ANT-I-THESIS: Study of the big-headed ant *Pheidole dentata* as a host of a novel species of inquiline *Solenopsis* at Brackenridge Field Laboratory

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INTRODUCTION

The Formicidae, an entirely eusocial lineage of Hymenoptera, are found on all continents but Antarctica. Though they make up only 1% of described insect species, they are speculated to make up 15-25% of the biomass for terrestrial fauna ((Gathalkar and Sen 2018; Moreau 2008). Their ubiquity, especially in urban and agricultural environments, makes them potentially valuable for assessing ecosystem health and biodiversity, and their relatively short lifespan and small size make them model organisms for morphological and behavioral research (Economo et al. 2018; Liu et al. 2019; Seid and Traniello 2006; Shukla et al. 2013; Venuste et al. 2018).

The eusocial ant colony can be described as a **superorganism**, where natural selection acts on the colony rather than the individual (Wilson 1984). With a brain a hundredth in size to that of a honeybee, a single ant would be incapable of the complexities of the colony (Muscedere 2017). Wilson and Holldobler (1988) suggest ant colonies are analogous to the nervous system, in which the limits of individual brain capacity and relatively simple physiology are made into a sophisticated entity by the existence of eusociality.

However, a highly successful superorganism, like any individual, may attract pathogens or parasites (Moyano and Feener 2014; Schmid-hempel 2017). In the broad sense, **myrmecophiles** are any vertebrate or invertebrate known to have a relationship with an ant species (Wojcik 1988). However, parasitism by other ant species does occur, and has resulted in an array of complex life history strategies. Some of these behaviors, like the nest-raids of an Amazon army ant, are conspicuous and dramatic (LaPolla et al. 2002). Other behaviors are so secretive and out of human eye that they remain unknown even in well-studied areas.

At Brackenridge Field Laboratory in Austin, Texas, two new species of parasitic *Solenopsis* ants have been discovered within the past two years. Here, I discuss the relationship between *Pheidole dentata* and an undescribed *Solenopsis* species in terms of host and parasite by reviewing their life histories and the evolution of parasitism in ants. I introduce a preliminary study into the undescribed species, as well as a habitat study of *Pheidole dentata* that may help find more specimens in the future.

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CHAPTER 1: The Host

1.1 *Pheidole* as a genus

Pheidole has been described as a "**hyperdiverse**" ant genus (Wilson 2003). With over 1,100 described species, the genus is globally the most speciose, rivaled only by *Camponotus* (Muscedere 2017). A phylogenetic study by Moreau (2008) described *Pheidole* as monophyletic with an origin in the New World 58.4-61.2 million years ago. They nest in a wide variety of habitats, from sandy deserts to rocky slopes (Cole 1956). Many species build medium-sized nests that allow the colony to migrate often to reduce predation (Traniello 2010). The relative ease in which *Pheidole* colonies can be raised *in vitro* has allowed for extensive study of their life history.

Development of worker caste system
One possible reason for the success of *Pheidole* is its completely dimorphic caste system (Gadagkar 1997; Traniello 2010). All *Pheidole* species (besides eight species with a supermajor caste, Moreau 2008) have a queen (or queens) and a worker caste made of major and minor workers that differ in both their morphology and task repertoire (Muscedere 2017). Separation of tasks allows for optimization of characters (Gadagkar 1997). This division of labor allows the colony to generate a large number of "low-cost" minors that are easily expendable yet highly effective abundant foragers, as well as keep a strong collection of major workers ("soldiers") that are energetically costly for the colony to produce but longer-lived and strong colony defense (Detrain and Pasteels 1991; Muscedere 2017).

Pheidole embryos are "**totipotent**," meaning they have the potential to give rise to any caste (Nijhout 2019). Castes are determined by pheromonal cues and nutritional conditions during their larval stages that alter the development of their imaginal disks and thus the scaling of their bodies (Anderson, Inksvayer, and Mith 2008; Gadagkar 1997; Muscedere 2017; Nijhout 2019). **Juvenile hormone** (JH), a multipurpose hormone also involved with insect metamorphosis and reproduction, is thought to be one of two hormones that determine caste by regulating allometry (De Menten et al. 2005; Nijhout 2019; Rajakumar et al. 2018). High levels of JH lead to development of rudimentary wing disks that create activate gene networks. These networks stimulate prolonged growth of the head and body disks, thus creating the big-headed major workers (Nijhout 2019, Rajakumar et al. 2018). Since healthy colonies with high nutrition may produce too many major workers, adult majors produce a cuticular "**Soldier-Inhibiting pheromone**" that blocks the effects of JH through contact with the brood once the majors reach a concentration of 5-10% (Nijhout 2019, Rajakumar et al. 2018).

Figure 1: *Pheidole floridana* egg to worker development, stack photograph using Canon T7. Taken by Jen Schlauch at UT Insect Collections.

Caste temporal polyethism

Temporal polyethism, or "age-related task performance" has been studied in *Pheidole* major and minor workers (Detrain and Pasteels 1991; Muscedere, Willey, and Traniello 2009; Wilson 1984). In most species, the minor workers are responsible for nursing of brood, foraging, and maintaining the nest, while major workers serve as a specialized minority reserve force for food processing and defense (Muscedere 2017). Recent research has revealed while colony plasticity varies between species, many species can adjust their ratio of minor to major workers in the event of environmental stress (Traniello 2010).

Understanding of temporal polyethism has expanded in recent years. First, majors, previously thought to have a very limited repertoire, are now known to expand their task capabilities to brood care in the case of minor worker removal, though their repertoire count and plasticity differs by species (Wilson 1984). Second, minors were thought to lose their initial brood care abilities in exchange for foraging as they aged (Wilson's early suggestions, as stated in Muscedere 2017). Newer studies suggest that minors expand, rather than replace, their repertoire from 5-17 tasks (called the "**repertoire expansion model**" Seid and Traniello 2006, Traniello 2010, Muscedere 2017). This expansion may be possible due to an increase in synapses and function of the mushroom body of the brain, and may be tied to increases in serotonin and dopamine (Traniello 2010, Seid and Traniello 2006).

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1.2 competition within the genus and how *Pheidole* makes a living globally

Because of its hyperdiverse nature and ability to thrive in a wide variety of habitats, *Pheidole* intra-genus competition has been well examined, particularly related to colony plasticity and caste morphology (Wilson 1984). Economo et al. (2019) inferred the phylogeny of 449 global *Pheidole* species and found that diversification rates were high at the instances in which the genus reached new continents and colonized, rather than supporting the **latitudinal diversity gradient hypothesis** (LDG), which states that diversity is highest at the equator (Economo et al. 2018). Further review suggested that temperate ant species are relatively young and clustered (Economo et al. 2019).

Pheidole have a significant level of heterospecific sympatry: over 40 species can be found in a single site (Tschá and Pie 2018). Ant colonies often maintain territories, and recognize their nestmates via cuticular hydrocarbon markers (Langen, Tripet, and Nonacs 2000). However, frequent exposure to neighboring colonies may lead to scent-recognition that results in reduced aggression, and the species may be sufficiently different in behavior and diet to allow for coexistence (Langen et al. 2000, Tscha and Pie 2018). That said, the potential for competition and inter-colony aggression may be high. Brown and Traniello (1998) regarded fitness in terms of the colony superorganism, as dependent on the ability of the individuals and caste structure to adapt. In their study of *Pheidole morrisi*, majors were successfully able to take over brood care if minor worker numbers were significantly reduced (termed "**coupled compensation**"). Colony behavioral plasticity like this varies between *Pheidole* species (Muscedere et al. 2017). In a study of ant communities at urban parks around Taiwan, Liu et al. 2019 found that ant diversity was highest in larger parks with more heterogeneity, and suggested that smaller ant species may be more effective foragers in complex habitats. This again supports the advantage in producing a caste of minor workers that excels at foraging tasks.

Diet and Foraging
Pheidole range widely in their diets, from granivory to carnivory. A forest ant community study in southeast Brazil found that unlike large attine ants, *Pheidole* foragers consumed seeds at the seed location (Christianini and Galetti 2007). A study by Wilson (2005) found that a number of smaller *Pheidole* species prey on oribatid mites, making them possibly a significant predator of this diverse (over 6,000 species) group.

Foraging success for ants is directly correlated with quantity of individuals, morphology, physiology, and behavior of the foragers. In a study of 10 species across two sites in central Texas, ants with more foragers, and foragers with relatively longer legs that could travel faster, appeared to have higher discovery success (Jessica M C Pearce-Duvet et al. 2011). Along with morphology, Pearce-Duvet et al. (2011) found that foraging patterns were species specific and could contribute to the complex niche partitioning seen in diverse ant communities. Salas-Lopez (2017) compiled a review of ant morphological features—particularly body size and leg segment length— and their possible functions in foraging success. However, a study of 10 *Pheidole* species by Tscha and Pie 2018 found that dominant species were those with faster walking speeds and higher tolerance to temperature and humidity extremes, while body size had no significant correlations to ecological dominance. Major workers had higher tolerance to environmental pressures, a finding supported by a study which found that larger ants had higher critical thermal maxima (Verble-Pearson, Gifford, and Yanoviak 2015). Small differences in behavior and diet support the existence of a "**limiting similarity mechanism**" which allows for long-term existence of similar species (Tscha and Pie 2018). Further, even diverse ant communities were defined by a minority of species (Tscha and Pie 2018). This has been called an "**ant mosaic**" model, in which a few dominant species determine the structure of the entire ant community, and has been documented within sites across several continents (Tschá and Pie 2018; Ward and Beggs 2007).

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1.3 life cycle that makes *P. dentata* susceptible to parasitism

Lack of suppression hydrocarbons
Ants are obligate eusocial insects whose perennial colonies contain queens that cannot successfully reproduce without workers (Boomsma, Huszár, and Pedersen 2014). In some eusocial species, like *Apis* honeybees, queens release suppression pheromones that prevent other females from laying eggs (Oldroyd 2018). In ants with non-sterile workers, ovary development may be suppressed by cuticular hydrocarbons produced by the queen—*S. invicta* queens produce two pyrones and a terpene that are involved in enforcing dominance (Leonhardt et al. 2016; Oldroyd 2018). *Pheidole* workers are sterile and lack ovaries (Muscedere et al 2009, Oldroyd 2018). This may reduce the need for the queen to produce suppression hydrocarbons, and may make the host queen a more vulnerable target to a species like an inquiline. Further, lack of suppression chemicals may allow the inquiline to lay her eggs uninhibited.

Competition between Pheidole dentata *and* Solenopsis
Competition between *Pheidole dentata* and the genus *Solenopsis*, particularly the red imported fire ant *Solenopsis invicta*, has been documented due to their dominance in ant communities throughout the southern United States (Johnston and Wilson 2015; Kaspari and Vargo 1994; Wilson 1976). Introduction of *S. invicta* polygyne colonies into Brackenridge Field Laboratory (see section 1.4) has altered the ant community competition dynamics by directly competing for resources and territory and significantly reducing soil arthropod diversity (Porter and Savignano 1990). Kaspari and Vargo 1994 found that *P. dentata* workers at Brackenridge Field Laboratory exhibited aggressive responses to alate *S. invicta* queens. *P. dentata* minors are documented to release alarm pheromones only for a small number of ant

species, primarily those within *Solenopsis* (Wilson 1976). Further, experimentally manipulated *S. invicta* foundresses were found to actively avoid nesting in soil tainted with refuse from *P. dentata* colonies. A study by Johnston and Wilson (1985) found that *P. dentata* alarm response was strongest against *Solenopsis* fire ants and thief ants. However, unlike some other species of *Pheidole*, *P. dentata* does not appear to be able to shift their proportion of majors in response to increased attack from *Solenopsis invicta* competitors (Traniello 2010, Johnston and Wilson 1985). Though this may be unrelated to inquiline susceptibility, it reminds us that general studies of the genus may not apply to all species.

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1.4 *P. dentata* at Brackenridge Field Laboratory, observations, experiments, long-term data, mapping

Brackenridge Field Laboratory (30.28 N, -97.78 W) is located along the Colorado River in Austin, Texas. Its 32 hectares are composed of a variety of habitats, primarily woodland dominated by juniper (*Juniperus ashei* and *viriginia*), cedar elm (*Ulmus crassifolia*), hackberry (*Celtis* spp.) and oak (*Quercus* spp.) (Feener 1981). The site has a history of anthropogenic impact, including limestone quarrying, housing developments, grazing, and local flooding (L. Gilbert, personal communications). These disturbances have created a community of invasive species and opportunists that have been relatively well documented. The variety of habitats within the property may have allowed for the coexistence of a diverse ant community (Tscha and Pie 2018).

Pheidole appear to be able to adapt to highly disturbed habitats, including regularly flooded areas and agricultural fields (Shukla et al. 2013). Kaspari and Vargo (1995) said that **Bergmann's rule** (which states that organisms tend to be smaller closer to the equator) applies not to individuals, but to colony size in ants. For social insects, this may be due to the need for larger nests and more individuals to serve as insulation during the winter months at colder latitudes. While this rule doesn't apply to all colonies, the relatively mild winters and warm summers of central Texas may have allowed for small, mobile colonies of *Pheidole* that are well adapted to disturbance.

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1.5 Observations and analysis of *Pheidole* at Brackenridge Field Laboratory

Moyano and Feener (2014) studied nest relocation of *P. dentata* at Brackenridge Field Laboratory as affected by air temperature, humidity, leaf-litter depth, nest type, and presence of phorid flies. They found that colonies relocated often (around every 16 days). While resource depletion, microclimate, or parasite predation could be factors encouraging nest migration, they found no significant correlations with rainfall, surface cover, phorid fly abundance, or competition (Moyano and Feener 2014). They concluded that *P. dentata* might prefer simple habitats with low cover (allowing for rapid food discovery), and that nest relocation cost for the species is relatively low. A study by Tscha and Pie in southern Brazil found that habitat heterogeneity, altitude, and productivity were not sufficient in explaining abundance between 10 *Pheidole* species (Tscha and Pie 2018). Studies in the monsoon Chihuahuan desert ecosystem found that *Pheidole* activity was highest in July, correlating strongly humidity and soil surface temperature (Whitford et al. 1981).

Methods: nest sampling and habitat analysis
Here, habitat surveys were done at Brackenridge Field Laboratory in Austin, Texas (30.28, -97.778). A cooler-than-average spring led to lower *Pheidole* activity than normal until after February. From 1 Mar 2018 to late May 2018, nest samples and collections were taken from two transects: Transect 143-146 behind the main building and Transect 102-106 toward the southeast end of the property. The locations

were chosen due to 1) *Pheidole* being previously documented in the area and 2) being relatively well-watered sections of the quarry with moderate to high canopy coverage. Unusual details were noted, as well as a general observation about other ant species seen during the survey. Occasional observation took place throughout Fall 2018 and Spring 2019, though heavy rains affected multiple observation days due to the tendencies of the colonies to retreat underground in extremely soggy conditions.

Methods: Brood collection
In mid- then late-May 2018, respectively, five different active colonies were selected from each the T140 and T101 transects (10 samples total) in order to look for male inquiline or eucharitid pupae. Rocks were flipped until an active colony was discovered. Initially, a trowel and chemistry scoop were used, but an aspirator proved more effective at collecting the most eggs with minimal dirt and damage. Eggs, pupae, and stray workers of *Pheidole dentata* from both the topsoil and rock face were collected into a small mason jar, which was then brought into the UT Insect Collection for sorting (Figure 2).

Figure 2: *Pheidole dentata* major worker, pinned. Collected by Dr. Alex Wild, photographed by Jen Schlauch using a Canon T7 at the UT Insect Collections

Methods: QGIS mapping
The QGIS 2.18.4 free mapping program was used to mark the sampling coordinates onto maps of Brackenridge Field Laboratory. BFL habitat and contour maps were acquired from BIO 373L as PDF files. PDF files were georeferenced using 8-10 Google Earth Pro coordinates and the GeoReferencer plugin within QGIS. Files were saved as "georeferenced" TIF files and added to QGIS as raster layers. Transparency was adjusted, and boundaries were confirmed using a Google Maps plugin in QGIS. Recorded coordinates were converted from UTM to latitude/ longitude and saved as a comma-delimited CSV file, then overlaid on the georeferenced maps of Brackenridge Field Laboratory.

Results: Transect Collection and Observations
Transect 102-106 had a much more consistent *Pheidole dentata* population throughout compared to Transect 143-146. The latter transect appeared to be dominated by other ant species, especially *Camponotus*, another diverse and highly successful genus. Other *Pheidole* species (*P. floridana*, *P. metallescens*) appeared to be as common as *P. dentata* in this area as well, while the T101 area contained mostly *P. dentata*. Solitary diurnal foragers like *Psueudomyrmex* and *Crematogaster* seemed to be equally common in both areas. The flipping of rocks appeared to attract small flies, possibly *Apocephalus* phorid flies (Feener Jr. 1981).

Elevation and coordinate analyses were done using the QGIS compiled maps and coordinates. Samples were taken between elevation 491-520 ft (149.7- 158.5 m). Transect 143-146 was located within the restoration meadow habitat but was still dominated by quarry rocks. Transect 102-106 lies just north of the east-side fish tanks in the old quarry south habitat (Figure 3).

Methods: habitat analysis and general observations
Ants were collected by flipping over rocks and using an aspirator to collect major worker, minor worker, and egg samples from every nest located on surveying. During collection and search for inquilines, the following data were taken: leaf litter cover, dicot cover, and canopy cover (all on a scale of 0 = no cover to 3 = fully covered); coordinate location, rock surface area (cm), nearest tree species, nearest tree distance (m), date, temperature, and start/ end time. Canopy cover was high in most cases, and very few nests were found in areas of direct sunlight. Transect 143-146 seemed to be more humid than T101. The right side of the trail (when walking away from the main building) was significantly more wet, with many rocks covered in lichen and moss. Here, there were almost no *Pheidole* colonies, but individual *Camponotus* queens were extremely common, located under nearly every rock.

In both transects, the same rock was flipped every few days, and very rarely did an occupied rock have a colony present after the next flip. Samples from colonies were collected regardless of whether that rock had previously been surveyed. In most cases, *Pheidole* were able to utilize the pores of the rock and, upon inspection with aspirator and trowel, did not appear to have very complex underground networks. This may contribute to how frequently colonies appeared to relocate. *Pheidole* were active throughout the survey period in the spring, and larger colonies were found as the season progressed. No flights or winged males and queens were observed using the cages set out in April 2018. Starting in early May 2018, numerous queen-sized pupae and eggs were present in many of the nests, especially in larger colonies. Several queen-sized pupae were a pastel orange and appeared to be more developed. Alate males were discovered in two nests as well. Colonies decreased in number in the fall, and became difficult to find especially as the weather cooled.

Results: vegetation type
Out of 48 samples, the highest combination of vegetation type was medium ground cover, low dicot cover, and medium canopy cover (ranked 2, 1, 2 respectively, 12.5% of total samples). The next two most frequent combinations were medium ground cover, low dicot cover, and high canopy cover (2,1,3) and high ground, no dicot, and high canopy cover (3,0,3) both at 8.33% of samples.

Results: coordinates
Locations were taken with the UTM MGRS Finder published by GVN Apps for Android, then checked and converted to coordinates with Google Earth and QGIS 2.14.12 (Figure 3). Data was collected primarily in the Old Quarry South habitat 100 transect, as well as in Old Quarry north.

Figure 3: Collection sites of *Pheidole dentata* samples from March through May 2018 at Brackenridge Field Laboratory. Habitat map of the field lab (from Dr. L.awrence Gilbert) was georeferenced onto a Google satellite map using QGIS 2.14.12. Dots indicate locations of *P. dentata* sampling sites, teal green dots indicate location where inquiline pupae were collected.

Results: rock data

Nests were found by flipping the limestone rocks in both quarry habitats to unearth active colonies and brood. Average rock surface area was 399.23 cm^2. Maximum rock surface area was 1,500 cm^2, minimum 49 cm^2. Rocks with colonies underneath were always directly touching the soil (no leaf litter underneath) and often contained other arthropod inhabitants, including spiders, springtails, millipedes, snails, and other ant species.

Results: nearest tree

The closest trees to each colony were either *Ulmus crassifolia* (68.89% of samples), *Celtis* spp. (22.22%), and *Juniperus ashei* (8.89%). Average distance of the nearest tree was 2.04 meters (range: 0 to 9 meters, median: 1.75 meters, mode: 3 meters).

Results: long-term Pheidole *species data*

Each semester during the BIO 373L field course at Brackenridge Field laboratory, students gather data on the ant communities present in selected acres around the property. Data from 2009 to 2018 from Dr. Rob Plowes was filtered to contain only *P. dentata, P. floridana,* and *P. tetra*. Entries missing information were omitted, and the spring and fall data were divided due to the large differences in weather and vegetation between these two semesters (Figure 4).

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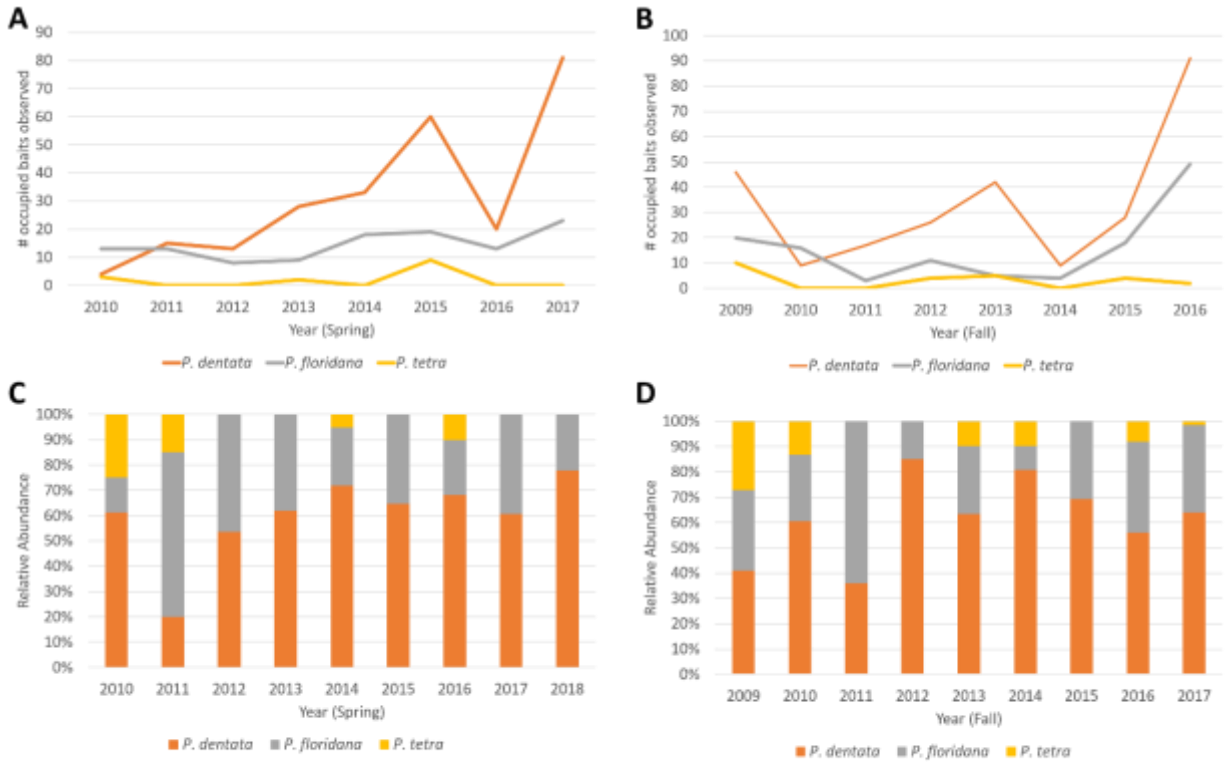
Figure 4: Three species of *Pheidole* at Brackenridge Field Laboratory over time. Graphs (A) and (B) show number of hot dog baits on which ants were observed by BIO 373L students during the ant community lab in the spring and fall semesters, respectively. Graphs (C) and (D) show the relative abundances of the three species, spring and fall semesters respectively.

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CHAPTER 2: THE INQUILINE (AN ANT-AGONIST)

2.1 parasitoid insects and myrmecophiles: a successful life strategy

Evolution of eusocial insects has allowed for the possibility of sympatric speciation of pathogens, predators, and parasites, especially for relatively ancient species and species that have intense brood care (Manna and Hauber 2016; Smith et al. 2007). **Emery's rule** for the origin of social parasitism states that the parasites are either the closest relatives of their host and have probably evolved sympatrically (strict interpretation) or closely related allopatric species that have similar life histories (loose interpretation (Manna and Hauber 2016; Smith et al. 2007). Both forms of Emery's rule have been documented, and the broad interpretation has been confirmed by molecular analysis (Buschinger 2009). A study of ant parasitism by Huang and Dornhaus (2008) found that Emery's rule applied to inquilinism and temporary parasitism, but did not apply to dulosis or xenobiosis (see below for definitions).

Broad effects of parasites on their host
Most parasites usually occur at much lower densities than their hosts (Robinson, Rothstein, and Peer 2013). Thus, they often have little impact in the total host populations and can subtly exist without causing major defense adaptations in the host. This is not true for all parasites: for example, *Apocephalus* phorid flies, all parasitoids of adult ants, may have strong direct impacts in host mortality at certain densities, and may have indirect effects by altering forager behavior (study of *Apocephalus* on *P. dentata*, Feener Jr. 1981). Nest depth and structure alteration in response to parasitism is documented in attine ants parasitized by the dulotic army ant *T. arizonensis* (LaPolla et al. 2002). Beyond behavior, parasitism may be a strong force in the evolution of chemical cue recognition (Martin, Helanterä, and Drijfhout 2011). Martin et al. 2011 studied the variation in chemical cues between populations of *Formica fusca*: two in Finland under high parasitic pressure, and two in the United Kingdom that was not. They found that the Finnish populations had much stronger egg and nestmate recognition, presumably due to the more intense parasite pressure. Thus, ant parasites and associates are still a significant component in studying ant community dynamics.

Cuticular hydrocarbons and other ways of hiding in plain sight
In order to interact long-term with the colonies, myrmecophiles and parasites must make morphological, behavioral, chemical, and acoustic adaptations (Leonhardt et al. 2016; Schonrogge et al. 2016; Wilson 1976). Phoretic parasites are often smaller, and mouthparts of parasitic ichneumon wasps and chalcid wasps are sometimes modified to receive trophallaxis from host ants (Wojcik 1988). Behavioral adaptations include grass-topping behavior of strepsipterans and egg-laying of eucharitids.

Because ants primarily rely on cuticular hydrocarbon recognition, parasitic species must develop some way to integrate or mimic the nest odors (Huang and Dornhaus 2008; Manna and Hauber 2016). These chemicals are often generated by a combination of genomic, environmental, social, and metabolic factors (Delattre et al. 2012). Beyond queen-worker caste recognition, ants typically have chemical cues identifying the colony. These cues can be complex, composed of over a hundred compounds (Menzel, Schmitt, and Blaimer 2017). Further, cues can be modified over time and can override one another, mainly by allogrooming or frequent contact between individuals, allowing for acceptance of an outsider (Leonhardt et al. 2016; Nehring et al. 2015). Larvae of the parasitoid *Orasema* that parasitize *Solenopsis* carry the cuticular mark of the host colony until they leave the nest (Wojcik 1989). In ants, Menzel et al (2017) found that phylogenetic relationships between species did not account for the quantitative traits

of cuticular hydrocarbon composition, and suggest that these cues may evolve in a saltational mode. This may help explain why inquilines may easily adopt their host cues (Johnson, Phelan, and Herbers 2008). Aron et al. (2004) suggests that inquilines mimic the chemical profiles of their host larvae.

Acoustic signaling is another form of communication that has been documented in nestmate recruitment and caste recognition in ants (Schonrogge et al. 2016). *Maculinea* butterfly and *Paussus* beetle myrmecophiles have been documented to use acoustic signals to disguise themselves in *Myrmica* and *Pheidole* nests, respectively (Schonrogge et al. 2016).

Myrmecophile diversity
There are an estimated 10,000 invertebrate obligate social parasites of ants (Schonrogge et al. 2016). Wojcik (1989) listed ant parasites and myrmecophiles belonging to four orders: Acarina, Strepsiptera, Hymenoptera, and Diptera, most of these parasitoid hymenopterans. Though over 500 wasp species have been documented as ant-associates, only a few have been confirmed as true parasites or parasitoids (Lachaud and Perez-Lachaud 2012). Knowledge of these secretive life histories is often limited, but the number of documented parasitoid wasps on Formicidae has risen to 138 known species in 9 families (Lachaud and Perez-Lachaud 2012). Within this, the hymenopteran family Eucharitidae is one of the most diverse groups of ant parasites (Torréns 2013). Ant parasitoids and parasites typically target ant brood (Lachaud and Perez-Lachaud 2012). In order to avoid the perils of entering a nest as an adult, eucharitid wasps deposit their larvae outside of the nest; this planidium (first larva instar) enters the nest by foraging worker collection (Torrens 2013, Wojcik 1989).

The Wasmann system categorizing myrmecophiles (into synechthrans, synoekeetes, symphiles, ectoparasites, endoparasites, and trophobionts) was simplified into ectoparasites and endoparasites by Wilson 1971, though a concrete categorization is still undefined (Wojcik 1989).

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2.2 Types of parasitism within Formicidae

The categories of parasitism within ants, by ants are less ambiguous. The evolution of parasitism within Formicidae is thought to follow the evolution of inbreeding, polygyny, and polydomy in certain ant groups, all of which allow for production of multiple queens and increased tolerance of non-nestmates (Buschinger 1986, 2009). Many parasitic species have two queen types: a larger "macrogyne" and smaller "microgyne," the latter of whom lack the reserves to found their own colony but may disperse over larger areas (see the "**found or fly hypothesis**" in Helms and Godfrey 2016). This could be the life strategy that eventually produced an obligate parasite species. As of 2009, 230 of 12,500 known ant species are described as "parasitic," primarily within Myrmicinae and Formicinae but including Dolichoderinae, Ponerinae and Psudomyrmecinae (Buschinger 2009). Global distribution of known species does not seem to be uniform, occurring primarily in colder latitudes: a study in Switzerland found about a third of the ant species to be facultative or obligate parasites (Buschinger 2009).

 Alfred Buschinger separates social parasitism into four categories: **xenobiosis**, where "guest ants" are dependent on another colony only for food; **temporary parasitism**, where a parasite queen replaces the original queen and eventually takes over the colony; **dulosis**, or slave-raiding, where parasite ants co-op workers from another colony or take over brood; and **inquilinism**, where a small parasite queen lives unnoticed in an ant colony and raises brood alongside the host (Buschinger 1986, 2009). Here, I focus on the latter two due to their similar need to integrate permanently with another species.

Dulosis
Dulotic (slave-raider) ants are usually fully dependent on the captive workers, but often have morphological adaptations for taking over host colonies, such as strong mandibles, powerful stings, and aggressive behavior (Buschinger 2009, Nonacs and Tobin 1992). Instead of chemical mimicry, raider *Formica sanguinea* ants adjust the chemical makeup of their slaves to their own profiles. These adjustments can be so effective as to make the parasite species and its host more similar than outside conspecifics (Włodarczyk and Szczepaniak 2017). While these species usually occur in low concentrations, they have relatively large geographic ranges (Nonacs and Tobin 1992). Most raids take place underground, and result in heavy brood loss, but not worker loss, since they only take brood (LaPolla et al. 2002). In cases of interspecific dulotic parasitism, a coevolutionary arms race occurs in which slaves may form "rebellions" against the raider ants, while the raiders adopt stronger chemical and brood mimicry (Delattre et al 2012). Previous studies suggested dulosis as a precursor to inquilinism, but newer studies suggest inquilines probably evolved from non-parasitic lineages (Buschinger 2009).

Inquilinism
Inquiline ants are thought to be evolutionarily older and agreeing with Emery's rule (Boomsma and Nash 2014; Buschinger 2009). The most species rich of the parasite complexes, they have evolved along multiple lineages, typically from genera with sterile workers (Bourke and Franks 1991; Buschinger 2009). The inquiline ant "syndrome" includes the development of a relatively small, slim queen (microgyne), whose nutritional threshold is lowered to align better with the host worker developmental period and whose size allows for easier concealment within the nest (Aron, Passera, and Keller 1999; Aron et al. 2004; Nonacs and Tobin 1992). Further, inquilines typically have widened, ventrally lobed petioles and postpetioles (Bharti, Radchenko, and Sasi 2016). Many inquilines produce only reproductives; Nonacs and Tobin (1992) suggest that inquilines that retain workers are in the evolutionary transition to losing the worker caste altogether. In true inquilinism, the parasite and host queen coexist, though some cases of "inquilinism" are documented where a small parasite queen eventually kills the host (Buschinger 2009).

Inquiline diversity
A 2016 review by Bharti et al. 2016 found 15 *Myrmica* inquilines in the Holarctic, but lack of life history data has left their status as "true" inquilines unclear. "The Ants" by Wilson and Holldobler 1990 lists 51 workerless inquilines, though this also contains insufficient knowledge of life history. I have attempted to compile a list of known "true" inquilines by modifying the list from Buschinger 2009 to include new or missing information (Table 1).

Associates with Pheidole dentata
A myriad of myrmecophiles and parasites have been documented associates with *P. dentata*. The genus *Orasema* has been documented in multiple *Pheidole* species (Lachaud and Perez-Lachaud 2012). A study by Van Pelt (1950) found *Orasema* parasitoid wasp larvae in *P. dentata* nests in Florida (one identified as *O. robertsoni*).

2.3 Life strategies by *Solenopsis*, an unusual genus (thief ants, fire ants, and inquilines)

As of 2018, there are around 190 described species of *Solenopsis,* split by life history into thief ants, fire ants, and inquilines (Pitts et al. 2018). The majority of *Solenopsis* is composed of tiny, secretive, monomorphic ants that belong to a group called the "thief ants" due to their strategy of stealing food from nearby species (Pitts et al. 2018; Pitts, Mchugh, and Ross 2005). Twenty of the *Solenopsis* species are polymorphic, aggressive ants that originate in the Neotropics. Four are native to North America, but several species but have colonized opportunistically with the aid of human dispersal (Pitts et al. 2018). There are a five *Solenopsis* species documented as social parasites (Pitts et al. 2018). *S. daguerrei* is a generalist workerless inquiline that parasitizes a number of *Solenopsis* species (Calcaterra, Briano, and Williams 2017).

2.4 The *Solenopsis* inquiline: a four-species enigma

Two inquiline *Solenopsis* have been formally documented. *S. phoretica* is described from a single individual queen found in a *Pheidole dentata* nest in Florida (Davis and Deyrup 2017). In the West Indies, *S. enigmatica* is described by two dealate queen and three worker specimens as a parasite of *Pheidole antillana. S. enigmatica* is possibly a temporary parasite, rather than a "true" inquiline (Buschinger 2009; Deyrup and Prusak 2017).

Known social parasites seem to be in higher ratios in colder climates. In temperate areas with seasons, colonies may have set temporal cycles of brood rearing and reproduction. No dulotic species have been found in the tropics, possibly because aligning to the host cycles may be more difficult with seasonality. Since permanent inquilines are in sync with the host brood cycle, they are probably least affected by seasonality (Nonacs and Tobin 1992). However, known *Solenopsis* inquilines are found only in tropical and subtropical regions.

Currently, there are two undescribed species of *Solenopsis* that are phoretic inquilines of *Pheidole* species at Brackenridge Field Laboratory: one associated with *P. tetra*, and one with *P. dentata*. Typically, inquilines parasitize a single, congeneric host with a mid-sized colony (Huang and Dornhaus 2008). *Pheidole* and *Solenopsis* are both myrmicine ants, but are in tribe Attini and Solenopsidini, respectively. In general, single-host parasites align closer to the strict interpretation of Emery's rule, but exceptions exist and whether these inquilines parasitize other *Pheidole* species is unknown (Huang and Dornhaus 2008).

2.5 Personal observations of inquiline

Nest with inquiline: spring 2018
The first rock collected (A8, rock 63) was a very active, previously marked *Pheidole dentata* colony that was flipped to confirm the presence of ants, then placed in an orange bucket. The majority of the colony was in the pores of the rock, and the queen was not originally sighted. The *P. dentata* queen, with an inquiline parasite, emerged after several minutes under the heat lamp. This added another observational facet to the project, and brief monitoring of the parasitized nest ensued. The second rock was home to an active colony and was collected using the same method as the first rock. However, the queen emerged without an inquiline.

Initially when the first colony (N001, with both host queen and inquiline) was in full health and numbers, the bright light of any camera or flashlight startled the colony into chaos and did not reflect usual colony activity. A red sheet to cover the nest tray was not acquired, but a red headlamp proved very successful in allowing full view of the colony without high disturbance. Inquiline and colony behavior were observed, documented, and analyzed.

When the inquiline was not seen on the host queen, the nest was carried over to the UT Insect Collection and photographed in the imaging room using a Canon T7 at 3x magnification (Figure 5). Potential male *Solenopsis* pupae were observed (Figure 5A). A high density of eucharitid wasp pupae were observed at various stages of development (Figures 5B). The dead inquiline queen was laying in the brood chamber and was partially eaten, with a missing antenna (Figure 5C). The *Pheidole dentata* queen was still alive at this time and was active in the brood chamber (Figure 5D). There was also a pupa that was possibly a queen *Pheidole*, though this is not certain at this point (Figure 5E).

Nest with inquiline: fall 2018
An inquiline *Solenopsis* pupae was collected with a small group of *Pheidole* workers and brood in Fall 2018 from Brackenridge Field Laboratory and contained in the UT Insect Collections with water, sugar water, and cockroach bait. However, months passed without the pupae hatching, and eventually the workers, as well as both *Pheidole* and *Solenopsis* brood died or were eaten. Though this rearing experiment was incomplete, it suggests that the inquiline brood may overwinter in areas with mild but colder winters.

Analysis of Pheidole *brood samples*
Brood samples were sorted under a dissection scope. Inquiline and eucharitid pupae were photographed and any additional notes were documented (Table 2, Table 3).

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Initially when the first colony (N001, with both host queen and inquiline) was in full health and numbers, the bright light of any camera or flashlight startled the colony into chaos and did not reflect usual colony activity. A red sheet to cover the nest tray was not acquired, but a red headlamp proved very successful in allowing full view of the colony without high disturbance. Inquiline and colony behavior were observed, documented, and analyzed.
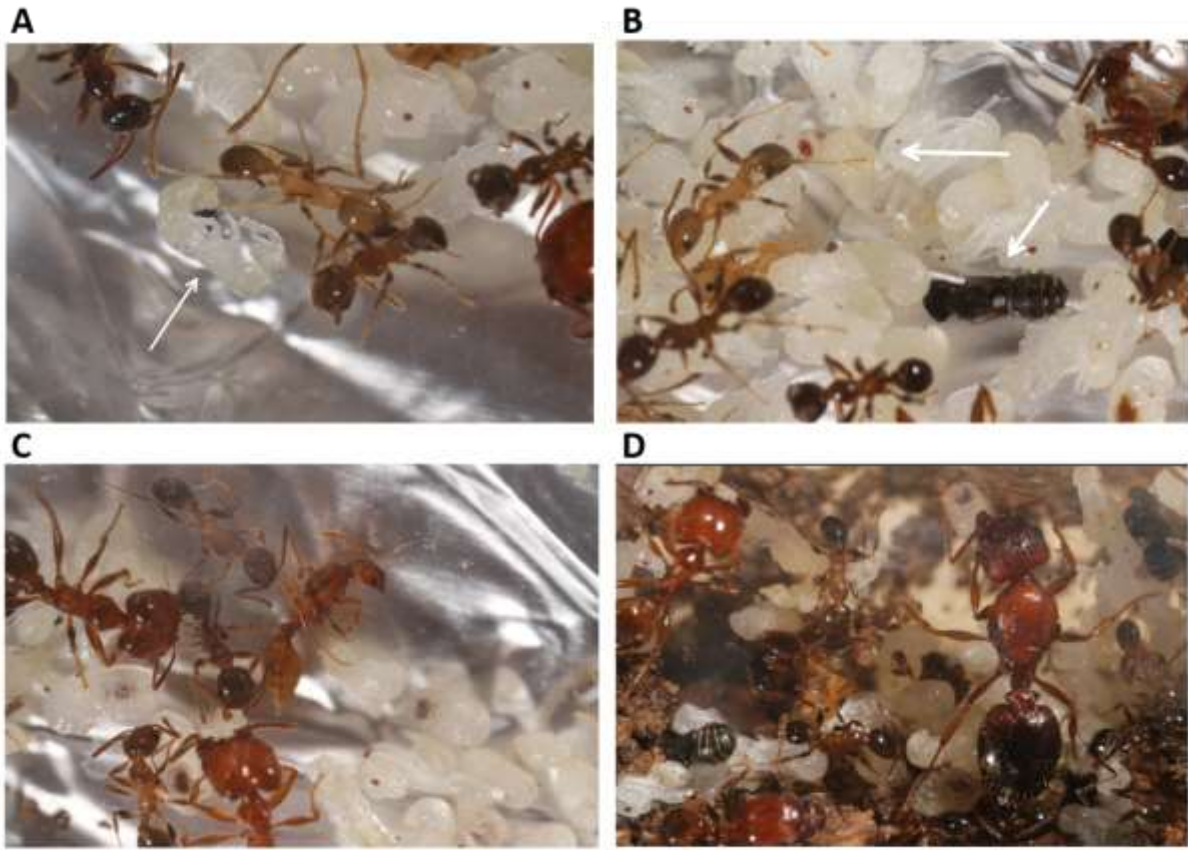
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Figure 5: Images of collected brood with inquiline and eucharitid parasites. Jen Schlauch N001 (collected 1 May 2018). Images by Jen Schlauch on Canon T7 at the UT Insect Collection.

A: possibly a male *Solenopsis* inquiline pupa

B: parasitoid eucharitid wasp pupae

C: dead *Solenopsis* inquiline queen, missing an antenna.

D: *Pheidole dentata* queen among brood

E: Possibly a queen pupa.

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2.7 Conclusion, the big picture, future steps

The host
Observations of *P. dentata* should continue with the goal of finding more inquiline adults for observation. The long-term student data was not normalized, and probably contained unreliable identifications and quantities. Abundance data was changed to presence/ absence for this reason. Number of baits observed may be affected by the number of students in the class in a given semester, and different acres were used each year. That said, the use of hot dog baits was constant, and the relative numbers of *Pheidole* can still provide a vague idea of the community dynamics over time. *P. tetra* appears to be in stable, low quantities across the years, sometimes completely absent from collections (though this can be due to misidentification). *P. dentata* and *P. floridana* appear to be increasing. While no inquilines are known for *P. floridana*, the increase over time in observed *P. dentata* suggests that neither eucharitids or inquilines are causing an observable decline in foraging populations. Genetic work should be done to estimate when the inquilines have arrived to the field laboratory, and more focused observations should continue to see if inquiline populations rise. Since a specimen was collected from every observed colony and preserved in 100% ethanol, genetic analysis can be done to see how often *P. dentata* relocate.

The inquiline
This project collected presence data, observations, photographs, and specimens of a novel, previously undescribed species. I attempted to create methods and a baseline of information for future studies. The life history and effects of these *Solenopsis* inquilines are still largely unknown, but this will hopefully change especially for species on a property heavily populated by ant researchers.

Though invertebrates are lacking in representation for the endangered or threatened listings in the United States, inquilines pose an interesting conservation issue. While their life histories and true densities are still largely unknown, their populations by nature will be much lower than their host species, and their small size and limited dispersal ability may put them in a smaller geographic range that is at higher risk of extinction (Nonacs and Tobin 1992). Public support and economic value of the species will probably not gain the traction necessary for listings of these individual species, but the existence of such elusive species in such a well-researched area experiencing increasing urbanization should be a humbling reminder of the valuable diversity that hides in plain sight.

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2.8 Acknowledgements

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Table 1: Updated list of inquiline species*, modified from Buschinger 2009. Hopefully future studies will fill in the gaps in knowledge.

Inquiline	Host(s)	Queen, male.workers	Brood raised by	Maximum	Range	Citation
3 other Mycocepurus iquilines described in past 20 years						
Acromyrmex insinuator	Acromyrmex echinatior, Acromyrmex octospinosus (rarely)				Gamboa, Panama	
Ectatomma parasiticum						
Epimyrma adlerzi (kills host queen, not true inquilne)	Temnothorax exilis (previously Leptothorax exilis)	y, y, n	Yes		Greece	Douwes et al 1988, Buschinger 2009
Epimyrma corsica (kills host queen, not true inquilne)	Temnothorax exilis (previously Leptothorax exilis)	y, y, no	Yes		Greece	Douwes et al 1988, Buschinger 2009
Leptothorax diversipilosus	Formica obscuripes, Formica haemorrhoidalis	y, y, y	separately	30%		Alpert and Akre 1973
Leptothorax goesswaldi ** (not true inquiline. kills host queen)	Leptothorax acervorum	y, y, no	yes	"rare"	Europe	Blatrix et al 2013
Leptothorax gredleri	Leptothorax acervorum				Europe (France)	Blatrix et al 2013
Leptothorax hirticornis	Formica integroides integroides				(European/ Asian)	Alpert and Akre 1973
Leptothorax kutteri	Leptothorax acervorum	y, y, no	yes	"rare"	Europe (France)	Blatrix et al 2013
Leptothorax pacis	Leptothorax acervorum	y, y, no	yes	"rare"	Europe (France)	Blatrix et al 2013
Leptothorax paraxenus (queen intolerant?)					North America	
Leptothorax wilsoni					North America	
Myrmica myrmicoxena	M. lobicornis	y, y, no	Yes		Switzerland/ Italy	Bharti et al 2016
Mycocepurus castrator	Mycocepurus goeldii					Boomsma and Nash 2014
Myrmica ereptrix	M. aimonissabaudiae? M. rugosa	y, y, no?	yes		South Asia (Himalayas)	Bharti et al 2016, Bolton 1988
Myrmica karavajevi				"rare"		Vankerhoven et al 2011
Myrmica latra	M. aimonissabaudiae	y, y, no?	yes		South Asia (Himalayas)	Bharti et al 2016
Myrmica schenckioides					Netherlands	
Oxyepoecus bruchi	Pheidole obtusopilosa, Pheidole rosae	y, ?,y		"rare"	Alta Gracia and La Grania. Córdoba.	Antwiki
Oxyepoecus daguerri	Solenopsis metanotalis picturata, Solenopsis tetracantha	y, ?,? 3 type specimens		Maybe if at all	Rosas, B. A., Argentina	Antwiki
Oxyepoecus inquilinus	Pheidole radoszkowskii	Workers only found		2 in 41 colonies.	Argentina	Antwiki
Pheidole inquilina	Pheidole pilifera coloradensis	y, y, y (including			CO (USA)	Cole 1965
Plagiolepis grassei	Plagiolepis pygmaea	Y, ye, yes			Europe (France, Spain. Italy)	Schifani 2017
Plagiolepis xene	Plagiolepis pygmaea	y, y, no	Yes			Aron et al 2004
Pogonomyrmex anergismus	Pogonomyrmex barbatus, Pogonomyrmex rugosus	y, y, no	yes	Only 7 of several	SW/NW TX, central NM. and south-	Anderson et al 2010
Pogonomyrmex colei	Pogonomyrmex rugosus	y, y, no	yes		NV, AZ	Anderson et al 2010
Pseudomyrmex inquilinus						
Solenopsis at BFL	Pheidole spp.		yes	14%?	Austin, TX	me, Dr. Alex Wild
Solenopsis daegurrei	Solenopsis richteri, Solenopsis invicta, Soleopsis saevissimia. Solenopsis macdonaghi. S.	y, y, n	yes	3.9-7%	Argentina, Brazil, Uruguay	Calcaterra et al 2000
Solenopsis enigmatica	Pheidole antillana	y,?,y	Yes		Dominica, West Indies	Deyrup and Prusak 2008
Solenopsis hostilis	Solenopsis spp.				Brazil	Antwiki 2019
Solenopsis phoretica	Pheidole dentata	y,?,?	Yes		Gilchrist Co., FL	Davis and Deyrup 2006
Temnothorax kinomurai	Temnothorax makora	Y, ?, no?			Japan	Imai et al 2003
Temnothorax minutissimus	Temnothorax curvispinosus	y, y, no			Eastern N. America (Indiana. Ohio)	Johnson, Phelan, & Herbers, 2008
Tetramorium inquilinum (previously Teleutomyrmex schneideri)	Tetramorium caespitum, Tetramorium impurum	y, y, no	Yes		France, Switzerland, Turkmenistan	Bushchinger 2009, Antwiki
Vollenhovia nipponica	Vollenhovia emeryi	y, y?, no			Central Japan	Satoh and Ohkawara 2008

*Additional citations from inquiline table not in text: (Alpert and Akre 2015; Anderson et al. 2010; Bolton 1988; Cole 1965; Douwes, Jessen, and Buschinger 1988; Johnson 1995; Satoh and Ohkawara 2008; Schifani 2017; Vankerhoven, Vanstraelen, and Dekoninck 2011)

Table 2: Samples in which inquiline brood were collected

specimen#	latitude	longitude	transect	rock_size_cm	leaf_cover	dicot_cover	canopy_cover	nearest_tree_sp	nearest_tree_m	date	notes	temp(F)	Start time	End time
A3	30.28384	-97.7788	T146	16x13	2	0	2	*Ulmus crassifolia*	1	3/1/2018	Active	70	4:00pm	5:30pm
A11	30.28102	-97.7761	T101	15x8	2	1	1	*Ulmus crassifolia*	3	3/12/2018	Very active, with one queen!	76	2:20pm	3:30pm
A24	30.28095	-97.77642	T101	15x15	2	1	3	*Ulmus crassifolia*	0	3/27/2018	Active, base of tree	74	11:00am	12:30pm
A44	30.28379	-97.77884	T146	25x10	3	0	2	*Ulmus crassifolia*	3	5/10/2018	Same as A33, collected brood	71	10:45am	12:15pm
B18	30.28101	-97.77629	T101	25x20	3	2	3	*Celtis*	3	9/19/2018	1 alate?	91	1:45pm	3:45pm

Table 3: Collected brood samples sifted for eucharitid and *Solenopsis* pupae. Samples that need further examination omitted; all samples stored in 100% Ethanol for potential genetic work.

Number	Accession#	Eucharitid	Solenopsis	Notes
A1	JNS559	0	0	
A2	JNS560	NA	NA	
A3	JNS561	0	1	at least 1 inquiline-esque larva. accidentally dumped 2 larvae from A4 into vial post-inspection
A4	JNS562	NA	NA	darker minor worker than A1-A3 collections?
A5	JNS563	0	0	at leats 8 springtails, 1 mite?? pupa
A6	JNS564	0	0	few springtails
A7	JNS565	0	0	
A8	JNS566	0	0	
A9	JNS567	0	0	
A10	JNS568		0	
A11	JNS569	0	1	SEVEN INQUILINES AT LEAST IN SAMPLE 1 grown Pheidole queen
A12	JNS570	0	0	
A13	JNS571	NA	NA	
A14	JNS572	0	0	
A15	JNS573	0	0	has some pretty tiny larvae/pupae; not sure if anything special but maybe inquiline larva?
A16	JNS574	0	0	
A18	JNS576	0	0	
A19	JNS577	0	0	*for imaging: has almost fully developed Pheidole pupa
A20	JNS578	0	0	mite
A21	JNS579	NA	NA	
A22	JNS580	NA	NA	
A23	JNS581	NA	NA	
A24	JNS582	0	1	1 inquiline! 1 weird pupa?
A25	JNS583	0	0	phorid fly pupae? pic on phone
A26	JNS584	NA	NA	
A27	JNS585	?	?	can't find
A28	JNS586	NA	NA	
A29	JNS587	?	?	can't find
A30	JNS588	0	0	
A31	JNS589	0	0	
A32	JNS590	0	0	
A33	JNS591	0	0	
A34	JNS592	0	0	WEIRD hemiptera larva

A35	JNS593	NA	NA	
A36	JNS594	0	0	3 Pheidole queen pupae
A37	JNS595	0	0	Pheidole queen pupae
A38	JNS596	0	0	Pheidole queen pupae
A39	JNS597	0	0	Pheidole queen pupae
A40	JNS598	0	0	1 alate 1 dealate Pheidole queen
A41	JNS599	0	0	
A42	JNS600	0	0	tetra?
A43	JNS601	0	0	
A44	JNS602	0	1	1 inquiline pupae
A45	JNS603	NA	NA	
A46		NA	NA	
B17	JNS620	1	0	one long grub
B18	JNS621		1	very active colony, lots of eggs, 2 inquiline pupae??