests. (a) Proportion of nests occupied ( $*P < 0.05$ ) with and without *Azteca*. (b) Deviation from expected colonization for the 10 most common TNAs. The line shows equal occupation in areas with and without *Azteca* and black bars show species that occupied significantly more nests without *Azteca*. *Proccryptocerus hyleaus*, Ph; *Pseudomyrmex simplex*, Ps; *Nesomyrmex echinatoidis*, Ne; *P. ejectus*, Pej; *Dolichoderus lutosus*, Di; *P. elongatus*, Ps; *Solenopsis picea*, Sp; *P. gracilis*, Pg; *Crematogaster carinata*, Cc; *Camponotus* sp. 1, C1.

competitive hierarchies using arena and field experiments (*H5*). In the lab, I will collect twig nests and will place all individuals (workers, alates, brood) collected from pairs of nests into plastic tubs with one bamboo twig. After 24 hours, I will record no. of individuals inside and outside nests for each species; the species with the higher fraction in the nest will be noted as the winner. Adding to the existing database, I will conduct trials for the 10 most common ant species at the site and will replicate trials for each species pair 10 times (440 trials overall). Following Lebrun (2005), I will create a two-way matrix of wins and losses and examine for a linear competitive hierarchy with Landau's  $h$  (de Vries 1995). To determine the significance of any linearity detected, and to organize the species into a competitive hierarchy, I will use the package MatMan 3.1; the program compares the observed matrix with a simulated matrix to see if the hierarchy generated is different than what would be expected at random. If the hierarchy is not linear, species will be ranked according to proportion of trials won.

Although lab experiments are useful in providing controlled tests between two species, the results from such tests need to be confirmed in the field. Thus, in addition to lab experiments, I will conduct a field experiment where pairs of species will be relocated to coffee plants devoid of twig ants and hollow twigs and monitored. I will collect entire colonies of twig nesting ants and re-attach them with twist-ties to coffee plants from which all dry twigs have been removed. I will place colonies of two species on each experimental plant. Nest identity will be marked at the start of the experiment and checked monthly for six months to observe any nest takeovers. I will use the eight most common ant species (for a total of 27 species pairs) and will replicate each species pair on 10 coffee plants. I will score plants for competitive dominance by one species over the other if a majority of nests within the colony are replaced. Where nests are abandoned, only some nests taken over by the paired species, or where nests are taken over by other species will be recorded as ties. The competitive hierarchy will be analyzed as for lab studies.

**Trade-offs in life-history traits:** The collected data will make it possible to test for a number of different life history traits among coexisting TNAs including a) fecundity-dispersal, b) fecundity-competition, and c) competition-dispersal. Trade-offs will be preliminarily explored using multiple correlations for individual species or for groups of species with similar life-history traits. The data, in combination with the data collected in the different habitat areas (see below) will facilitate examining how these trade-offs may change with environmental variation by examining colony size, fecundity, and dispersal in the different habitat types. Specifically, these data will also allow testing whether dispersal limitation for certain species is stronger in disturbed habitats compared to undisturbed habitats (Palmer *et al.* 2002).

## **B. Canopy disturbance: Changes in relative abundance and correlates of life-history traits**

**TNA surveys:** Building on a two-year data set, I will resample TNAs in areas that were affected by the large-scale canopy manipulation and nearby control areas (that will remain uncut in perpetuity; W. Peters, farm owner, *pers. comm.*). I will sample ants in 2 hectares in the uncut 6-ha plot (the long thin shape of the plot precludes other 100 x 100 m sample areas), in 3 hectares in the area cut in 2007, and in 3 hectares cut in 2008. In each ha, I will sample ants at two distinct spatial scales: the full ha scale, and in 20 x 20 m plots randomly located within each larger plot. Full ha surveys will consist of sampling ants on the coffee bush nearest to each tagged tree. During each sampling period, I will sample the coffee bush nearest to each tree (alternating by year to N, S, E) by removing all dry twigs, counting hollow and occupied twigs, and identifying all occupant ants. In the 20 x 20 m plots, each coffee plant (~100-150 plants) will be sampled in the same manner. Twigs are an ephemeral resource, with relatively high turnover at small spatial scales. Thus removing the twigs once per year is unlikely to affect the censusing during later sample periods. Previous work on arboreal twig-nesters will facilitate identification of collected ants. Reference samples will be collected as needed to add to the existing collection. After destructive sampling of twig nests, all ants and nest pieces will be left at the exact sample location to facilitate recolonization of nearby nest sites. However, during the first year of surveys fifty colonies of each ant species will be collected for examining numbers of workers, brood, and alates. Based on previous work I estimate sampling the full ha plots and 20 x 20 m plots will take 4 trained technicians 4 weeks.

I will collect data on canopy cover, coffee density, and microclimate to add to existing data on stand basal area, and identity, size, location, richness, density, and species composition of trees. Twice per year,

I will sample canopy cover at 25 evenly spaced points in each ha using a concave spherical densiometer. This will take roughly 4 days. To estimate coffee density I will randomly select 3 trees ha<sup>-1</sup> and count coffee plants within a 10 m radius. It will take 15 min. per tree or ~4 days of work per year. I will place data loggers at a random location in each ha to record relative humidity and temperature at 4-hour intervals. I will create a vegetation complexity index (Mas & Dietsch 2003) to assess habitat quality in each ha. I will use canonical correspondence analysis controlling for spatial autocorrelation in the data to find significant relationships between environmental variables and relative abundance of TNA species. This data will allow testing whether relative abundance of particular ant species varies with environmental gradients (H12).

**Nest availability:** During plot surveys, I will record the number of hollow dry twigs on each coffee plant surveyed to examine H11. To assess the overall relative species abundances I will generate a rank-abundance distribution fitted with a normal curve. I will use linear regressions to test for correlations between number of hollow twigs per plant and colony size, and number of twigs occupied by a species. I will use an ANCOVA Test for Homogeneity of Regressions to test whether species show similar responses (with respect to proportion of community occupied) to increasing numbers of hollow coffee twigs. This information will add to the existing database and inform whether variation in nest site abundance demonstrates a pattern consistent with changes in assembly.

**Changes predominant life-history traits with time since disturbance:** For each species, information will be collection on life-history traits related to colony size, fecundity, dispersal, and competitive ability. This information will be used to examine H4 and H8 and will test whether species with particular life-history traits (high fecundity, dispersal, or high competitive ability) decrease or increase with time with disturbance in the different habitat areas. I will use correlations to examine change in relative abundance of species and of traits with time since disturbance. I will also use rank clocks (Collins *et al.* 2008) where temporal data on rank abundance can be quantified to examine rank shifts over time.

### **C. Recruitment Processes in Twig-Nesting Ants**

**Dispersal and recruitment:** Because ant colonies can be highly persistent once established, recruitment may be very important in community assembly (Andersen 2008). A suitable habitat patch for twig-nest establishment may depend on number (Philpott & Foster 2005) or diversity of nest sites (Armbrecht *et al.* 2004), or presence of *Azteca* ants. To examine recruitment, I will place artificial twig nests (bamboo) in coffee plants every 50 m at the same locations as queen traps and for the same duration. With the data I can examine recruitment limitation and compare composition of ants found in the traps with those recruiting into available nests. At each trap point, I will affix 5 artificial nests to the nearest coffee plant, and will harvest and replace nests every 3 months for one year (1045 nests per census, 4180 total). Two people can harvest and replace 150 nests and identify all ants each day. Thus the project will take 2 people one week every 3 months. To examine the importance of environmental gradients on nest recruitment (see Q1), I will categorize nest placement sites according to the following conditions within a 25 m radius of the site: a) surrounding percent canopy cover, b) tree density and species richness, c) stand basal area, d) percent of trees in the genus *Inga*, e) mean temperature, and f) mean relative humidity. To examine the influence of *Azteca* (see Q3), I will count the number of *Azteca*-occupied trees within 25 m. I will examine the influence of each factor on nest occupation with linear regressions.

I will also compare composition of dispersing ants (from traps) and recruiting ants (from nests) from the same locations. I will average the data from queen arrivals and nest recruits over a 1-year period to avoid problems associated with temporal variation in arrival and survival of ants (Nathan & Muller-Landau 2000). Then, using partial Mantel tests I will examine for correspondence between the species composition of queens found in traps and successfully recruited nests at the same habitat patches (Legendre & Fortin 1989). If disperser and recruiter compositions significantly overlap, this will provide evidence that dispersal is a more important process driving twig-nesting assembly. By contrast, if significant differences in disperser and recruiter composition are found at certain trap localities, those patterns will be consistent with habitat differences or interspecific competition driving ant assembly.

**Recruitment experiment:** I will conduct a nest addition experiment to examine the influences of three

major factors on recruitment processes in TNAs a) environmental variability at local and regional scale, b) presence of TNAs, and c) presence of a dominant arboreal ant species. I will conduct a simple, extensive experiment that will allow direct comparisons of the relative influences of local environmental variation (nest availability on a single coffee plant) (*H10*), plot-scale environmental differences (canopy characteristics) (*H13*), and competitive influences from TNAs (*H6*) and the aggressive dominant, *Azteca instabilis* (*H7*). I will establish 30 blocks of 6 coffee plants in each of three habitat types: 1) uncut 6-ha plot with high vegetation complexity, 2) area of 45-ha plot cut in 2008, and 3) area of 45-ha plot cut in 2007. The three habitat types will vary in terms of large-scale environmental factors such as canopy cover, tree density, tree diversity, and canopy thickness all of which will be measured with standard vegetation surveys around each sample block. In each habitat type, 15 blocks will be placed near *Azteca* nests, and 15 blocks will be placed at least 30 m from the nearest *Azteca* nest. Within each block, I will remove all dry twigs from half of the coffee plants to minimize any competitive interactions from other twig-nesting ant species. Finally, within each block, I will attach artificial nests in three densities: 5 nests (on 2 plants), 10 nests (2 plants), and 15 nests (2 plants). Artificial nests will be cut from bamboo sized to resemble the range of natural nests sizes. Bamboo grows abundantly and is easily harvestable. Previous studies demonstrate the feasibility of this method (Philpott & Foster 2005, Friedrich & Philpott 2009). Coffee plants have on average 1 hollow twig available for nest colonization (Philpott & Foster 2005), thus the numbers added will substantially increase available nesting sites. This will yield a total of 540 coffee plants and 5400 artificial nests. Nests will be placed on plants in all habitat types within a 2-week window in April, before the onset of the rainy season and the time at which many tropical ants found nests (Kaspari *et al.* 2001), and will all be harvested within a 2 week period in August. Upon harvesting, all bamboo twigs will be cut open and the resident ants will be identified. In addition I will count the numbers of individuals (queens, males, workers, and brood). Based on previous capture rates in artificial nests in areas with and without *Azteca*, I predict this will yield ~1900 occupied nests.

In order to compare the composition of the colonizing ants with ants in the natural nests in the surrounding area, I will also harvest all dry coffee twigs from 12 plants surrounding the experimental plants. For half (6), I will collect all dry twigs prior to the start of the experiment, and the other half will be collected after the experiment. In addition, I will collect nests from experimental plants either at the start of the nest experiment (no-TNA-plants) or after harvesting artificial nests (with-TNA-plants). I will use Chi-square tests to compare the relative frequencies of abundance in the surrounding community and in the colonizing community.

I will examine differences in nest colonization by TNAs in two ways. First, I will use MANOVA to examine whether the colonization of specific ant species was affected by any experimental treatment with proportion of nests occupied by individual species as the dependent variable, and nest availability, TNA presence, *Azteca* presence, and habitat type as independent variables. Then, I will examine the relative importance of different factors on colony founding using multivariate regression trees (MRT) (Breiman *et al.* 1984, De'ath 2002). MRT is a non-parametric approach that uses a recursive data-partitioning algorithm to choose a best single predictor variable, and then divides the data based on that variable. The iterative process then repeats to find the next most important predictor. I will build regression trees using the rpart package in R (R Development Core Team 2005).

Adding nests and examining recruitment processes to those nests will help elucidate several assembly processes. For example, examining differences in relative abundance in artificial and natural nests, combined with information on life-history traits of occupant ants may elucidate factors driving assembly at the recruitment stage. For example, because nests are presumably the limiting resource for twig-nesting ant communities, I will use dominance rank (win: loss ratio) derived from nest competition trials as an index of competitive ability. I will examine the relationship between dominance rank and relative abundance on coffee plants varying in nest abundance using methods employed by Harpole and Tilman (2006) to examine the relationship between  $R^*$  (ability to deplete nitrogen levels) and relative abundance in plots varying in nest abundance. Similarly, I will examine the relative abundance of species varying in dispersal ability and nest availability to examine potential tradeoffs between competition and dispersal ability in co-existing species. Further, the MRT will allow examining which factor is most important in

determining relative abundance of different species in the artificial nests.

#### **D. Colony Survival of Twig-Nesting Ants**

**Reciprocal transplant experiment:** As implications of both competitive and dispersal abilities will be confounded in nest addition/ recruitment experiment above, I will also conduct transplant experiments and place pre-assembled ant communities (including 1 nest each of 5 species differing in competitive ability). I will conduct transplants of these entire ant communities to examine the influences of three major factors on survival processes in TNAs a) environmental variability regional scale, b) presence of TNAs, and c) presence of a dominant arboreal ant species. This experiment will allow direct comparisons of the relative influences of environmental variation (canopy characteristics) (*H13*), and competitive influences from TNAs (*H6*) and *Azteca* (*H7*). I will establish 30 blocks of 8 coffee plants in each of three habitat types: 1) uncut 6-ha plot with high vegetation complexity, 2) area of 45-ha plot cut in 2008, and 3) area of 45-ha plot cut in 2007. The three habitat types will vary in terms of large-scale environmental factors such as canopy cover, tree density, tree diversity, and canopy thickness all of which will be measured with standard vegetation surveys around each sample block. In each habitat type, 15 blocks will be placed near *Azteca* nests, and 15 blocks will be placed at least 30 m from the nearest *Azteca* nest. Within each block, I will remove all dry twigs from half of the coffee plants to minimize any competitive interactions from other twig-nesting ant species. I will harvest 2 plants from each block every other month (2, 4, 6, and 8 months after placement) to look at survival ranges of different species in different habitats. I will compare composition of communities in different treatments using non-metric multi-dimensional scaling. I will examine species richness with species accumulation curves generated in EstimateS (Colwell 2005). Additionally, I will examine the relative importance of different factors on colony survival using multivariate regression trees (MRT) (Breiman *et al.* 1984, De'ath 2002) as above. I estimate the experiment will take 3-4 weeks to set up, and 1 week for each harvest. The experiment will enable examining changes in the ant assemblage over time. Introducing entire colonies of competitively disparate species will allow for examining the role of competition in assembly processes in the absence of dispersal differences. If competition for nest sites is an important process driving community assembly, I predict that the relative abundance of ants with high dominance rank in nests will increase with time. More broadly, convergence of the relative abundance of species of high dominance rank in nest-limited communities or of species with high dispersal ability in the least limiting communities will demonstrate evidence for local interactions dominating assembly (Harpole & Tilman 2006).

### **VIII. SUMMARY AND SIGNIFICANCE OF THE PROPOSED RESEARCH**

The proposed research will determine whether life history trade-offs, competitive exclusion, disturbance, dispersal, and recruitment processes as well as environmental variation at two distinct scales affect twig-nesting ant assembly. I will examine both local and regional drivers of community assembly in a spatially explicit context using a well-defined insect community in a well-studied research site. The suggested observations, manipulations, and analyses will mechanistically test the importance of different factors in ant community assembly, and will enable examining the relative importance of each factor. Comparing species composition of dispersers (in queen traps) and recruits (in artificial nests) at identical locations will enable examining at which life stage compositional differences exist and thus which assembly process or processes primarily drive community differences. Examining changes in species relative abundance in differently managed areas, coupled with detailed information on life-history traits will enable examining the role of disturbance on community assembly of TNAs. It is possible and likely that several assembly processes are important. The detailed experiments outlined will allow examining how the various assembly mechanisms interact to produce co-occurrence in the system, and to evaluate the relative importance of different factors. Very few experimental tests of insect assembly processes have been conducted, and none at the level of diversity at which I propose to examine the twig-nesting ant assemblage. This coupling of survey data and manipulative fieldwork make this an ideal system in which to advance understanding of community dynamics and assembly.



## IX. BROADER IMPACTS OF THE PROPOSED RESEARCH

### I. Training of graduates and undergraduates in ant taxonomy.

The amount of ant material collected from this project will be significant. Although some previous collecting has been conducted in Chiapas, little material from this state in particular is available in international museum collections. I will hire one undergraduate per semester to prepare and photograph ant collections and to add to online reference materials for the ants of Chiapas. Training at the undergraduate level is critical for maintaining interest in taxonomy in younger scientists.

### II. Training of minority groups, women, and international students.

I am strongly dedicated to training of minorities and women scientists, and will actively recruit minority and women students to the project. I currently advise four undergraduate researchers, three of whom are women. I have led several groups of Mexican students in field projects, and co-taught field courses primarily for Nicaraguan students for several years. I currently advise one Hispanic MS student (one of two minority graduate students in my department). I am directing a PhD Thesis of a Mexican student at ECOSUR in Chiapas and have served on committees of graduate students in Colombia. I have requested status as a research affiliate of ECOSUR, the nearest university to the research site, so that Mexican undergraduates can conduct their theses within the proposed project.

### III. Contribution to management decisions in coffee agroecosystems.

Coffee is the second most traded commodity thus the importance of understanding the ecology of this agroecosystem cannot be overstated. Coffee agroecosystems are consistently cited as important for conservation at the landscape level due to the high levels of diversity that they maintain. Yet coffee management intensification reduces biodiversity and can lead to increased pest problems. Coffee's two major insect pests are the berry borer and the leaf miner. The leaf miner, usually a pest in sun coffee farms, is an emerging pest in this shaded plantation. Several species of ants, including *Azteca instabilis*, prey on the borer (Perfecto & Vandermeer 2006, Armbrecht & Gallego 2007) and the abundance of TNAs negatively correlates with leaf miner attacks (De la Mora *et al.* 2008). Investigating relationships between TNAs and the leaf miner will likely be a PhD thesis topic within the scope of the project. An understanding of the factors governing twig-nesting ant assembly may benefit management decisions for promotion of conservation biological control within shaded coffee plantations. Further, better understanding the ecological underpinnings of community structure in insect communities is generally important for sustainable agricultural management.

### IV. Dissemination of research findings.

Publications resulting from the research will be published in leading scientific and ecological journals, and I will actively encourage publications from students working on the project. The project will also contribute to an online guide to the "Ants of Chiapas". Both previously collected specimens and ants that will be collected during the project will be mounted, photographed, and organized along with natural history information available from various sources. The PI, postdoc, graduate, and undergraduate students will present research findings at conferences. I will also present annual seminars at ECOSUR to share information with local scientists and to recruit Mexican undergraduates to the project.

## X. RESULTS FROM PRIOR NSF SUPPORT

### a. NSF Graduate Research Fellowship, Amount: \$90,000: Period: 09/01/99-04/30/03

### b. Title of project: Ant biodiversity and biological control in coffee and cacao agroecosystems

c. Summary of work completed: Previous support was awarded to study biodiversity and ecology of ants in coffee agroecosystems. The work provides much of the background outlined in the proposal. Additionally, I have a) described the species richness and composition of arboreal ants, b) outlined mechanisms of species loss with coffee management intensification, c) detailed impacts of ants on arthropods, coffee damage, and yield in sites varying in management intensity, d) examined the roles of ant behavioral diversity and vegetation complexity in modifying trophic interactions, e) documented spatial patterns of numerically dominant arboreal ants, and f) examined trait-mediated effects of phorid



parasites on *Azteca instabilis*, the most common ant in the study sites.

**d. List of publications from previous NSF support:** The GRF resulted in 13 publications (those marked with asterisks in the references section).

**e. Brief description of available data, collections, etc.** Research from the NSF GRF resulted in a large collection of arboreal ants. This collection has been sorted to morphospecies, and has been deposited at a) at ECOSUR in Tapachula and b) the PI's lab at U. Toledo. Undergraduates in the PI's lab have photographed collection using automontage software and photographs are available on the PI's website – a work in progress leading to a guide, “Ants of Chiapas”.

### XI. TIMELINE OF PROEJCT ACTIVITIES

Year 1	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Queen traps	Select trap locations	Construct traps	Map trap locations	Collect traps bi-weekly, sort for TNAs, ID TNA ants								
Recruitment around Queen Traps			Prepare bamboo	Place nests		Prepare bamboo	Harvest, place nests	ID, count indiv.	Prepare bamboo	Harvest, place nests	ID, count indiv.	Prepare bamboo
1-ha & 20x20 m plot surveys								Survey large plots, 20 x 20 m plots, canopy, coffee density data				
Examine colony sizes, worker:alate								Examine colonies collected in surveys			Data analysis	
Year 2	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Queen traps	Collect traps bi-weekly, sort for TNAs, ID TNA ants			Finish ID of TNAs collected in traps			Data analysis					
Recruitment around Queen Traps	Harvest, place nests	ID, count indiv.	Prepare bamboo	Harvest, place nests	ID, count indiv.	Data analysis						
1-ha & 20x20 m plot surveys								Survey large plots, 20 x 20 m plots, canopy, coffee density data				
Recruitment experiment						Select sites	Prepare bamboo, harvest natural twigs	Place nests, ID			Harvest artificial nests, ID	Harvest natural twigs, ID
Competition trials				Arena experiments								
Year 3	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
1-ha & 20x20 m plot surveys								Survey large plots, 20 x 20 m plots, canopy, coffee density data			Data analysis	
Recruitment experiment	ID ants,	Data analysis										
Competition trials			Set up field transplant experiments	Monitor nests once per month								Data analysis
Reciprocal transplant experiment	Select sites, remove natural nests	Collect, transplant colonies		Harvest ¼ artificial nests		Harvest ¼ artificial nests		Harvest ¼ artificial nests		Harvest ¼ artificial nests	Data analysis	