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Biology and Behavior of the Asian Needle Ant, *Brachyponera chinensis*

Hamilton Ross Allen
Clemson University

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BIOLOGY AND BEHAVIOR OF THE ASIAN NEEDLE ANT, *BRACHYPONERA CHINENSIS* (EMERY)

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Entomology

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by
Hamilton Ross Allen
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Accepted by:
Dr. Patricia Zungoli, Committee Chair
Dr. Peter Adler
Dr. Eric P. Benson
Dr. Patrick Gerard

ABSTRACT

Ants are among the most successful invasive organisms in the world. To curb the spread of invasive ants in non-native environments, their ecological, biological, and behavioral characteristics must be identified. The Asian needle ant, *Brachyponera* (= *Pachycondyla*) *chinensis* (Emery) is an emerging invasive pest in urban areas and forests in the southeastern United States. However, general basic biological information on *B. chinensis* and subsequent management options are deficient. This work contributes to the standing biological information on *B. chinensis* by examining the seasonal life cycle, social nesting habits, and transport behaviors of *B. chinensis* colonies.

To determine the seasonal life cycle of Asian needle ants, nests were collected monthly for ten months from infested locations in South Carolina. During the study 40 nests were collected and the number of workers, queens, male/female alates, eggs, larvae, and pupae was determined. The ants follow a seasonal cycle of production. Worker ant numbers are highest in May, August, and October while the majority of male and female reproductives are produced in July and August. Colonies also produce more males than reproductive females during the year. The number of workers and male ants found in a nest is positively correlated with the number of queens.

To determine if Asian needle ants are polydomous, I investigated the spatial organization and aggression between *B. chinensis* nests. Spatial distribution of nests was determined in four *B. chinensis* infested locations. Monte Carlo tests for compete spatial randomness revealed that three nests followed a pattern of uniform distribution suggesting that the ants are not polydomous. However, nests in one of the plots did follow a clumped distribution. Furthermore, workers originating from the same experimental plot and separate locations did not display aggression in 3 x 3 nestmate recognition assays, suggesting that *B. chinensis* are a polydomous ant species

In a laboratory study, the recruitment behaviors of *B. chinensis* ants during nest emigration were determined. Before recruitment to the new nest location began, *B. chinensis* ants organized into three distinctive groups: queen-tending, brood-tending, and scouting. Once the new nest site was identified, scout ants began physically transporting nestmates into the new harborage via tandem carrying or adult transport. Transport rates increased in the first 30 minutes and did not change during the 30-55-minute time interval when brood was transported. However, the adult transport rate increased again after brood transport was completed and decreased after 90 minutes.

Results presented in the current study contribute to the growing body of biological knowledge on invasive *B. chinensis* populations in the U.S. The information presented here are the first to address the colony attributes of this species. I identified adult transport as a recruiting method during emigration. Further the information presented here, highlight the life cycle and polydomous nature of *B. chinensis* and support the development of a seasonally based and area-wide management strategies for this nuisance ant species.

DEDICATION

I would like to dedicate this work to everyone who has helped me along the way. Life is a series of attempts to accomplish specific projects and without certain individuals, I would have never left 119 May St. One love to Mama Lean and to Aunt Sandra.

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Lastly this goes out to anyone that took the time to read this, just know that the world is huge and that there is more than one way to skin a cat. Keep your head up and ride this thang called life till the wheels fall off!

TABLE OF CONTENTS

Page

CHAPTER

Table of Contents (Continued) Page

LIST OF TABLES

List of Tables (Continued)

LIST OF FIGURES

List of Figures (Continued)

List of Figures (Continued)

List of Figures (Continued)

List of Figures (Continued)

CHAPTER ONE

RESEARCH JUSTIFICATION

The occurrence and potential threat of biological invasions continue to plague the world. Biological invasions can be detrimental to biodiversity and ecosystems, depending on the biology and behavior of the newly introduced species (Blight et al. 2017). Invasive ants have several common characteristics and are regarded as some of the most prolific invasive species causing ecological damage while impacting normal human behaviors in many environments (Lester and Gruber 2016).

The Asian needle ant, *Brachyponera (Pachycondyla) chinensis* (Emery), is an invasive ant that is becoming a dominant species in the southeastern U.S. (Nelder et al. 2006). Management options for the pest are limited because the invasive characteristics of the ant are unknown. To date, the most comprehensive biological study on *B. chinensis* was completed by Murata et al. (2017) who documented the ants' seasonal life cycle in Japan. However, the biology, ecology, and behaviors of introduced species can differ from their native counterparts (Blight et al. 2017).

The presence of multiple, connected nests in an ant society is a condition known as polydomy. Polydomy is a characteristic common to many invasive ant species (Holway et al. 2002). *Brachyponera chinensis* is identified as a polydomous ant species but the extent of polydomy in invasive populations is unknown. Moreover, polydomous ant colonies participate in budding during nest emigrations which contributes to the success of invasive ants. However, studies documenting the nest emigration behaviors of *B. chinensis* have not been identified.

The purpose of the current research is to identify biological characteristics of *B. chinensis* populations in the southeastern U.S. The identification of these characteristics contributes to the growing knowledge on invasive organismal biology and may also influence the creation of management techniques for *B. chinensis*.

The goals of this research were to:

1) Monitor the seasonal life cycle of *B. chinensis* in South Carolina.

   Documenting the ants' life cycle assisted in identifying the differences between native Japanese and U.S. populations and may also identify patterns that could be exploited by pest management professionals.

2) To determine the extent of polydomy in *B. chinensis* by identifying spatial nesting distribution patterns and measuring agonistic behavior between colonies originating from separate locations. Results from this study will determine if colonies are polydomous and may also provide insight into the propagation patterns of *B. chinensis.*

   Hypothesis: *B. chinensis* colonies are polydomous and nestmate aggression increases with origin distance.

3) 3) To determine if *B. chinensis* workers participate in adult transport during nest emigrations

   Hypothesis: *Brachyponera chinensis* workers employ adult transport during nest emigrations

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CHAPTER TWO

LITERATURE REVIEW

Life History

Brachyponera (= Pachycondyla) chinensis (Emery), the Asian needle ant, belongs to the primitive subfamily Ponerinae. The Ponerinae are a diverse ant group originating in the Tropics, but presently found in sub-tropical and temperate regions on all continents excluding Antarctica (Fisher and Cover 2007). As a group, the Ponerinae possess similar behavioral and morphological characteristics including worker caste monomorphism, small colonies consisting of several hundred adult workers, and solitary foraging behavior (Creighton 1950, Guénard and Dunn 2010, Schmidt and Shattuck 2014). Additional characteristics common to the Ponerinae include engaging in adult transport during colony emigration, prey immobilization by stinger, and a preference for termitophagy (Schmidt and Shattuck 2014). The Ponerinae are morphologically and phylogenetically separated into two tribes, Platythyreini and Ponerini. Platythyreini are represented by one species while the Ponerini tribe contains the remaining Ponerinae species.

Asian needle ant workers were initially described and named by F. Smith in 1874 (Creighton 1950) but have since undergone several name changes and descriptions. *Ponera solitaria* (Smith), *Ponera nigrita chinensis* (Emery), *Euponera chinensis* (Emery), *Pachycondyla chinensis* (Emery), and *Brachyponera chinensis* (Emery) have all been used as acceptable names for the ant. Although F. Smith is credited with the initial identification of *P. solitaria*, Emery is currently credited as the identifier of *B. chinensis* because *chinensis* is the junior synonym of *solitaria* (Brown 1958). The genus *Brachyponera* originates from the neotropics, and is one of the most diverse ant groups. However, Schmidt (2013) identified the *Brachyponera* as a paraphlyletic group. Thus, *Brachyponera* was divided into 19 genera and the Asian needle ant was placed into the genus, *Brachyponera* (Emery). The genus *Brachyponera* contains 24 species, with *B. chinensis* being the most studied, due to its invasive nature.

The Asian needle ant is also known as the "giant needle ant" or "oo-hari-ari" in its native Japan and colonies have been documented in Eastern and Southeast Asia, (Yashiro et al. 2010). Yashsiro et al. (2010) reported that *B. chinensis* ants from the Japanese and Ryukyu archipelagoes display great morphological variation. A phylogenetic tree based on CO1 region mitochondrial sequence data revealed that the *B. chinensis* species complex is composed of *B. chinensis* and *Brachyponera nakasujii* (Yashiro, Matsuura, Guénard, Terayama & Dunn) (Yashiro et al. 2010). Although, similar in appearance *B. chinensis* workers possess larger morphological traits when compared to *B. nakasujii* but the latter species has a larger dorsal petiole width. Further, *B. chinensis* males are lighter in color with less developed mandibles.

Morphology

Brachyponera chinensis workers are less than 4-5.0 mm in length and uniformly black in color but, possess brown mandibles and orange-brown legs (MacGown et al. 2013). They possess 3-segmented antennae with 9 flagellomeres with the antennal scape extending beyond the posterior margin of the head by the length of the second antennal segment. Mandibles are composed of six or seven apical teeth and several less conspicuous teeth basally. The petiole is one segmented, large, erect, and convex anteriorly (Smith 1934, MacGown 2009). *Brachyponera chinensis* workers do not climb smooth surfaces or vegetation because they possess reduced tarsal arolia (Guénard and Dunn 2010). The ants have well-defined stingers capable of injecting prey or potential predators with venom composed of several different histolytic and neurotoxic peptides (Nelder et al. 2006).

Brachyponera chinensis queens are dark-brown to black in color, measure between 5.0 and 6.5 mm in length, possess 12-segmented antennae, and may possess two pairs of wings or thoracic dealation scars (MacGown 2009). Male ants are yellow-brown to dark-brown in color; 3.5 to 4.0 mm in length; head and mouthparts are reduced; and the antennae are 13-segmented (MacGown 2009).

History in the U.S.

The first U.S. documentation of *B. chinensis* occurred in 1932 when H.T. Vanderford of the United States Department of Agriculture collected specimens from forests near Decatur, GA (Smith 1934). Since 1932, *B. chinensis* complete colonies and singular specimens have been documented in Alabama, Connecticut, Florida, Georgia, New Jersey, New York, North Carolina, South Carolina, Tennessee, Washington State, and Virginia (Smith 1934, Nelder et al. 2006, Pecarevic et al. 2010, MacGown et al. 2013), becoming a common invasive insect species in its southeastern U.S range (Nelder et al. 2006).

Asian needle ants are prevalent in the southeastern U.S., but could establish populations outside their introduced range. Bertelsmeier et al. (2013) used current global distribution data for *B. chinensis* in addition to global climatic predictors (mean annual temperatures, annual precipitation) to determine the potential range increases for the insect. According to climate model predictions, *B. chinensis* can increase its range and survive in Northeast America, Southeast Asia, and South America. Specifically, by 2080 the North American range of *B. chinensis* may increase by 1,972,781 km^2 or 74.9% with continual climate change increases.

Colony Life Cycle:

Brachyponera chinensis colonies are often composed of a few hundred workers (Creighton 1950) but Zungoli and Benson (2008) documented colonies consisting of several thousand workers. Colonies tend to establish nests near mature forests in termite galleries within logs or under pavement tiles and lawn ornaments in urban environments (Paysen 2007, Gúenard and Dunn 2010, Pecarevic et al. 2010).

Zungoli and Benson (2008) reported the seasonal colony activity of *B. chinensis* in South Carolina. *Brachyponera chinensis* worker ant activity was first observed in January but consistent activity did not occur until March when ambient temperatures remained at or near 15°C. Ant activity peaked in August and began to decline until activity ceased in November. Reproductive alates were collected from light trap samples beginning in May and ending in September.

Information on the sociometric attributes of many ant species are unavailable (Tschinkel 2011) and *B. chinensis* is one of the least studied species, considering its pest status in the U.S. However, in 2008, Gotoh and Ito (2008) attempted to identify the seasonal life cycle of *B. chinensis* in Japan. However, after reviewing *B. chinensis* morphological data provided in the 2008 study, Yashiro et al. (2010) proposed that Gotoh and Ito conducted their studies on a newly described cryptic species, *B. nakasujii*. It should be noted that Yashiro et al. (2010) did not compare physical specimens collected from their study with specimens identified by Gotoh and Ito (2008) as *B. chinensis*. Therefore, some of the information presented by Gotoh and Ito (2008) could pertain to *B. chinensis*. Murata et al. (2017) built on the work of Gotoh and Ito (2008) and documented the seasonal difference between the number of workers and queens in Japanese *B. chinensis* populations. The authors collected *B. chinensis* nests in October 2011 and in May 2012. The mean number of queens and workers per nest were not significantly different between months of May and October. Their results do contribute to the amount of biological information on *B. chinensis* but further studies must be conducted over a longer period to fully elucidate the seasonal life cycle of this invasive ant species.

Polydomy

Many eusocial insects build one nest containing all colony members. However, some ant and termite species build several spatially separated yet connected nests, a phenomenon known as polydomy (Debout et al. 2007). Characteristics common to polydomous ant colonies include a high density of ant colonies within an area, decreased intraspecific aggression, and decreased distances between nests (Ellis et al. 2017, Debout et al. 2007). Benefits of polydomy may include increased brood production (Gotoh and Ito 2008), territorial dominance, decreased occurrence of predation (Debout et al. 2007), and increased foraging efficiency (Buczkowski 2011). Further, polydomous ant colonies are associated with polygynous queen social structure. However, select monogynous ant colonies also display polydomous behavior(s). Debout et al. (2007) characterize polydomy by polygynous species as a secondarily evolved trait following polygyny expressed by a monogynous ancestor. Polydomy has been observed in 166 ant species belonging to 49 genera (Debout et al. 2007).

Despite being a common phenomenon, the origins, mechanisms, and adaptive significance of polydomy have not been elucidated. Understanding the social organization of a colony provides insight on the behavioral and evolutionary ecology of an ant species (Robinson 2014). Although, polydomy is poorly understood, it may contribute to the success of invasive ant species (Holway et al. 2002). Five ant species including *Anoplolepis gracilipes* (Smith), *Linepithema humile* (Mayr), *Pheidole megacephala* (Fabricius), *Solenopsis invicta* (Buren), and *Wasmannia auropunctata* (Roger) are listed on the world's worst invasive species list (Robinson 2014) and each of these species exhibit polydomy.

Brachyponera chinensis is characterized as a polydomous ant species but experimental studies have not been conducted. However, anecdotal evidence supports the notion that *B. chinensis* exhibit polydomy. Paysen (2007) collected thirteen *B. chinensis* nests from the Great Smoky Mountain National Park and three of the nests did not contain dealate female reproductive, suggesting that the colonies participate in budding. Also, colonies did not display inter-colonial aggression, and nine of the colonies were polygynous. These results suggest that *B. chinensis* colonies could be unicolonial or polydomous. Gotoh and Ito (2008), conducted studies to identify the seasonal nesting cycle of *B. nakasujii* in Japan. During June 2004, 78 of the 104 *B. nakasujii* nests collected did not contain any queens, but workers and brood were present. These findings in conjunction with Paysen's (2007) observations indicate that *B. chinensis* could exhibit a polydomous nesting system.

Nestmate Recognition

For an ant colony to flourish, nest members must be able to distinguish nest mates from non-nestmates (Hölldobler and Wilson 1990, Sturgis and Gordon 2012). Ants use visual cues and cuticular hydrocarbons to distinguish nestmates from non-nestmates (Sturgis and Gordon 2012). Closely related ants produce and share the colony odors through the sharing of space, food, and through grooming (Sturgis and Gordon 2012). Nestmates and non-nestmates can be distinguished by comparing cuticular hydrocarbon profiles by chemical analysis (Wagner et al. 1998) or by conducting nestmate recognition assays (Roulston et al. 2003). Nestmate recognition assays are used to measure the amount of aggression displayed by ants originating from the same or different colonies (Roulston et al. 2003).

Murata et al. (2017) conducted nestmate recognition studies to determine if native *B. chinensis* populations displayed conspecific aggression. Their results show that regardless of nesting origin, non-nestmates did not display aggression towards one another. Nestmate recognition studies have not been conducted with non-native *B. chinensis* populations. Nestmate recognition studies could be performed to support or dispel *B. chinensis*' status as an invasive and functionally polydomous ant species.

Foraging

The foraging activities of ants are well documented (Hölldobler and Wilson 1990, Showler et al. 1990, Pearce-Duvet et al. 2011). Studies have reported ants employ variable foraging behaviors. Foraging type is largely determined by dietary choice, food, and location (Hölldobler and Wilson 1990). According to Hölldobler and Wilson (1990), ants exhibit three different foraging/hunting categories including solitary foraging, recruitment, and group searching.

Ant colonies employ different recruitment methods during foraging expeditions. Foraging recruitment methods include tandem running, trail following (Hölldobler and Wilson 1990), and the recently described behavior of tandem carrying (Guénard and Silverman 2011). Tandem running occurs when a worker ant leads a nestmate to a known food item. During the process, the lead ant walks toward the item, stopping every so often allowing the trailing ant to catch up and strum the body of the lead ant with its antennae (Franklin 2014). The process continues until the pair reaches the desired food item. During trail following worker ants lay pheromone trails from a food source back to the nest. Workers follow the deposited trail to the food item and upon return reinforce the trail with pheromonal secretions (Hölldobler and Wilson 1990, Franklin 2014).

Bednar and Silverman (2011) hypothesized that polydomy, or multiple nesting sites may influence foraging behavior. In laboratory studies, they reported that *B. chinensis* employ several different foraging behaviors to collect food including termitolesty, group recruitment, and solitary foraging. The recruitment processes of *B. chinensis* were initially described by Takimoto (1988) but Guénard and Silverman (2011) recently re-described this behavior. Workers participate in a context specific adult transport behavior known as tandem carrying. Tandem carrying occurs when a food item is too large for a single worker to collect and return to the nest. A worker unable to retrieve food items returns to the nest and drums its antennae on potential recruits. The recruited ant folds its legs inward towards its venter. According to Guénard and Silverman (2011) the carrying ant grasps the recruit with its mandibles between the first and second pairs of legs of the ventral mesothorax. They reported that studies to determine the presence and usage of pheromones during tandem carrying were inconclusive. However, visual and chemical signals may still solicit tandem carrying behavior.

Medical Importance

Brachyponera chinensis use their sting to stun their prey before capture but stinging is not limited to prey items. The ants are known to sting humans (Nelder et al. 2006). Sting victims experience local reactions persisting from several hours to two weeks after the initial sting. Symptoms arising from the sting may include urticaria, skin redness, swelling, and in extreme cases anaphylaxis may occur (Yun et al. 1999, Nelder et al. 2006, Lee et al. 2009). In fact, anaphylactic reactions were documented in 2.1% of persons stung by *B. chinensis* in a South Korean town (Lee et al. 2009). Allergens present in the venom are responsible for the variety of symptoms. Lee et al. (2009) determined that a 23 kDa and 25 kDa protein belonging to the antigen 5 family are the major reactive *B. chinensis* venom components. Leath et al. (2006) reported the case of a 67-year-old male suffering from anaphylaxis stemming from stings delivered by an unknown insect at his home. Several colonies around his home were collected and identified as *B. chinensis*. This case served as the first documentation of anaphylaxis due to *B. chinensis* stings in the United States.

In 2004, the first *B. chinensis* stings reported in South Carolina were made by Greenville Zoo workers who first observed the ants on their campus in 1997 (Nelder et al. 2006). Since 2004, multiple *B. chinensis* stinging events were reported in South Carolina, and three reports of anaphylaxis due to stings also were reported in the U.S. (Leath et al. 2006, Nelder et al. 2006).

Ecological Impact

One of the most important characteristics contributing to successful establishment of invasive ant colonies is the ability to outcompete other species for resources, specifically, the competitive displacement of native ant species (Hölldobler and Wilson 1990, Holway et al. 2001). For example, the ability of two of the most successful invasive ant species, *Linepithema humile* (Mayr) (Human and Gordon 1996) and *Solenopsis invicta* (Buren) (Tschinkel 2006), have been extensively studied. Mechanisms contributing to invasive success include nestmate recruitment ability and increased interspecific aggression (Holway et al. 2002).

Guénard and Dunn (2010) were first to report negative impact of *B. chinensis* presence on species richness and abundance of native ant populations in mature North Carolina hardwood forests. The abundance of *B. chinensis* workers was higher than native ants at all collection sites, accounting for more than half of all collected ant specimens. Furthermore, overall species density of native ants was 30 to 40% lower in *B.*

chinensis invaded areas. Moreover, *B. chinensis* presence was negatively correlated with smaller-sized Formicine, Myrmicine, litter foraging ant species, and *Aphaenogaster* spp. ants. On the other hand, the abundance of larger ants such as *Camponotus* and *Formica* species were not affected by *B. chinensis* presence. The reasons for this occurrence are currently unknown.

The ability of invasive ants to outcompete interspecific ant species from primary food resources is suggested as a key to success (Holway et al. 2002). Bednar and Silverman (2011) conducted food preference studies determining that *B. chinensis* workers prefer *Reticulitermes virginicus* (Kollar) termites over other offered items (*Parcoblatta spp*., Elaterid larvae, Chilopoda, and Collembola). Also, *B. chinensis* workers preferred to reside in harborages previously occupied by *R. virginicus*. Results suggest that *B. chinensis* may be able to outcompete ants for nesting space and resources. Bednar et al. (2013) conducted additional studies to determine if *B. chinensis* workers outcompete *Aphaenogaster rudis* (Emery) for *R. virginicus*. Asian needle ant workers discovered termite nests faster than *A. rudis* and killed *A. rudis* workers in the presence of *R. virginicus*. Rodriguez-Cabal et al. (2012) reported that *B. chinensis* workers negatively impact the seed dispersing ability of *A. rudis* in invaded plots. The number of *A. rudis* workers was 70% lower in plots containing *B. chinensis* and the number of seeds removed from these plots was 70% lower than in non-invaded plots.

Management

Outside their native range invasive ants may achieve high local abundance, disrupt native ecological assemblages, and may cause public health concern (Tsutsui and Suarez 2003, Holway et al. 2002). As a result, control measures are necessary but may be difficult (Holway et al. 2002). Current viable control methods for invasive ants in urban environments include biocontrol (Tschinkel 2006) and cultural control methods (Silverman and Brightwell 2008), but residual liquid insecticide applications and toxic bait treatments remain the most widely used methods (Buczkowski et al. 2014). However, liquid insecticide applications provide short term control, inadequate control of subterranean ant nests, may negatively affect non-target ant populations, and may not control large unicolonial ant populations (Silverman and Brightwell 2008, Buczkowski et al. 2014) resulting with ant colonies reestablishing uninvaded areas.

Insecticidal baits are recommended in many situations. Baits are non-repellent, express a delayed toxicity, and exploit the food sharing behavior of ants (Hooper et al. 1998). Baits are delivered in liquid, gel, and granular formulations. Liquid formulations usually consist of an active ingredient suspended in a sugar based matrix. These baits are useful for ants preferring sweet items (Buczkowski et al. 2014) but may not be useful for ants subsisting on a diet dominated by protein-based items such as *B. chinensis*. Granular baits consisting of protein or oil-based matrices are instead used to control these ants (Tschinkel 2006, Buczkowski et al. 2014). Spicer-Rice et al. (2012) conducted studies to determine the efficacy of scattered and discrete applications of Maxforce Complete® (AI = hydramethylnon, 1.0%) (Bayer CropScience, Monheim, Germany) a granular hydramethylnon bait to control *B. chinensis* populations in North Carolina. Results from the study indicate that granular applications of hydramethylnon provide rapid and effective control of *B. chinensis* colonies for twenty-eight days. However, during the baiting study the *B. chinensis* worker abundance also began to decline in control plots 14–28 days after treatment in September and October. The continued suppression of *B. chinensis* worker in treatment plots may have been an artifact of seasonal changes in ambient temperature.

Although Spicer-Rice et al. (2012) reported that protein-based bait can provide adequate control of *B. chinensis*. Mo (2013) reported that *B. chinensis* workers did not display a preference for agar/sucrose or vegetable oil. However, *B. chinensis* workers preferred canned tuna in field trials. Mo (2013) also compared the efficacy of several baits currently used for ant control. Results indicated that Advion® gel (AI = Indoxacarb, 0.22%) (DuPont, Nemours, and Company, Wilmington, Delaware) performed better than MaxForce Complete® in field trails. The results are inconsistent with those reported by Spicer-Rice et al. (2012), so further comparisons are necessary.

Buczkowski (2016) conducted mortality studies with *R. flavipes* termites exposed to 25 ppm fipronil (Termidor SC®, BASF Corp, Raleigh, NC) treated sand. A single-contaminated termite killed 100 *B. chinensis* workers in 9 hours. Using contaminated termites as bait in field situations is difficult because termites will only remain viable for 60 minutes following exposure. Also, additional termite feeding organisms could be negatively affected by exposure to fipronil treated termites. Further, Termidor SC® label directions instruct the user to apply the product to the exterior of structures to control ants. Using fipronil treated termites is an off label use and therefore illegal application, so the proposed “trojan horse” technique is not a viable method of control at this time.

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CHAPTER THREE

SEASONAL LIFE CYCLE OF *BRACHYPONERA CHINENSIS* (EMERY)

Introduction

Invasive biology is the study of human-mediated transport and the subsequent introduction of species to areas outside their native range (Davis 2009). Transported species residing outside their natural environment are often removed from the threat of competition, disease, parasitism, and predation (Porter et al. 1997). Consequently, invasive species thrive in exotic locations and in some cases, cause negative impacts on economies, community assemblages, and biodiversity (Sanders et al. 2003).

Given their colonial structure, social insects are perfect models for invasion (Moller 1996). Ants are currently the most successful group of social insects. Their colonies are prominent members of an ecosystem and can become aggressive colonizers when populations are located outside their natural range (Tsutsui and Suarez 2003). Due to their propagation potential and ecological impacts, invasive ants cost the United States more than one billion dollars in damage and management costs annually (Pimentel et al. 2005).

Invasive ants often possess shared characteristics that allow them to efficiently exploit environments outside their native range including polygyny, reproduction by budding, unicoloniality, and decreased intraspecific aggression (Holway et al. 2002, Tsutsui and Suarez 2003). Oftentimes, the biology and behavior of introduced ants differs from native populations. Red imported fire ants, *Solenopsis invicta* (Buren), native to the Pantanal region of Brazil, have a higher number of single queen colonies, low intraspecific aggression and, colonies with defined territories (Porter et al. 1997). Conversely, *S. invicta* populations in the United States are more likely to be polygynous, display decreased intraspecific aggression, and have multiple colonies residing in close proximity. (Porter et al. 1997). Another successful, invasive ant is the Argentine ant, *Linepithema humile* (Mayr). Argentine ants are native to Argentina, Brazil, and Paraguay (Suarez et al. 1999). Native *L. humile* colonies have defined territories and are genetically distinct from one another (Buczkowski et al. 2004). Invasive *L. humile* populations are genetically similar, unicolonial, forming supercolonies in California (Buczkowski et al. 2004, Suarez et al. 1999) and Europe (Berville et al. 2013).

Another invasive ant species in the United States is the primitive ponerine ant, *Brachyponera chinensis* (Emery) (Nelder et al. 2006, Yashiro et al. 2010). *Brachyponera chinensis* is native to Japan (Gotoh and Ito 2008). The ants are currently established in the southeastern United States (Nelder et al. 2006) and a few workers have been collected in Washington State, Connecticut, New Jersey, New York, and Virginia (Pecarevic et al. 2010, MacGown et al. 2013). Although primarily restricted to the southeastern U.S. the ant may naturally increase its range in the next 20 years according to climate-based population models (Bertelsmeier et al. 2013). Since its introduction, *B. chinensis* has displayed several characteristics in its native and introduced ranges common to invasive ant species. The ants exhibit polygyny in both ranges but polydomy and decreased intraspecific aggression has only been identified in Japanese populations (Murata et al. 2017). Invasive ant populations are known to cause ecological damage by impacting the success of native ant species (Porter and Savignano 1990). United States *B. chinensis* populations are associated with outcompeting and subsequently displacing the native, seed-dispersing ant, *Aphaenogaster rudis* (Enzmann) (Bednar et al. 2013, Warren et al. 2015).

Life cycle studies provide information on a species' capability to flourish within an environment. A study conducted by Murata et al. (2017) determined the social structure of *B. chinensis* in Japan. Results from the study indicate that *B. chinensis* is functionally polygynous and the number of workers does not differ significantly between the spring and fall. The colony characteristics of *B. chinensis* have been studied in Japan (Murata et al. 2017), but information regarding the seasonal colony life cycle of the ant in the U.S. are absent.

The research presented here documents, the seasonal life cycle of *B. chinensis.* Specifically, the number of workers, reproductives, and brood were sampled over the course of nine months. I anticipated observing a fluctuating pattern of colony member with respect to month of nest censure. Yielded information can be used to determine differences between native and invasive populations, provide insight into reasons for *B. chinensis'* invasive success, and aid future management efforts.

Materials and Methods

Nest Sampling

Brachyponera chinensis nests were collected twice monthly from sites and locations within the Clemson Experimental Forest (CEF) (34^{0}43'54" N, 82^{0}51'06" W). The CEF is located in Anderson and Pickens Counties, South Carolina and is a 17,500-acre mixed-hardwood and pine forest.

Brachyponera chinensis colonies establish nests in fallen timbers, stumps, beneath stones, and under pavement tiles (Smith 1934, Creighton 1950). All nests collected for the study were found within fallen timber, under timber, or within older tree stumps. Four *B. chinensis* nests were collected monthly from March 2014 to December 2014. In total, 40 *B. chinensis* nests were collected. At each sampling, fallen timber containing a *B. chinensis* nest was manually extracted with a shovel and all accompanying soil/materials were transferred to a plastic tub. Collected nests (n=40) were returned to the lab and placed in a freezer until counting. When processed in the laboratory, nests were censused for number of workers, dealate queens, alate queens, males, eggs, larvae, and pupae. Worker, queen, male, and alate *B. chinensis* ants were deposited in the Clemson University Arthropod Museum in November of 2017.

Statistical Analysis

Separate one-way ANOVAs were conducted to determine if the number of collected workers, dealate queens, alate queens, males, eggs, larvae, or pupae was influenced by collection month (SAS 9.2; SAS Institute Inc., Cary, NC). Post hoc analyses of the means were analyzed using a Tukey's HSD (JMP 12 Statistical Analyses). The inter-relationship between the presence of workers, queens, alate queens, males, eggs, larvae, and pupae was analyzed and determined by Pearson's partial correlation (SAS 9.2; SAS Institute Inc., Cary, NC). Relationships were considered significant when $P < 0.05$.

Results

Forty *B. chinensis* nests were censused providing the monthly mean number of workers, dealate queens, alate queens, males, eggs, larvae, and pupae (Figures 3.1-3.8). ANOVA indicates there was a relationship between month and the number of workers produced ($F_{9,30} = 6.12$; $P < 0.0001$). The mean number and standard deviation of *B. chinensis* workers collected monthly during the study is shown in Figure 3.2. There was considerable variation between the number of workers collected from each nest during the study. The number of workers from a single nest ranged from 26 to 741 workers. The average number of worker ants in a nest was highest in May (488 ± SD 179.95) and August (402 ± 196.76) while the lowest number of workers was collected in December (50.7 ± SD 26.23). Worker numbers increased from March to May but decreased while male and female alates were produced in June (265.5±79.4) and July (312.5±78.8). However, there is not a significant relationship between worker presence and male ants (Pearson correlation; $r = 0.010$, $P = 0.954$) or alate queens (Pearson correlation; $r = 0.210$, $P = 0.253$) (Table 3.1). The number of workers began to decrease significantly after October.

Overall, ANOVA results indicate there was a relationship between month and the number of workers produced ($F_{9,30} = 4.78$; $P = 0.0005$). Four of the forty collected nests were queenless, while 36 were queenright. Queen numbers per month ranged from 0 to 14. The highest average number of dealate queens was collected in August (9.5 ± SD 3.69) and the lowest number in December (0.75 ± SD 1.5) (Figure 3.3). A seasonal change in the number of queens was observed. The number of queens increased from

March to August, peaking in the latter month. Queen numbers declined after August and continued into December. Queen presence was negatively correlated with male presence (Pearson correlation; $r = -0.481$, $P = 0.006$).

The number of alate *B. chinensis* queens collected from nests differed significantly between months ($F_{9,30} = 14.65$; $P = 0.0001$). Average female alate numbers were highest in July (24.75 ± SD 8.84) and zero in March, November, and December (Fig. 3.4). Female alates were not collected because reproductive females are not produced in early spring, late fall, or during winter months.

The number of males found in nests was significantly different between months ($F_{9,30} = 13.88$; $P = 0.0001$). Male ants first appeared in May and were collected until October (Fig. 3.5). The highest number of males was collected in July (108 ± SD 41.04); males were not collected in March, April, November or December. Similar to alate queens, reproductive males are not produced in early spring, late fall, or during winter months.

The number of eggs collected from nests differed significantly between months ($F_{9,30} = 9.93$; $P = 0.0001$). Eggs were laid from March to October (Fig. 3.6). The highest number of eggs was collected in May (154.5 ± SD 64.66) and June (147.5 ± SD 55.09) and eggs were not collected in November or December.

Larvae were collected from April to September. Average larval collections were highest in June (76.25 ± SD 94.02) and zero in March, October, November, and December (Fig. 3.7). The number of larvae collected monthly did not differ significantly ($F_{9,30} = 1.95$; $P = 0.08$). Larval production increased from March to August. Larval increases coincide with the production of workers and female alates observed in Figures 3.1 - 3.4. However, the relationship between larval occurrence to worker and female alate numbers was not statistically significant (Table 3.1). The average number of pupae collected was highest in August (151.5 ± SD 34.15) but pupae were not collected in March, November, or December (Fig. 3.8). The average number of pupae collected monthly differed significantly ($F_{9,30} = 7.06$; $P = 0.0001$).

Discussion

This study serves as the first to document the seasonal cycle of *B. chinensis* nest members in the United States. The mean number of workers collected from *B. chinensis* nests was highest in May, August, and October (Fig. 3.2) suggesting three different worker production periods. Worker numbers were lower in June, July, and September and a few factors may contribute to the worker number oscillations. Reproductive ant (females and male alates) counts were highest in July when the colony may have switched its production from workers to reproductive ants. Higher worker numbers observed in October could be an artifact of seasonal polydomy. Some polydomous ant colonies fuse their nests before the winter months (Gordon and Heller 2014). Japanese, *B. chinensis* populations exhibit seasonal polydomy (Murata et al. 2017). Seasonal spatial patterns have not been studied in U.S. *B. chinensis* populations, but the evidence could be used to determine if *B. chinensis* exhibit patterns of seasonal polydomy in the U.S. (Ellis et al. 2017).

During the study, queen ants were collected from nests monthly but queenless nests were collected in November (1) and December (3). Queenless nests are a common feature of polydomous ant species (Hölldobler and Wilson 1990, Denis et al. 2007); queens are present in primary nests but are absent in associated secondary nests. In the case of secondary nests, workers can function sometimes as secondary reproductives (Hölldobler and Wilson 1990). However, *B. chinensis* workers are incapable of producing eggs because the workers lack ovarioles (Murata et al. 2017). Murata et al. (2017) also collected queenless *B. chinensis* nests from locations in Japan. Queenless colonies were prevalent after overwintering. I did not find any *B. chinensis* nests in the months of January/February and I did not find any queenless nests when the study began in March. Additional studies need to be conducted to determine if queenlessness follows a seasonal pattern similar to native Japanese *B. chinensis* populations. Also, queen numbers appear to be higher in U.S. populations than Japanese populations. Murata et al. (2017) reported average queen numbers in May (~ 2.5) and October (~3) but they did not follow nests over the course of one season so a true comparison is still needed. I hypothesize that *B. chinensis* queen number is higher in the U.S. Invasive ants tend to lean towards polygyny and possess higher queen numbers when compared to native populations (Tsutsui and Suarez 2003).

Like the seasonal fluctuations in worker production, alate production within an ant species follows a distinctive pattern (Hölldobler and Wilson 1990). Female alate production began in April but peaked in May and again in July. Winged queens were found in *B. chinensis* nests until October, ceasing in November and December. After September, the number of female alates decreased significantly. Zungoli and Benson (2008) collected female *B. chinensis* alates, using light traps and determined that female numbers were highest in June/July. Although there is a discrepancy, the differences can be attributed to mating flight dates. Alates may be produced early in the year but are not released until colony and environmental cues stimulate flight. The initial collection of male *B. chinensis* ants occurred in May, peaked in June/July, and ceased after September. The peak average number of males and winged females occurred in July and the calculated male: female sex ratio for July was 4.4:1 (Figure 3.1). Zungoli and Benson (2008) also collected more *B. chinensis* males than females (19:1) in their study. The presence of female alates is negatively correlated with the presence of males (Pearson correlation; r = -0.002, P = 0.9924) but the relationship is not significant. The specific mating methods of *B. chinensis* are unknown, but male-biased production ratios are common in polygynous ant species (Crozier and Pamilo 1996). Studies documenting the mating behaviors of *B. chinensis* are lacking, but given the preponderance of males collected, the ants may participate in budding and the adoption of newly mated queens into established colonies (Brown and Keller 2000) which are two behaviors common to polygynous ants (Franks 1987).

The collection of *B. chinensis* eggs and larvae proved to be difficult. During the study, nests were placed in the freezer before counting. Nest counts were not always performed immediately and some of the brood material was not preserved well. *Brachyponera chinensis* larvae are attached to the walls of their nests but some larvae were damaged during the removal and thawing process. Instead of emerging as a distinct specimen, some larvae appeared as gel-like masses. Egg to adult developmental time is unknown but the first eggs appeared in March and pupae were initially collected in April.

Development from egg to pupa may take 5-6 weeks but additional studies are necessary. Pupae were collected from April to October but the cases were not dissected to determine the ratio of workers to reproductives.

The research reported here focused on studying the seasonal life cycle of *B. chinensis* at the nest level. To effectively study the sociometry (quantitative measurement of all parts of an insect society) (Tschinkel 2011) of *B. chinensis* the boundaries and territory of a colony must be identified. Understanding the invasive biology of *B. chinensis* can serve as a basis for the design of management practices for this invasive ant. The information presented here provides preliminary data on the sociometry of *B. chinensis* in the United States.

Table 3.1 Pearson's partial correlation coefficients as tests of the null hypothesis that there is no association between *Brachyponera chinensis* (Emery) nest member demographic relative to monthly nest samples. Each table entry presents (in descending order); correlation coefficient (*r*), the probability (*P*) that there is a significant interrelationship between nest members.

	Nest Members						
	Workers	**Queens**	**Alate Queens**	**Males**	**Eggs**	**Larvae**	**Pupae**
Workers							
r	1.000	0.345	0.210	0.010	0.561	-0.235	-0.078
P		0.057	0.255	0.954	0.001	0.203	0.675
Queens							
r		1.000	0.231	-0.480	0.473	-0.032	-0.233
P			0.210	0.0062*		0.863	0.206
					0.007*		
Alate							
Queens			1.000	-0.002	0.350	-0.056	0.243
r				0.992	0.053	0.746	0.186
P							
Males							
r				1.000	0.058	0.300	0.311
P					0.755	0.101	0.088
Eggs							
r					1.000	-0.178	0.087
P						0.338	0.641
Larvae							
r						1.000	0.328
P							0.071
Pupae							
r							1.000
P							

Probability values followed by * indicate significance P < 0.05.

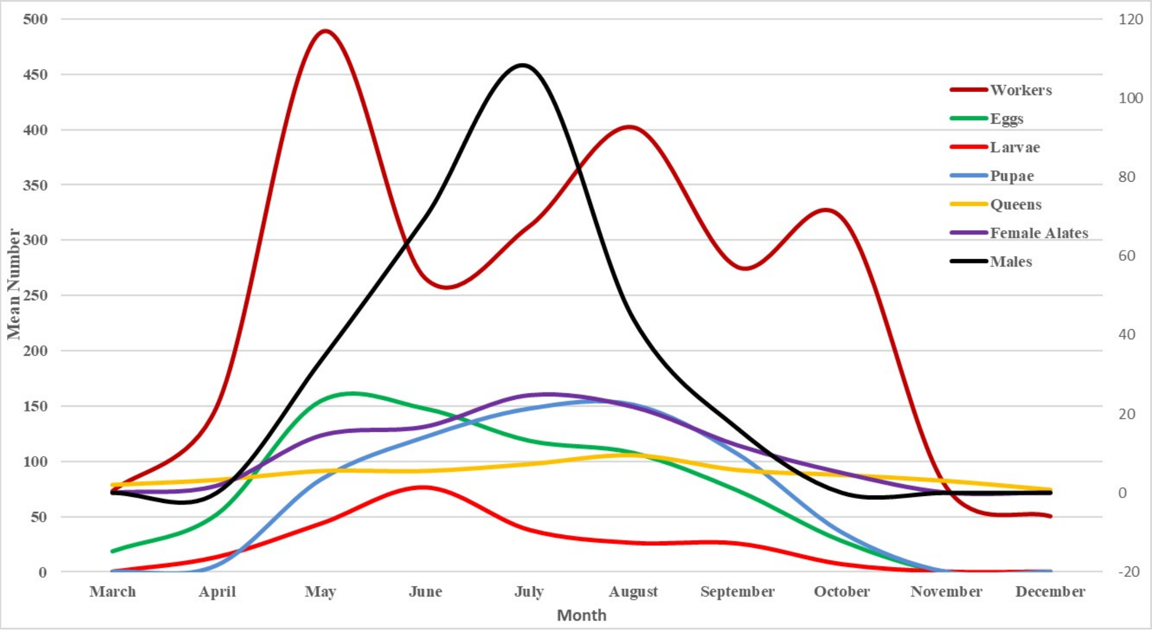

Figure 3.1. Total mean number of *Brachyponera chinensis* (Emery) workers, queens, males, female alates, eggs, larvae, and pupae collected from nests per month. Each monthly statistic was derived from four censused nests. Statistical analysis was not conducted on these data.

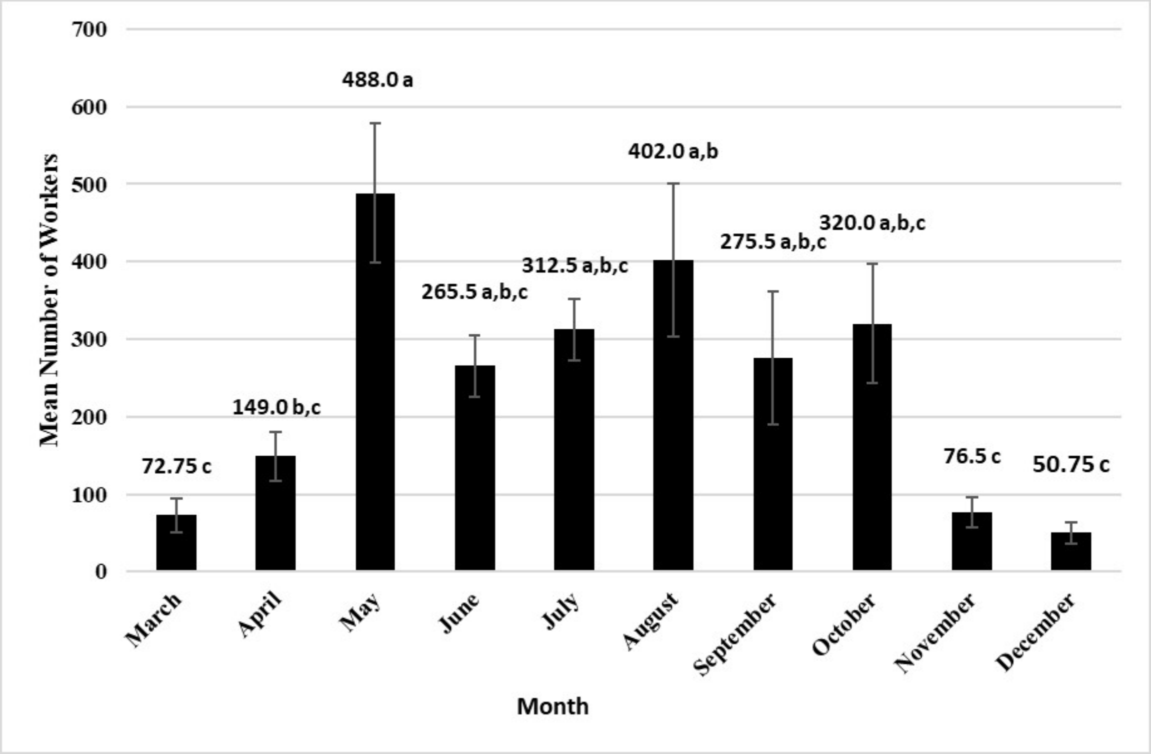

Figure 3.2. Mean number of *Brachyponera chinensis* (Emery) worker ants collected from nests per month. Columns show mean number and the SD is represented by whiskers. Each monthly statistic was derived from four censused nests. One-way ANOVA of the mean number of workers by month ($F_{9,30} = 6.1179$; $P < .0001$). Mean values connected by same letter are not significantly different.

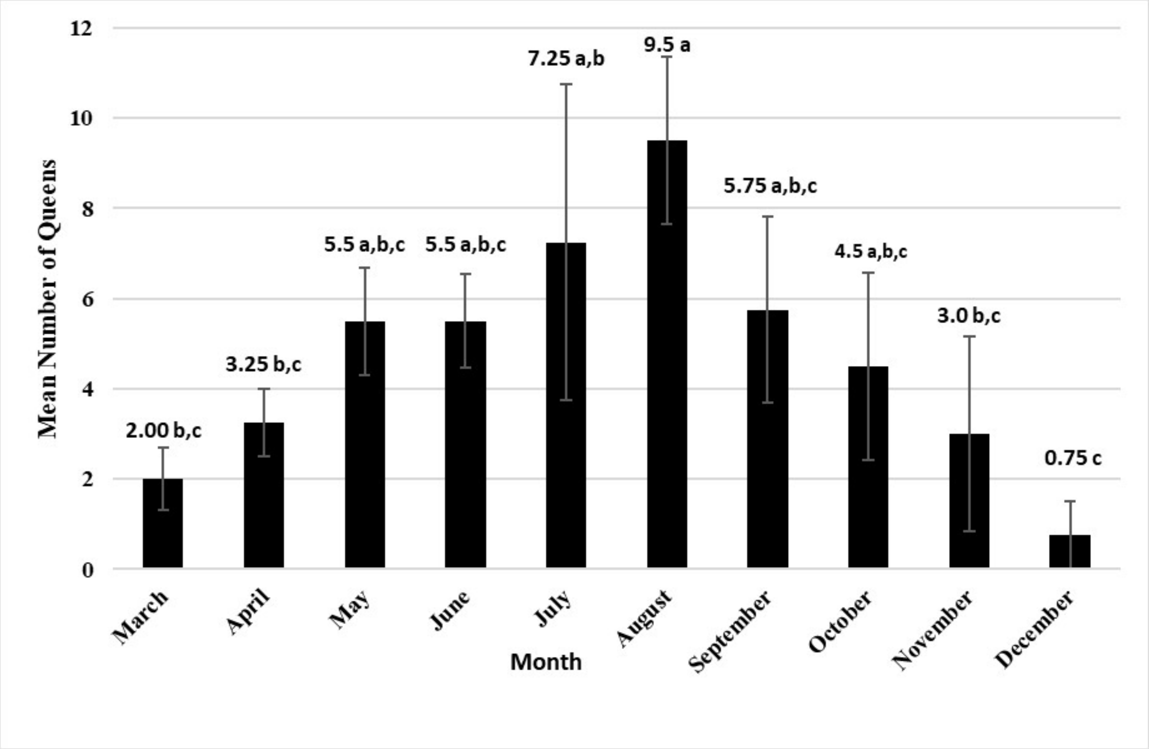

Figure 3.3. Mean number of *Brachyponera chinensis* (Emery) queens collected from nests per month. Columns show mean number and the SD is represented by whiskers. Each monthly statistic was derived from four censused nests. One-way ANOVA of the mean number of queens by month ($F_{9,30} = 4.7784$; P = .0005). Mean values connected by same letter are not significantly different.

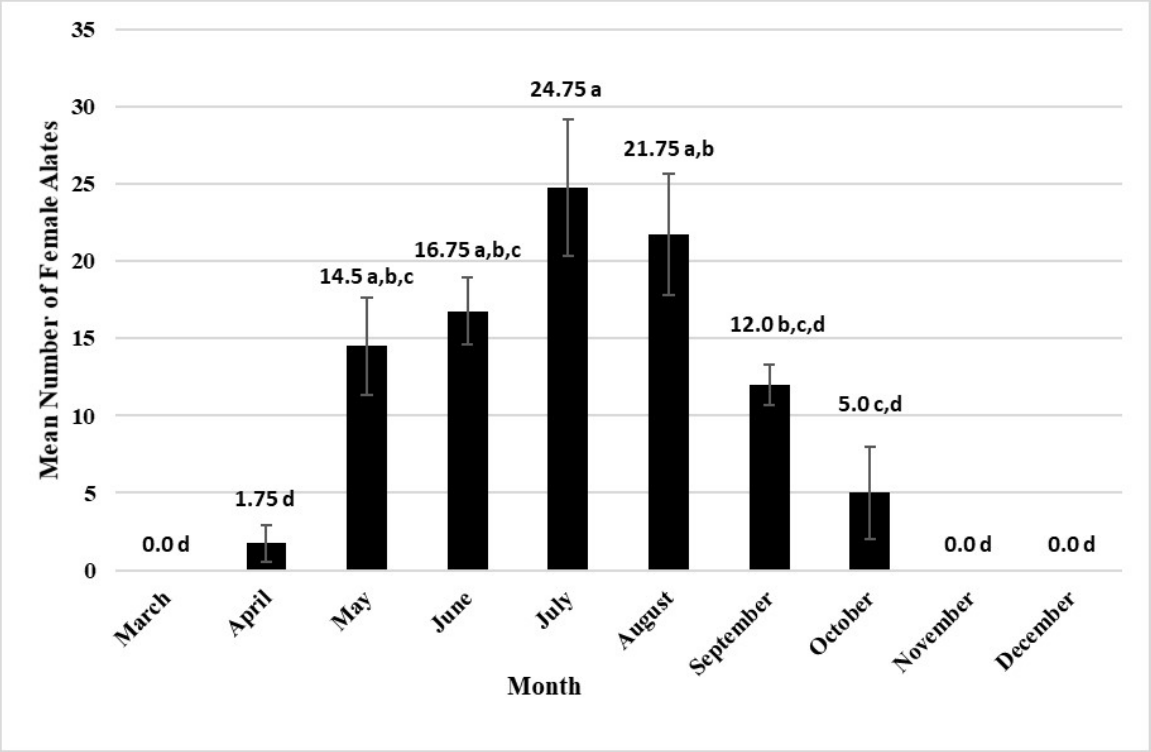

Figure 3.4. Mean number of *Brachyponera chinensis* (Emery) female alates collected from nests per month. Columns show mean number and the SD is represented by whiskers. Each monthly statistic was derived from four censused nests. One-way ANOVA of the mean number of female alates by month ($F_{9,30} = 14.65$; $P < .0001$). Mean values connected by same letter are not significantly different.

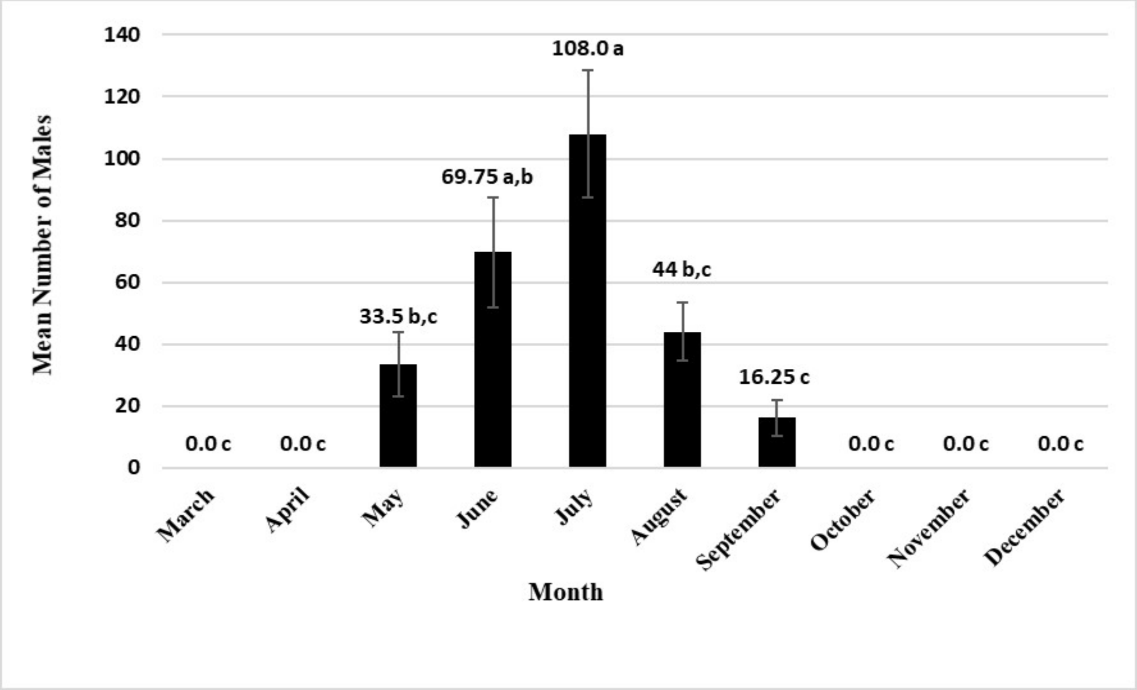

Figure 3.5. Mean number of *Brachyponera chinensis* (Emery) males collected from nests per month. Columns show mean number and the SD is represented by whiskers. Each monthly statistic was derived from four censused nests. One-way ANOVA of the mean number of males by month ($F_{9,30} = 13.88$; $P < .0001$). Mean values connected by same letter are not significantly different.

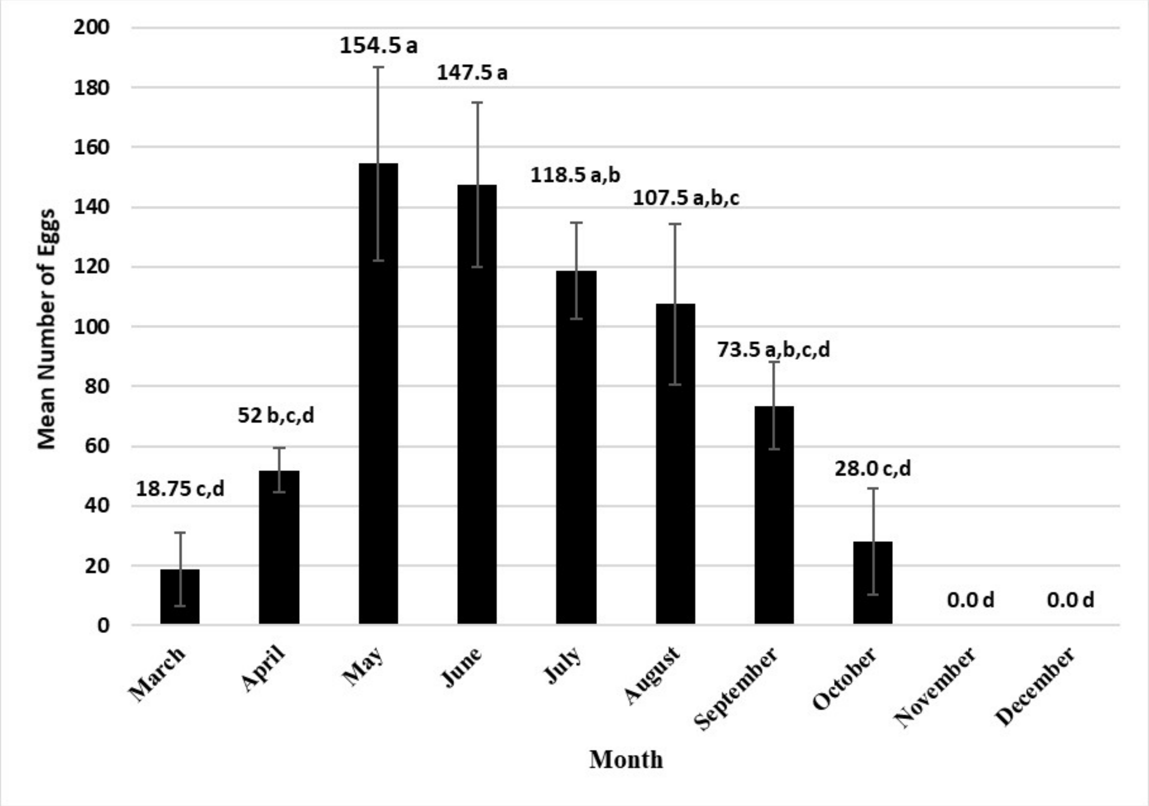

Figure 3.6. Mean number of *Brachyponera chinensis* (Emery) eggs collected from nests per month. Columns show mean number and the SD is represented by whiskers. Each monthly statistic was derived from four censused nests. One-way ANOVA of the mean number of eggs by month ($F_{9,30} = 9.93$; $P < .0001$). Mean values not by same letter are not significantly different.

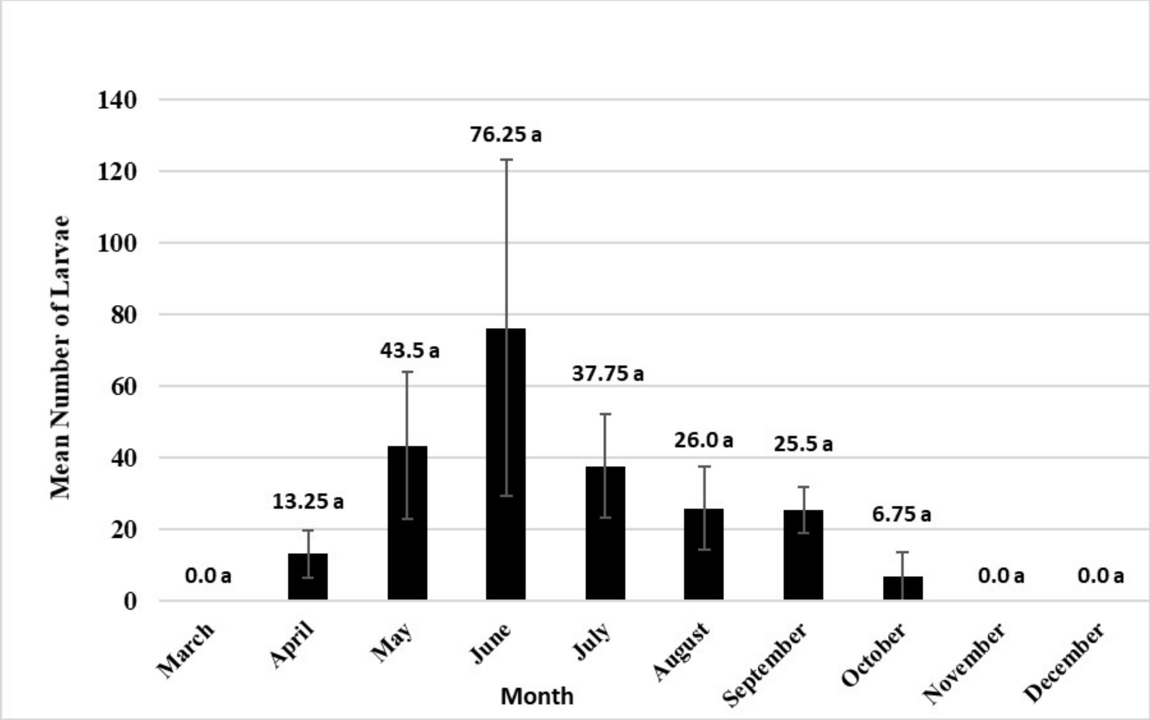

Figure 3.7. Mean number of *Brachyponera chinensis* (Emery) larvae collected from nests per month. Columns show mean number and the SD is represented by whiskers. Each monthly statistic was derived from four censused nests. One-way ANOVA of the mean number of larvae by month ($F_{9,30} = 1.95$; $P < .08$). Mean values connected by same letter are not significantly different.

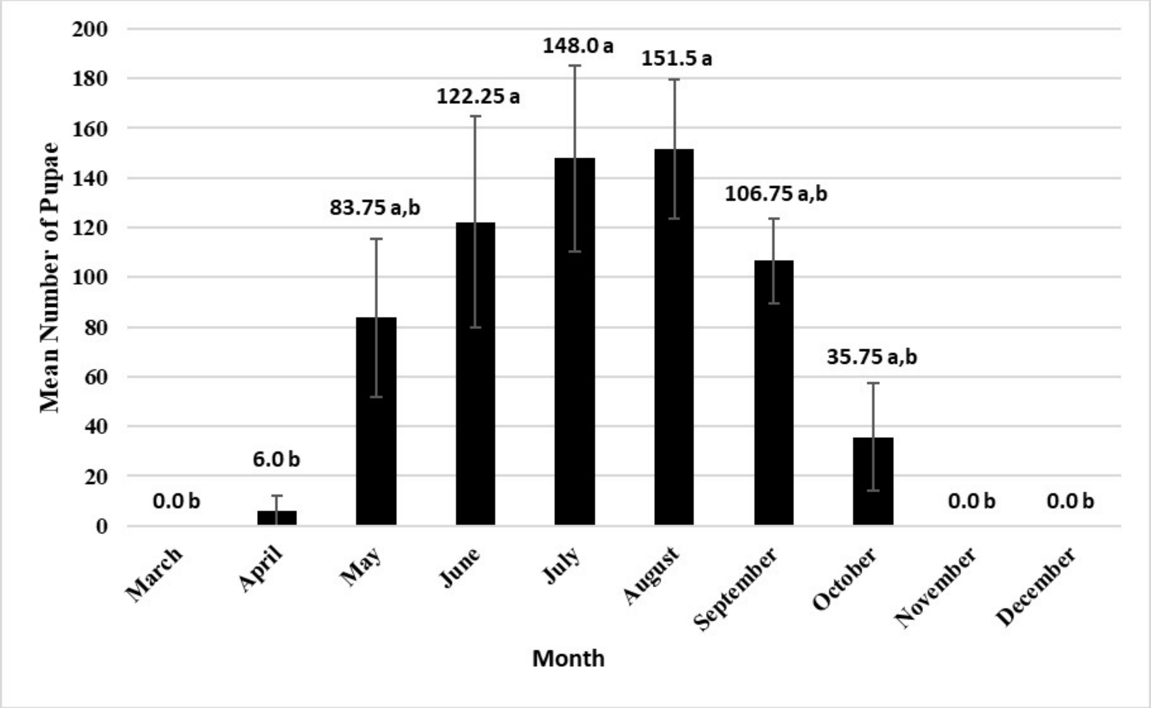

Figure 3.8. Mean number of *Brachyponera chinensis* (Emery) pupae collected from nests per month. Columns show mean number and the SD is represented by whiskers. Each monthly statistic was derived from four censused nests. One-way ANOVA of the mean number of pupae by month ($F_{9,30} = 7.06$; $P < .0001$). Mean values connected by same letter are not significantly different.

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CHAPTER FOUR

INTRASPECIFIC AGGRESSION AND POLYDOMY IN THE ASIAN NEEDLE ANT, *BRACHYPONERA CHINENSIS* (EMERY)

Introduction

An ant colony is a cooperative group of closely related workers, reproductive ants, and brood (Robinson 2014) residing in a harborage or cooperatively functioning as a moving unit (Hölldobler and Wilson 1990). A singular colony may inhabit one nest, a phenomenon identified as monodomy, or a colony may be polydomous and inhabit two or more spatially separated nests (Debout et al. 2007). Although spatially separated, polydomous ant colonies participate in the exchange of workers, queens, brood, and food between nests (Buczkowski and Bennett 2008). Independent ant colonies freely participating in the exchange of materials are unicolonial. Unicolonial colonies have lower genetic diversity between nests than monodomous colonies and nest members perform as one large colony unit (Debout et al. 2007, Robinson 2014).

The causes and consequences of polydomy have not been fully elucidated and the characteristics associated with polydomy vary among species (Debout et al. 2007, Buczkowski and Bennett 2008). However, the social organization of a colony can be determined experimentally through comparison of inter-colony cuticular hydrocarbon chemical profiles (Newey 2011), resource exchange between nests (Dahbi et al. 2008), inter-nest aggression assays (Roulston et al. 2003), genetic relatedness (Steinmeyer et al. 2012), and spatial nesting patterning (Dillier and Wehner 2004).

Overall, more than 150 ant species have a polydomous nesting structure (Debout et al. 2007). Additionally, polydomy is a social trait expressed by invasive or tramp ant species (Holway et al. 2002). For example, five ant species; *Anoplolepis gracilipes* (Smith), *Linepithema humile* (Mayr), *Pheidole megacephala* (Fabricius), *Solenopsis invicta* (Buren), and *Wasmannia auropunctata* (Roger) that are on the world's worst invasive species list exhibit polydomy (Robinson 2014, Lowe et al. 2000). Holway et al. (2002) attributes the invasive success of the five species to polydomy and unicoloniality.

The Asian needle ant, *Brachyponera (=Pachycondyla) chinensis* (Emery) is a recent invader of the southeastern U.S. It is associated with the suppression of native ant populations specifically disturbing the seed dispersing capabilities of *Aphaenogaster rudis* (Emery) (Rodriguez-Cabal 2012, Guénard and Dunn 2010). Bednar and Silverman (2011) reported that *B. chinensis* workers can exclude native ants from feeding on *Reticulitermes virginicus* (Kollar) termites. In each study, the authors state that *B. chinensis* are polydomous. However, these postulations have not been confirmed through experimentation in the United States. Murata et al. (2017) determined that Japanese populations of *B. chinensis* workers did not display aggression toward nestmates or non-nestmates in aggression assays and aggression did not increase with distance between nests.

Because *B. chinensis* is increasing its U.S. range, determining its colony structure is important to understanding its potential for invasive success and for designing potential management programs. The current study focused on using inter-nest/intra-nest agonistic behavior and the spatial distance between colonies as a measure of determining polydomy in *B. chinensis* colonies. In addition to using aggression and spatial information as measures of polydomy, cuticular hydrocarbon profiles of different *B.*

chinensis colonies were compared. I hypothesized that *Brachyponera chinensis* colonies are polydomous but nestmate aggression increases with origin distance.

Materials and Methods

Study Areas

Field studies were conducted in four Asian needle ant infested locations in upstate South Carolina (Agricultural Service Station 34° 39’ 29.219” N, 82°49’56.241” W; Sturkey 34° 38’ 42.78” N, 82°48’46.799” W; Lawrence Bridge 34°44’1.608” N, 82°52’25.895” W; Pendleton 34° 40’21.247” N, 82° 48’ 38.75” W). In July 2014, one 10 x 10 m plot was established at each site (Figures 4.1, 4.2, 4.3, and 4.4).

Visual inspections were performed at each location and all identified *B. chinensis* nests were flagged. Ant aggregations designated as nests, contained worker ants accompanied by at least one queen and brood. *Brachyponera chinensis* nests were shallow and composed of small galleries usually within or under fallen timber. GPS coordinates for each nest were recorded but, nests separated by < 2.0 m could not be distinguished using geographic coordinates. As a result, plots were broken up into 1 x 1m squares and nests were identified with X, Y coordinates.

Field Collection of Nests

Two nests separated by a maximum distance within each plot at each location were collected and used in field and laboratory nestmate recognition assays. Agricultural Service (AgSer) nests were separated by 7.62m, Lawrence Bridge (LB) by 8.6m, Pendleton (PD) by 8.2m, and Sturkey (ST) by 8.8m. On average, the nests were separated by 8.3m. Nests separated by the maximum distance were selected to account for potential unicoloniality. After identification, two nests from each site were physically removed with a shovel and transferred to separate plastic tubs. A total of eight nests (AgSer-1, AgSer-2, LB-1, LB-2, PD-1, PD-2, ST-1, ST-2) were collected.

Field Intra-nest Nestmate Recognition Assays

To determine if worker ants originating from the same nest displayed aggression, I conducted intra-nest recognition assays in the field. Before conducting the assays, the bottoms of 44.3 ml plastic, medicine cups were removed with scissors. The “open” cups were inverted and placed into a 60 x 15mm polystyrene petri dish (Becton, Dickinson and Company, 1 Becton Drive; Franklin Lakes, NJ). Six worker ants were removed from each nest with featherweight forceps. Contamination was minimized during the study. A different pair of forceps was used for each nest and forceps were washed with 70% isopropyl alcohol between each rep. Three ants were transferred to the inside of the inverted cup and three ants were placed on the exterior of the cup. Ants acclimated to the medicine cup/petri dish for one minute. New petri dishes were used for each rep to minimize contamination. After acclimation, the cup was removed allowing interaction between both groups. Nestmate recognition interactions were scored according to an aggression scale created by Suarez et al. (1999). Scoring is outlined as: 0 = ignore, 1 = touch, 2 = avoid, 3 = aggression, and 4 = fighting (Suarez et al. 1999). Trials lasted for a maximum of five minutes and were replicated five times for each nest from each location.

Field Inter-nest Nestmate Recognition Assays

To determine if worker ants originating from different nests within the same plot displayed agonistic behavior, I conducted inter-nest recognition assays in the field. The inter-nest aggression assays were conducted using the previously collected nests. For each location, three ants were removed from each collected nest using featherweight forceps. Contamination was minimized as described before. A group of three ants from one nest within a location was randomly placed into the inside of a bottomless medicine cup or placed directly onto a petri dish as previously described. After acclimation, the cup was removed allowing interaction between the groups. Nestmate recognition interactions were scored in the same manner as the intra-nest assays (Suarez et al. 1999). Trials lasted for a maximum of five minutes and were replicated five times at each location. Scores were not evaluated via statistical analysis.

Laboratory Nestmate Recognition Aggression Assays – Trial 1

To determine if ants originating from separate locations displayed aggression, 3x3 nestmate recognition assays were conducted. *Brachyponera chinensis* nests used in the field assays were brought to the Cherry Farm Insectary (Clemson, SC; 34° 39.108’N, 82°50.328’W), and maintained in an environmentally controlled room at 21°- 24°C; RH: 40% with a 12:12: L/D cycle. Collected nests (n=8) were allowed to acclimate to lab conditions for 24 hours. All assays were conducted after the acclimation period. During assays, one nest was randomly designated as the “primary nest” and the seven additional nests were identified as “secondary” nests. The “primary” nest was used in each round of assays while the “secondary” nests were used in one round. For example, if AgSer-1 was selected as the primary nest, AgSer-2, LB-1, LB-2, PD-1, PD-2, ST-1, and ST-1 were designated as secondary nests. All assay combinations (within nests, within nests from the same plot, or between nests from separate locations) were explored during experimentation. An experimental trial consisted of selecting 21 workers from the primary nest and 21 workers from the secondary nests. Laboratory assay procedures were the same as field assays. Three worker ants from each nest were randomly placed inside an inverted bottomless 44.3 mL medicine cup or placed directly onto the 60 x 15mm polystyrene petri dish. Contamination was minimized as described before. Ants were allowed to acclimate for one minute. After acclimation, the medicine cup was removed allowing interaction between both groups. Assays were replicated seven times. Nestmate recognition interactions were scored according to an aggression scale created by Suarez et al. (1999). A total of 1,336 worker ants were used in the assay.

Laboratory Nestmate Recognition Assays – Trial 2

To determine if *B. chinensis* originating from alternate locations displayed aggression, 3 ant x 3 ant nestmate recognition studies were conducted. Ant colonies were collected from locations included in Trial 1 (Agricultural Service Station 34° 39’ 29.219” N, 82°49’56.241” W; Sturkey 34° 38’ 42.78” N, 82°48’46.799” W; Lawrence Bridge 34°44’1.608” N, 82°52’25.895” W; Pendleton 34° 40’21.247” N, 82° 48’ 38.75” W). Ant colonies also were collected from three additional sites in South Carolina; Westminster (W) 34° 36’45.644” N, 83° 4’ 57.302” W, Pickens (P) 34° 52’ 16.783” N, 82° 42’ 6.245” W, and Anderson (A) 34° 33’ 19.516” N, 82° 40’ 42.176” W. One colony was collected from each site on May 6, 2015. Colonies were physically removed with a shovel and transferred to separate plastic tubs. *Brachyponera chinensis* nests were brought to the Cherry Farm Insectary (Clemson, SC) for experimentation. A total of seven nests were collected. Assays were conducted on the same day as nest collection. Twenty-seven worker ants were manually selected from each nest and placed into separate plastic medicine cups. Contamination was minimized as described before. Ants were held in the cups until experimentation. Assay procedures performed in Trial 1 were replicated in Trial 2. All within nest and between nests from separate locations pairwise combinations were explored. Assays were scored according to the Suarez et al (1999) aggression scale. Assays were replicated three times for each nest pairing. A total of 189 worker ants were used in the assay.

Statistical Analyses

To evaluate the distribution of *B. chinensis* nests at each location a Monte Carlo test for complete spatial randomness (Besag and Diggle 1977) using the quadrat method was applied (SAS 9.1; SAS Institute Inc., Cary, NC). A one-way analyses of variance (ANOVA) on ranks test was conducted to determine if nestmate recognition assay scores differed within nests, within nests from the same plot, or between nests from separate locations (SAS 9.1; SAS Institute Inc., Cary, NC) in Trial 1. A one-way analyses of variance (ANOVA) on ranks test was conducted to determine if nestmate recognition assay scores differed within nests or between nests from separate locations (SAS 9.1; SAS Institute Inc., Cary, NC) in Trial 2.

Cuticular Hydrocarbon (CHC) Collection

To determine if *B. chinensis* ants originating from separate locations displayed similar cuticular hydrocarbon profiles, worker ants were collected from four different nests in the Clemson Experimental Forest. Locations include: Agricultural Service (AgSer), Lawrence Bridge (LB), Pendleton (PD), and Sturkey (ST). Twenty workers from each nest were manually removed, transferred to four different glass vials (1 dram), and stored in a freezer at -20°C until use. Cuticular components were obtained from each ant sample (n=4) by rinsing the ants in 1mL of n-hexane for 10 minutes.

GC-MS Analysis

Seven hundred microliters of each sample (n = 4) and a hexane control were dried by RapidVap for five minutes and reconstituted in 100 µL of hexane. Cuticular extracts were examined by GC-MS on an Agilent 7890A GC with a DB=5 bonded phase column connected to an Agilent 5975C mass selector. Helium was used as carrier gas and samples were injected in splitless mode at 2 µL per minute. Analyses were done using temperature programming, at an initial oven temperature of 80°C, a final temperature of 300°C, a program rate of 8°C /min, and a 2-minute final hold at 300°C. Total run time was 31 minutes. Hydrocarbons were identified by their mass spectra and their retention indices.

Results

Spatial Distribution

A total of forty-nine *B. chinensis* nests were identified in the four experimental plots (Figures 4.1 – 4.4). The highest number of nests was found at the AgSer location

(14) and the fewest number of nests were located at LB (9) (Table 4.1). Regardless of location, all flagged nests were located under or within fallen timber. Results from the complete spatial randomness test for randomization revealed that nests located at LB (P = 0.99), PD (P = 0.376), and ST (P = 0.888) were not clumped in distribution (Table 4.1). Nests studied at the AgSer location were found to have a clumped distribution (P = 0.034).

Field Inter-nest Nestmate Recognition Assays

Inter-nest nestmate recognition assays were conducted in the field to determine if ants originating from different nests within the same plot displayed aggression. Because aggressive behavior was not observed, the results from the assay were not analyzed (Table 4.2). Average aggression scores ranged from 0.00 to 1.00, indicating that the ants were docile towards one another.

Laboratory Nestmate Recognition Assays – Trial 1

Brachyponera chinensis workers originating from the same nest or plot did not display aggression towards one another in field assays. To determine if these field results were consistent, I conducted nestmate recognition assays in a laboratory setting. The lab tests consisted of intra-nest and inter-nest assays with all pairwise combinations. Regardless of treatment level, mean aggression scores indicate that *B. chinensis* workers did not behave aggressively regardless of nest location; Within Nest (0.98 ± 0.13 SD), Same Location (1.00), and Between Location (1.05 ± 0.34 SD). As expected, assays conducted with ants from the same colony or from the same plot produced results similar to those observed in field assays. Further, inter-colony pairings produced comparable results. Kruskall-Wallis results (Table 4.2) indicate that aggression levels did not differ between assays conducted with ants from the same nest, same plot, or different plots ($P = 0.1571$).

Laboratory Nestmate Recognition Assays – Trial 2

Mean aggression scores from nestmate recognition assays with ants from the same nest (0.41 ± 0.51 SD) and ants originating from separate locations (1.45 ± 0.82 SD) did differ significantly from one another ($F_{1,82} = 18.02$; $p < 0.0001$).

Cuticular Hydrocarbon Analyses

A total of 42 different compounds were obtained between the four samples and 18 different cuticular hydrocarbons (CHC) peaks were identified by GC/MS (GC/MS data can be found in Appendix A). The most commonly observed cuticular hydrocarbons were tetradecane, hexadecane, heptadecene, 8-heptadecene, and tricosane (Table 4.5). However, the number and type of CHCs varied between sample locations.

Discussion

Results from the current study indicate that three of the four *B. chinensis* nests were uniformly distributed and workers originating from different colonies do not display aggression towards one another. Nestmate recognition assay results suggest that *B. chinensis* colonies are polydomous.

Nests located at the LB, PD, and ST plots had a uniform distribution while nests within the AgSer plot had a clumped distribution. Although ANOVA results indicate that nests located at LB, PD, and ST are not clumped, the results should be scrutinized. A general observation of the plots in (Fig. 4.1 - 4.4) shows that nests are grouped together in aggregations of two to three nests but the total number of nests within each plot may have influenced the statistical outcome. The number of nests at the LB (9), PD (12), ST (13) locations was lower than the number observed at the AgSer (14) location. Each field site was 10 m^2 but larger sites may be required to identify *B. chinensis* nesting patterns and subsequent colony boundaries. Scale has a direct impact on the outcome of spatial studies involving ants (Ellis et al. 2017). As the study area and number of analyzed nests increase in number, there may be an increase in the amount of ecological information obtained on an ant species.

The distance between the four plots used in the first laboratory nestmate recognition assay ranged from 4.3 km to 18.5 km. Because I did not observe significant aggression interactions in the assay, I located three more field sites separated by a distance greater than 18.5 km. Theoretically, intra-specific aggression increases as the distance between two nests increases (Lehmann and Rousset 2010). The three new sites A, P, and W were separated from the AgSer, LB, PD, and ST sites by at least 26.9 km. During the additional trials, aggression was not observed in assays conducted with ants from the same nests and nests from separate locations.

Results of the nestmate recognition assays (Trial 1) demonstrate that *B. chinensis* workers originating from different colonies within the same location or separate locations did not display aggression towards non-nestmates. However, ANOVA results produced in Trial 2 indicate that same nest (0.45) and different location (1.45) scores differed significantly. The scores are different but the average difference location score is below 3 (aggression). Therefore, I speculate that agonistic behavior did not occur. The lack of aggression between colonies could be the result of a single introduction of *B. chinensis* in the U.S. The invasive Argentine ant, *Linepithema humile* (Mayr) is unicolonial and forms supercolonies spanning hundreds of meters in California (Tsutsui and Suarez 2003), Heller et al. 2006). Lack of aggression between colonies is attributed to a genetic bottleneck influenced by the within nest mating and budding dispersal behaviors of *L. humile*. Asian needle ant nests are not separated by great distances but may participate in nuptial flights. Zungoli and Benson (2008) collected *B. chinensis* reproductives in light traps and observed a male to female ratio of 19:1 indicating the possibility of nuptial flights. However, *B. chinensis* workers use adult transport during nest emigrations (unpublished data) which may be an artifact of budding; another characteristic of polydomous ant colonies (Debout et al. 2007).

The data suggest that colony boundaries are not well defined and colonies are polydomous but the nestmate recognition studies may be flawed (Roulston et al. 2003, Buczkowski 2012). Sometimes, nestmate recognition assays fail to produce measurable aggression potentially due to lack of ecological or social cues. In the current study, three vs. three assays were used but I experimented with one vs. one, three vs. three, five vs. five, and ten vs. ten ants in preliminary trials. Roulston et al. (2003) determined that aggression increases with the number of ants used but I did not observe any increase in behavior during preliminary lab trials. Aggression responses in ants are also influenced by a colony’s seasonal life cycle (Katzerke et al. 2006). *Formica exsecta* (Nylander) workers did display aggression towards non-nestmates in the spring but not in the summer or fall. *Brachyponera chinensis* aggression studies were conducted in the spring when resources were available, but aggression may increase as resources become less available in the fall.

A preliminary cuticular hydrocarbon analysis was conducted with worker ants collected during the first laboratory nestmate recognition trial. Cuticular profiles were analyzed via GC-MS with tetradecane (AgSer, PD), hexadecane (AgSer, LB, ST), heptadecane (LB, ST), 8-heptadecene (LB, ST), eicosane (AgSer, PD), and tricosane (AgSer, ST) appearing in more than one sample. However, changes to the analytical methods should be performed before the results can be verified in additional studies. Hexane was used as the solvent but the compound has a propensity to maintain impurities; pentane or iso-octane should be used in further studies. Also, additional hydrocarbon standards should be used to identify unknown peaks and to substantiate GC/MS library findings.

Spatial analyses, nestmate recognition assays, and cuticular analyses were the three methods used to explore polydomy in *B. chinensis*. I found that *B. chinensis* nests are uniformly distributed suggesting monodomy and that colonies separated by a distance less than 34 km are not aggressive suggesting polydomy. The two outcomes are competitive but I hypothesize that *B. chinensis* is a polydomous ant species based on results in this study in conjunction with nesting behaviors like its sister species *Brachyponera nakasujii* (Yashiro, Matsuura, Guénard, Terayama & Dunn) (Gotoh and Ito 2008). To further understand the social organization of *B. chinensis* colonies, mark and recapture studies (Katzerke et al. 2006), genetic analyses (Tschinkel 2006), and observations of resource sharing between nests (Ellis et al. 2017) should also be conducted but, each method used to identify colony characteristics has its advantages and disadvantages (Ellis et al. 2017, Debout et al. 2007). Polydomy is a complex behavior and may be a context dependent behavior (Cao 2013) and to determine the proper study methods questions regarding *B. chinensis*' seasonal cycle, colony demographics, reproductive behaviors, and ecology must be answered.

Determining colony boundaries and nesting behaviors are integral to ecological and pest management programs. The impacts of polydomy on the current and future invasive success of *B. chinensis* in the United States are unknown but could explain how the ants outcompete *A. rudis* for resources (Rodriguez-Cabal et al. 2012, Bednar and Silverman 2011), displace *L. humile* (Spicer-Rice and Silverman 2013a, 2013b), and continue to increase their populations across the United States (Guénard and Dunn 2010).

Table 4.1. Monte Carlo test for complete spatial randomness analysis for *Brachyponera chinensis* (Emery) nests. The values in the table represent the location, total number of nests, and probability that nests from a specific location are randomly distributed. Values > 0.05 indicate random distribution. Values < 0.05 indicate clumped distribution.

Location	**Total Number of Nests**	**P – value**
AS	14	0.034
LB	9	0.99
PD	12	0.376
ST	13	0.888

Table 4.2. Field inter-nest nestmate recognition assays. Three by three nestmate recognition raw scores and mean score for assays conducted with *Brachyponera chinensis* (Emery) worker ants originating from the same location. Nestmate recognition scores ranged from 0 to 4; 0 = ignore, 1 = touch, 2 = avoid, 3 = aggression, 4 = fighting (Suarez et al. 1999). Ant behavior was measured up to 5 minutes after the assay began. Average scores < 3 indicate that aggression was not observed between ants originating from the same location.

Location	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**	**Avg.**
AS	0	0	0	0	0	0.00
LB	1	1	1	1	1	1.00
PD	1	1	1	1	1	1.00
ST	1	1	0	1	1	0.80

Table 4.3. Cuticular hydrocarbons identified in *Brachyponera chinensis* (Emery) by location through GC/MS. Location abbreviations are as follows; Agricultural Service = AgSer, Lawrence Bridge (LB), Pendleton (PD), and Sturkey (ST).

Cuticular Hydrocarbons	**Locations**			
	AgSer	**LB**	**PD**	**ST**
Cyclohexane	X			
4-Octene	X			
Nonane			X	
2,5 Nonadiene	X			
Tetradecane	X		X	
Hexadecane	X	X		X
Heptadecane	X			
8-heptadecane	X			
Heptadecene		X		X
8-Heptadecene		X		X
Heneicoasane	X			
Docosane	X			
Eicosane	X		X	
Tricosane	X			X
E-7 Octadecene				X
Z-5 Nonadecane		X		
9-Nonadecene	X			
3-Undecene			X	

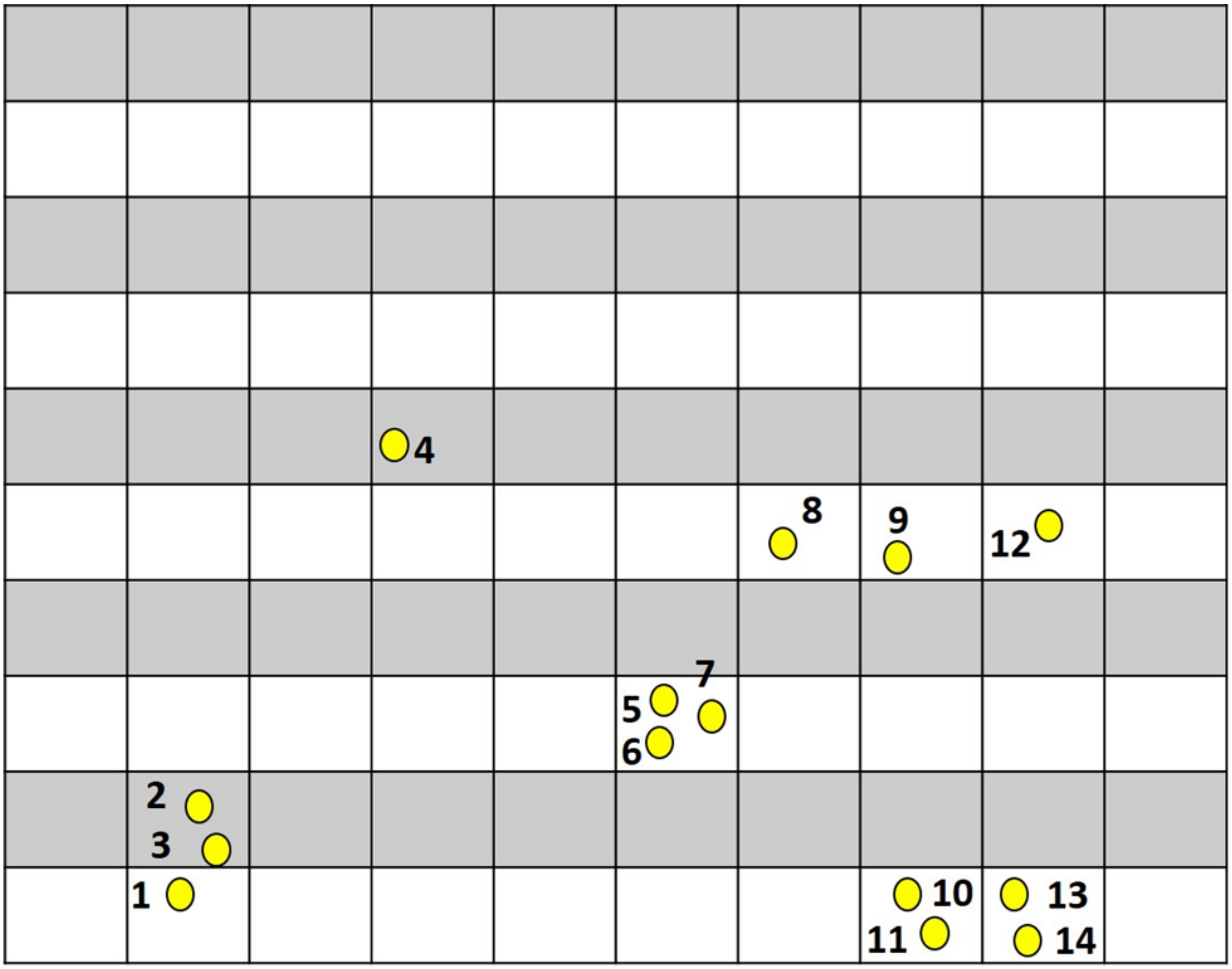

Figure 4.1 Spatial distribution of *Brachyponera chinensis* (Emery) nests located in a 10m x 10m Agricultural Service Station (Pickens County, SC) plot. Yellow circles represent *B. chinensis* nests (n = 14). Monte Carlo test for complete spatial randomness analysis of nests indicated a clumped distribution (P = 0.034).

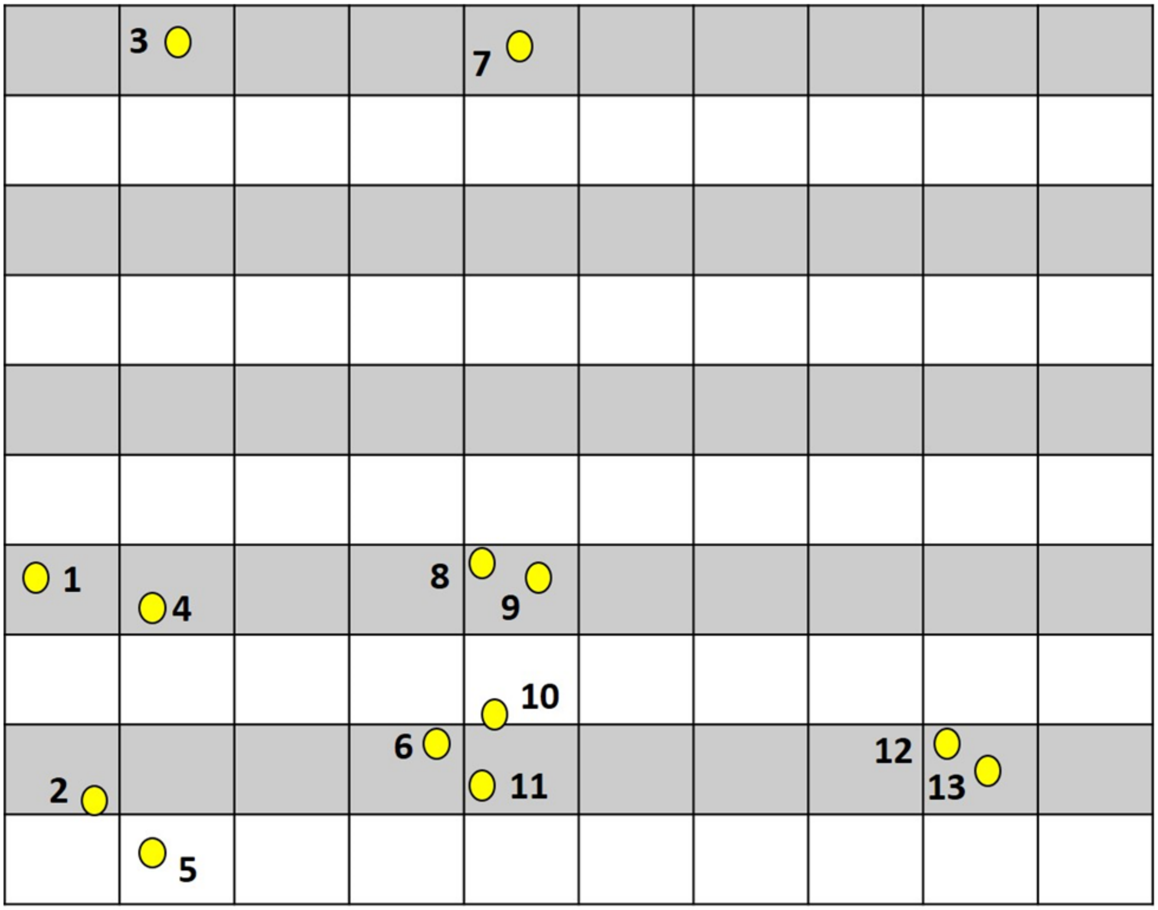

Figure 4.2. Spatial distribution of *Brachyponera chinensis* (Emery) nests located in a 10m x 10m Sturkey (Pickens County, SC) plot. Yellow circles represent *B. chinensis* nests (n = 13). Monte Carlo test for complete spatial randomness analysis of nests indicated a random distribution (P = 0.888).

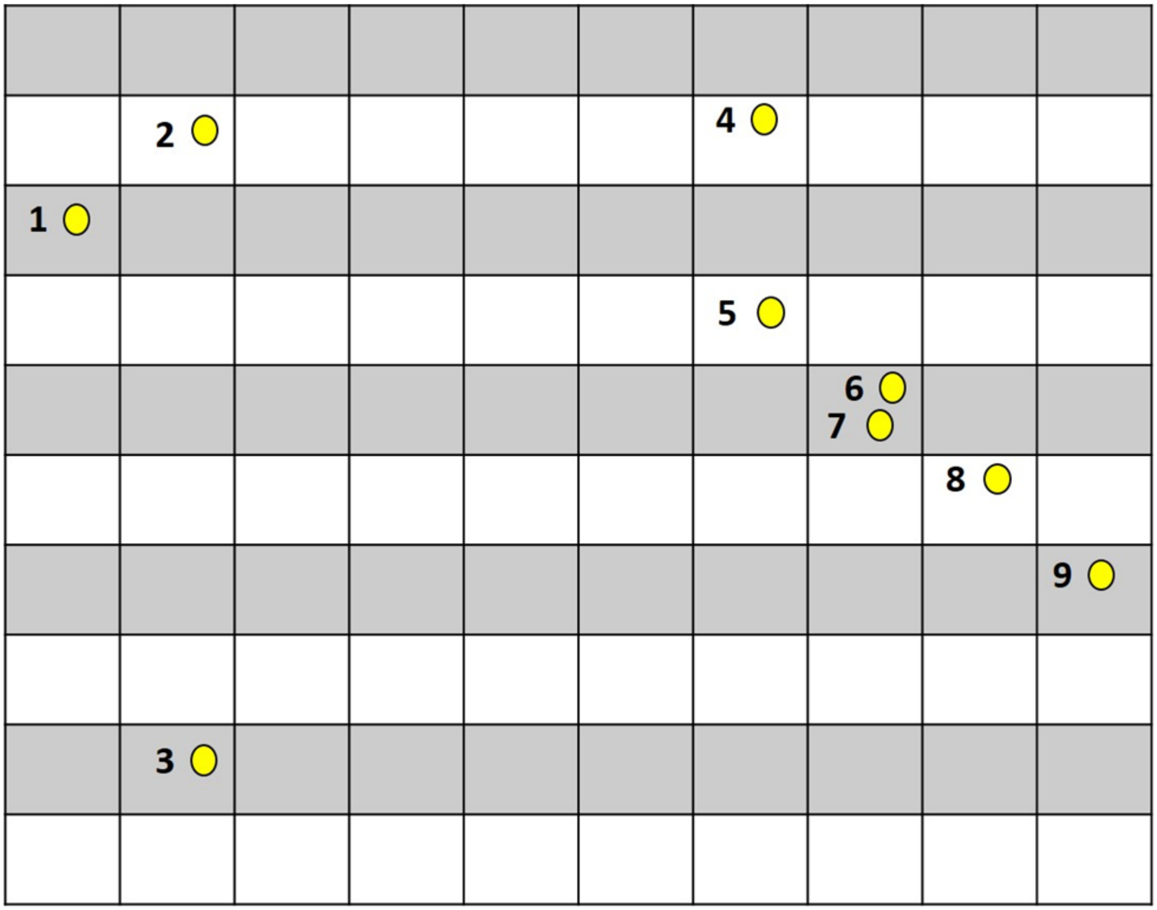

Figure 4.3. Spatial distribution of *Brachyponera chinensis* (Emery) nests located at 10m x 10m Lawrence Bridge (Oconee County, SC) plot. Yellow circles represent *B. chinensis* nests (n = 9). Monte Carlo test for complete spatial randomness analysis of nests indicated a random distribution (P = 0.99).

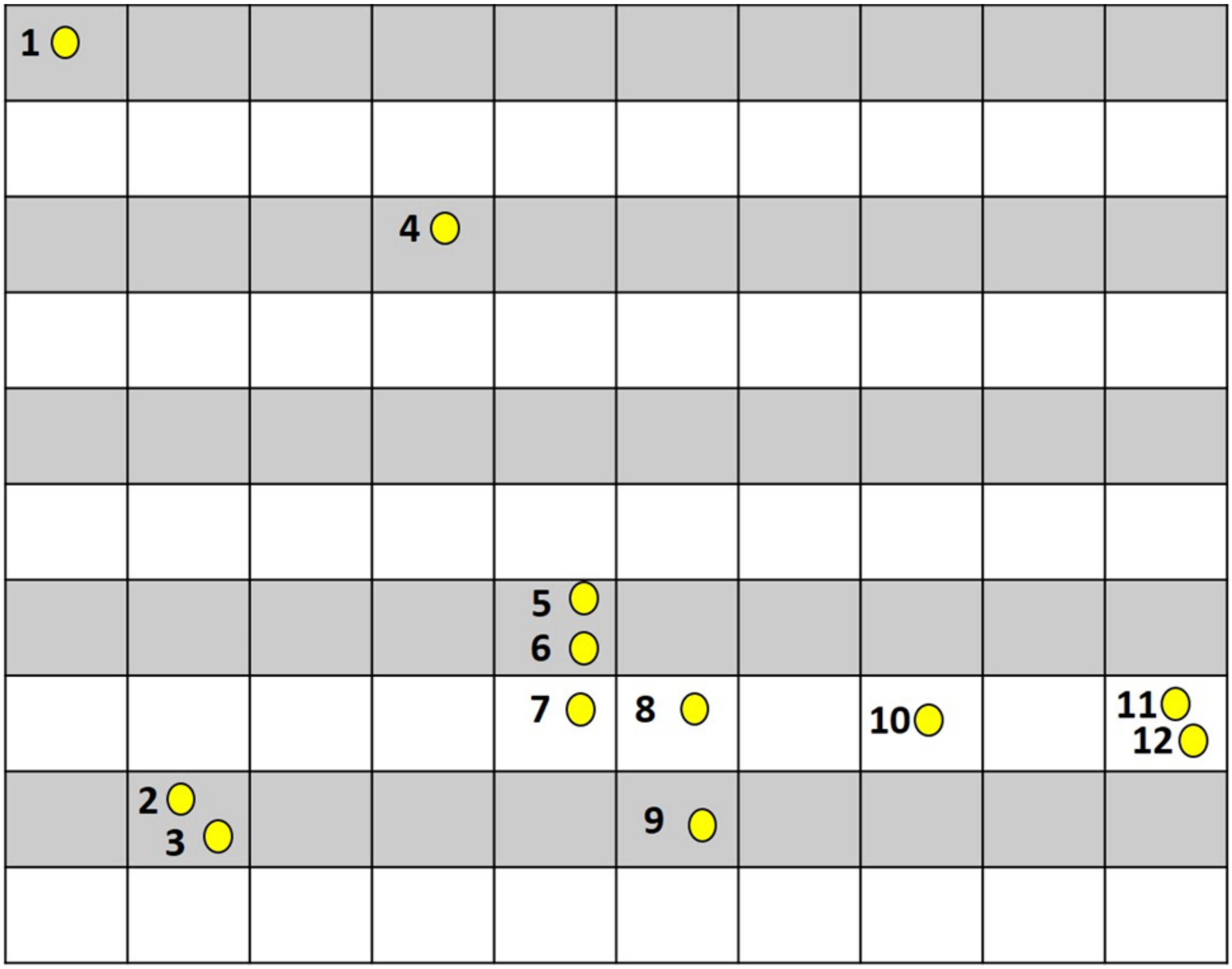

Figure 4.4. Spatial distribution of *Brachyponera chinensis* (Emery) nests located at 10m x 10m Pendleton (Anderson County, SC) plot. Yellow circles represent *B. chinensis* nests (n = 12). Monte Carlo test for complete spatial randomness analysis of nests indicated a random distribution (P = 0.376).

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CHAPTER FIVE

CARRY ON: NEST EMIGRATION BEHAVIORS OF *BRACHYPONERA CHINENSIS*

Introduction

The process of nest emigration is essential to the propagation and establishment of ant colonies. Many ant colonies are labile with members changing nest location in response to drought, flooding, predation, competition, budding, and nest disturbance (Fowler 1981, Tay and Lee 2015). Although emigration is a familiar process, recruitment methods during emigration differ between and within ant species (Hölldobler and Wilson 1990; Planque et al. 2010). Recruitment methods include pheromone trail following, tandem running, and physical adult transport (Maschwitz et al. 1986, Beckers et al. 1989). A single species may employ a variety of recruitment methods during an emigration event. For example, *Bothroponera tesseronoda* (Emery) uses tandem running and trail following to complete emigration (Jessen and Maschwitz 1986), whereas tandem running is preceded by adult transport in *Neoponera obscuricornis* (Emery) (Traniello and Hölldobler 1984) and *Leptothorax albipennis* (Curtis) (Pratt et al. 2002).

Adult transport by ants was initially characterized solely as a nest emigration recruitment method (Haskins and Haskins 1950). However, Guénard and Silverman (2011) re-described the physical transport methods of the invasive Asian needle ant, *Brachyponera (=Pachycondyla) chinensis* (Emery), during foraging expeditions (Takimoto 1988). The foraging recruitment method, tandem carrying, is a newly described context-dependent recruitment behavior, occurring three to ten times more often when a food item is too large to be moved by a single worker. In addition to being a new foraging strategy, tandem carrying was further distinguished from other forms of adult transport because the carrying posture differed from previously described transport postures (Guénard and Silverman 2011). In other ant species, worker ants are grasped and/or transported by the mandibles, the neck, or on their side (Möglich and Hölldobler 1974), whereas ants being transported during tandem carrying are grasped between their first and second pairs of legs on the ventral mesothorax. Guénard and Silverman (2011) described this region as the mesometasternum but this designation is incorrect, as the sternites on ants are internal invaginations and not external features.

Research on *B. chinensis* has increased due to its potential as a public health threat (Cho et al. 2002; Nelder et al. 2006; Lee et al. 2009), negative impact on native and introduced ant species populations (Guénard and Dunn 2010; Bednar et al. 2013; Spicer-Rice and Silverman 2013), spread in the southeastern U.S., and potential spread into areas outside its current range (Bertelsmeier et al. 2013). *Brachyponera chinensis* typically nests in downed timber or under objects in contact with the soil, usually in areas where termites are abundant, contributing to their success (Bednar and Silverman 2011). Identifying and determining the potential for geographic spread and nesting behavior are central to understanding the invasive ecology of *B. chinensis*.

During field and laboratory observations, *B. chinensis* workers engaged in adult transport. The observed transport took place in the absence of immovable food items. The ants were physically transporting nestmates to various locations in space but I did not identify any subsequent tasks. Because adult transport occurred outside of foraging recruitment and is associated with nest emigration, I hypothesized that *B. chinensis* employs adult transport to complete nest emigration using the posture assumed during tandem carrying reported by Guénard and Silverman (2011). In the laboratory study reported here, field-collected colony fragments were subjected to identical nest emigration trials elicited by physical disturbance to determine the recruitment methods of *B. chinensis* during emigration. In a separate study ants were marked with paint and subsequently subjected to nest emigration trials to determine the extent of task allocation during nest emigration in *B. chinensis*.

Materials and Methods

Twenty queenright *B. chinensis* nests were collected from separate locations within the Clemson Experimental Forest, a mixed hardwood-pine forest in Pickens County, SC (34^{0}43’55.018” N, 82^{0}51’6.654” W) between August-October 2012 and April-June 2013. Each nest used in the study contained at least one queen, brood, and 200 worker ants. Laboratory trails were conducted between September-November 2012 and April-June 2013. Collected nests were subjected to experimental trials within one week of collection. Ants nests were housed in 20-gallon uncovered plastic tubs (40.89 cm x 39.06 cm) and provided with a glass test tube (250 mm x 25 mm) wrapped in red transparent cellophane with a piece of moistened cellulose sponge inside as a water source (Figure 4.1). Test tube harborages were used to mimic *B. chinensis* galleries in fallen timber. Colonies were fed *Reticulitermes* sp. workers, and *Tenebrio molitor* (Linnaeus) larvae *ad libitum,* and maintained at 21°C at a 12:12 L/D cycle with 70-80% RH.

All studies were conducted in arenas consisting of a plastic container (60 cm x 42 cm x 16 cm) divided into equal halves by a 37-cm acrylic glass insert (Figure 4.1). Before testing began, an uninhabited glass test-tube harborage was placed in one half of the arena, and the other half remained empty. Because the acrylic glass insert was not completely flush with the bottom of the arena, 37-cm strips of Play-Doh® (Hasbro Corporation, Pawtucket, RI) were used to seal openings. Worker ants inspected Play-Doh® strips but did not attempt to feed on or remove the inserts.

Colony emigration studies were conducted concurrently in separate treatment (n = 10) and control (n = 10) arenas. Overall, ten colonies were used per treatment. On the day of experimentation, a colony subset consisting of 200 workers, one queen, and 20 brood items (eggs, larvae, or pupae) were manually removed from two separate colonies and transferred to two different glass test tubes (250 mm x 25 mm) prepared as previously described (Figure 4.1). After removal, the test subjects were allowed to acclimate to the new environment for two hours. Treatments consisted of a physical nest disturbance, defined as manually removing all nest members and associated materials from a test tube harborage. Specifically, during disturbance the sponge insert was removed and the test tube was lightly shaken to dislodge any ants remaining in the test tube. Any ants latching onto the sponge insert were removed using a paintbrush. Test tube harborages were not physically disturbed in control treatments. In each round of experimental trials two colonies were selected. Selected colonies were randomly designated as “treatment” or “control” and were used once during the study. In both treatments, the Play-Doh®

barrier and plexiglass inserts were removed from the arenas after a one-hour acclimation period following the disturbance, permitting unrestricted ant movement.

Data collection began after the first successful carrying event was observed. Successful transport consisted of the carried individual being released inside the new harborage. All carrying events were visually observed, time of carry was recorded, as well as the total number of successful carries. Data collection ceased after 90 minutes. Preliminary data indicated, after this time, the interval between carrying events was greater than five minutes. A one-way Wilcoxon test was conducted to determine differences between total number of successful transports occurring in treatment and control arenas during nest emigration trials (JMP® Pro 10, SAS Institute, Inc. 2012. Cary, NC). An alpha error of < 0.05 indicated statistical significance. The difference in number of ants being carried in 15-minute intervals was compared using a Repeated Measures MANOVA in SAS 9.3 (SAS Institute, Inc. 2012. Cary, NC). An P-value < 0.05 indicated statistical significance.

Task Allocation

To determine if *B. chinensis* workers performed repeated adult transport episodes during an emigration, worker ants were marked with paint and subjected to physical disturbance to elicit nest emigration. Ants used in the current study were obtained from four of the nests used in the previous study. As part of the study, two hundred worker ants were removed from a colony along with one queen and brood. Queen ants and brood items were not marked with paint during trials. Before marking, five ants were selected, placed into a 1.5 mL plastic medicine cup, and transferred to a freezer (-18 ^{0}C) for 2 minutes. After removal from the freezer, the ants were transferred from the medicine cup onto a chilled metal panel. To mark an ant, a single ant was placed onto a foam platform with a single strand of hair taped down as a loop. The hair loop served as a restraint for the ants. Worker ants were marked either on the head, abdomen, thorax, or a combination of the body parts with Testors® modeling paint (Testors, Vernon Hills, IL) to distinguish workers. After marking, each ant was placed into a plastic container (60 cm x 42 cm x 16 cm). The queen ant and brood items were transferred to the arena after the worker ants were distributed. A glass test tube (250 mm x 25 mm) wrapped in red transparent cellophane with a piece of moistened cellulose sponge inside as a water source was added to the arena to elicit emigration. Observational data were recorded but statistical analyses were not performed.

Results

Brachyponera chinensis workers used adult transport as a recruitment method during nest emigration. In total, I observed 396 successful transport events in treatment arenas and 42 in control arenas (Table 5.1). The mean number of successful transports occurring in treatment arenas (39.6 ± 6.94 SD) was significantly more than the number of successful transports observed (4.2 ± 2.2 SD) in control arenas. Treatment had a significant effect on the number of successful transports occurring during the study (Wilcoxon Test, $x^2 = 14.35$, $p = 0.0002$). On average, 19% of ants were physically transported inside the new harborages. The remaining 81% of ants walked into the new harborage without worker assistance.

I observed workers in treatment arenas organizing into task-associated groups before plexiglass inserts were removed. Groups consisted of brood retrievers (Fig. 5.2), members of the queen's tending group (Fig. 5.3), and scouts. Queen-tending ants surrounded the queen, remaining with the group until the queen walked into the new harborage. Scouting groups consisted of transporting and non-transporting worker ants. Non-transporting scouts moved around the arena but not in a particular pattern. After the insert was removed, ants either remained in groups or began walking into or under the new test tube harborage. Adult transport began after several scouts explored the new test tube harborage 12-15 minutes after the trial began (Fig. 5.4).

The number of successful transports occurring every fifteen minutes in treatment arenas was compared. Results indicate that the number of transports did change with time (F = 62.58, $p = 0.0002$). Once initiated, transport continued at a steady rate for 30 min. (Fig. 5.4). Transport rates remained constant from 35 to 55minutes. During this period, retrieval and placement of brood from the open arena into the new harborage became the focus of non-transporting ants. Queen ants were also moved during the 35-55 minute period. After 55 minutes, the number of successful transports increased again for 15 minutes on average.

When transporting scouts encounter other ants, the pair interacts by drumming their antennae together. In each successful case, the ant being transported lowers its head, allowing the transporting ant to grasp it by the mandibles. From this position, as defined by Guénard and Silverman (2011), the carried ant assumes a pharate pupal posture and eventually is grasped on its venter. The pair then walked toward the harborage; however, the path to the new nest site may not be direct. Once the pair reaches the new nest, the carrier ant releases its nest mate within the harborage. The carrier may remain inside the nest or return to the arena after releasing its nestmate.

The marking experiment was not successful but one ant was observed performing two consecutive transports. The marked ant picked up a nestmate dropped her off inside of the harborage and immediately entered the arena to retrieve another nestmate. Consecutive transporting was not observed in any of the other trials.

Discussion

Social carrying in *B. chinensis* was initially characterized as a context-dependent behavior performed only to recruit nestmates to food items too large to be carried by individual ants, a process known as tandem carrying (Guénard and Silverman 2011). The data presented in the current study indicate that *B. chinensis w*orkers also employ adult transport during nest emigration.

In some ant species, adult transport during emigration is sometimes preceded by tandem running (Möglich 1978; Traniello and Hölldobler 1984; Pratt 2005). *Brachyponera chinensis,* however, do not employ multiple methods during emigration. On average in this study, 19% of workers were physically transported into the new harborages during trials and the remaining 81% traveled alone to the nest site. Consequently, the question of how non-transported ants locate new nest sites remains unanswered. Non-transported ants were observed walking directly into the harborage without exhibiting tandem-running or trail-laying behavior. However, for a worker ant to locate the new nest site, directional cues must be present. Guénard and Silverman (2011) attempted to determine if *B. chinensis* use trail pheromones during tandem carries, but their experimental results were inconclusive. However, the use of trail pheromones should not be discounted. To exclude pheromones as a contributing factor, gaster positions during emigration must be analyzed and extractions of glands commonly associated with pheromone production, such as the Dufour's gland or pygidial gland should be made (Hölldobler et al. 1982). In addition to chemical signaling, tactile and visual signaling should also be evaluated as directional cues used by *B. chinensis* during emigration.

Although adult transport is an effective recruitment strategy for *B. chinensis*, each carrying attempt is not successful. The "transporting" ant always initiated the process in this experiment, but the "transported" ant may resist. Resisting ants pull away from the transporter or place their thorax/abdomen on the floor of the arena, preventing the other ant from gaining the leverage needed for carrying. This observation is in accordance with Langridge et al. (2008) who documented comparable behaviors in *Temnothorax albipennis* Curtis during colony emigration. An unsuccessful transport could be the result of a "transporter" ant encountering an individual that previously experienced visiting the new nest, another "transporter", or encountering an ant responsible for protecting brood or the queen.

Before initiating transport, *B. chinensis* worker ants organized into groups. Groups consisted of scouts, brood tenders, and the queens' retinue. Scouting ants spent time exploring the foraging arena and were the first to locate the new nest. It appeared that worker ants were protecting the queen during emigration. Queen protective behavior during emigration was also observed in *Oecophylla longinoda* (Latreille), weaver ants (Hölldobler and Wilson 1983). *Oecophylla longinoda* queens exude pheromones produced by the exocrine gland that attract workers to the queen during emigrations, allow workers to produce trophic eggs, but prevent workers from producing viable developing eggs. The loss of worker egg production ensures that queens are the sole producer of life within a colony making her a vital asset to the longevity of the colony. Worker ants of *B. chinensis* (Ito and Ohkawara 1994) and its sister species *B. nakasujii* do not possess ovarioles (Gotoh and Ito 2008). As a result, *B. chinensis* colonies solely depend on the queen for egg production. The presence of queen retinue in *B. chinensis* and absence of ovarioles in *B. chinensis* worker ants may be linked to queen produced pheromones. Brood items and the queen were transported during the middle (35-50 min.) of the study. Queen movement during this period is consistent with Pezon et al. (2005) in which *N. obscuricornis* queens were transported mid-way through emigration presumably to optimize their protection.

The number of potential transporters was lower than the number of potential transportees suggesting that a small proportion of the total workforce is allocated towards adult transport. Previous studies show related results (Langridge et al. 2008, Sendova-Franks and Franks 1995), but studies attempting to identify if carrying behavior was relegated to a specific group of ants during emigration have been inconclusive (Sendova-Franks and Franks 1995). However, physical marking caused workers to devote more time to grooming than to colony tasks. Yet, in one marking trial, I documented one marked ant carrying two nestmates into the new harborage. In view of repeat transports, I anticipate that *B. chinensis* workers can perform multiple carries, but additional marking studies are needed to determine the worker ant carrying frequency.

Colony duties may also be associated with a worker ant’s age, a phenomenon known as temporal polyethism (Robinson et al. 1994, Sendova-Franks and Franks 1993). In some studies, younger ants tend to work within the nest whereas older ants usually take on tasks outside the nest, such as colony defense, foraging, and recruitment. During trials, we observed workers transporting dusky-yellow colored callow workers into the nest, but callow workers never behaved as transporters. Abraham and Pasteels (1980) reported similar behavior in *Myrmica rubra* (L.). Recognition of this phenomenon raises the possibility that temporal polyethism also may play a role in task allocation during adult transport in *B. chinensis*.

As *B. chinensis* continues to increase its geographic range (Guénard and Dunn 2010), thorough documentation of colony movements will be more important. The dispersal abilities of invasive ant species are affected by dispersal type. After establishment, invasive ants may naturally increase their range by mating flights (Markin et al. 1971) or increase their foraging range through an emigration process known as budding (Holway et al. 2002). During budding a portion of a colony leaves the original nest to found a new nest a few meters away. *Brachyponera chinensis* use adult transport during foraging (Guénard and Silverman 2011) and during nest emigrations (current study) so it is possible to suggest that ants also use adult transport to increase their range.

My studies serve as the first to provide insight into the nest emigration recruitment behaviors of *B. chinensis* workers. In this laboratory study, *B. chinensis* employed adult transport during emigration to move colony members to new nesting locations, but only a subset of the entire colony was relocated in this manner. These results suggest that *B. chinensis* may disperse through budding. However, Zungoli and Benson (2008) collected winged males and females in a light trapping study, suggesting that mating flights may also occur; although, males alates were trapped more frequently than females (19:1). Future studies of *B. chinensis* emigration should attempt to address colony propagation and task allocation during emigrations. Studies of this nature will help us understand dispersal factors contributing to the invasive success of *B. chinensis* in the United States.

Table 5.1. Total number, mean, and standard deviation of successful carries performed by *Brachyponera chinensis* (Emery) workers during nest emigration trials. Means followed by different letters are significantly different ($p < 0.05$) based on one-way Wilcoxon test. Total number, mean, and standard deviation of successful carries performed by *B. chinensis* workers during nest emigration trials. Means followed by different letters are significantly different ($p < 0.05$) based on one-way Wilcoxon rank sum test

	N	**Total**	**Mean**	**Standard Deviation**
Control	10	42	4.2^{a}	± 2.2
Physical Disturbance	10	396	39.6^{b}	± 6.94

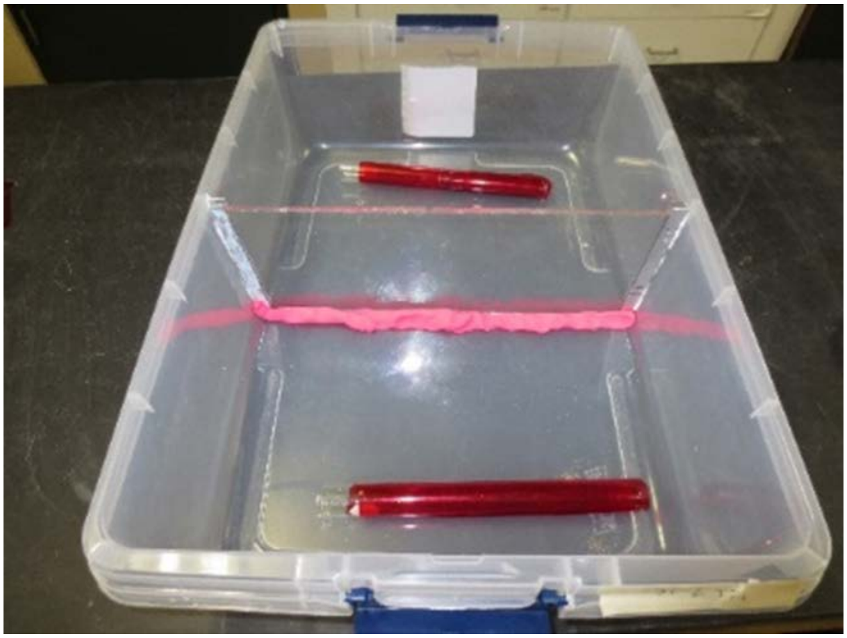

Figure 5.1. *Brachyponera chinensis* (Emery) nest emigration arena (60.9 cm x 42.6 cm x 16.7 cm) with test tube (250 mm x 25 mm) harborages, plexiglass insert, and Play-Doh® lining. Test tubes are wrapped with red cellophane paper with a moistened 8-cm sponge inserted. During experimental trials, the plexiglass insert, Play-Doh ®, and one harborage were removed allowing the ants to freely move in the arena.

Figure 5.2 Group of *Brachyponera chinensis*(Emery) worker ants retrieving and hoarding brood during nest emigration trials.

Figure 5.3. Queen (red circle) *Brachyponera chinensis* (Emery) and her retinue during a nest emigration trial.

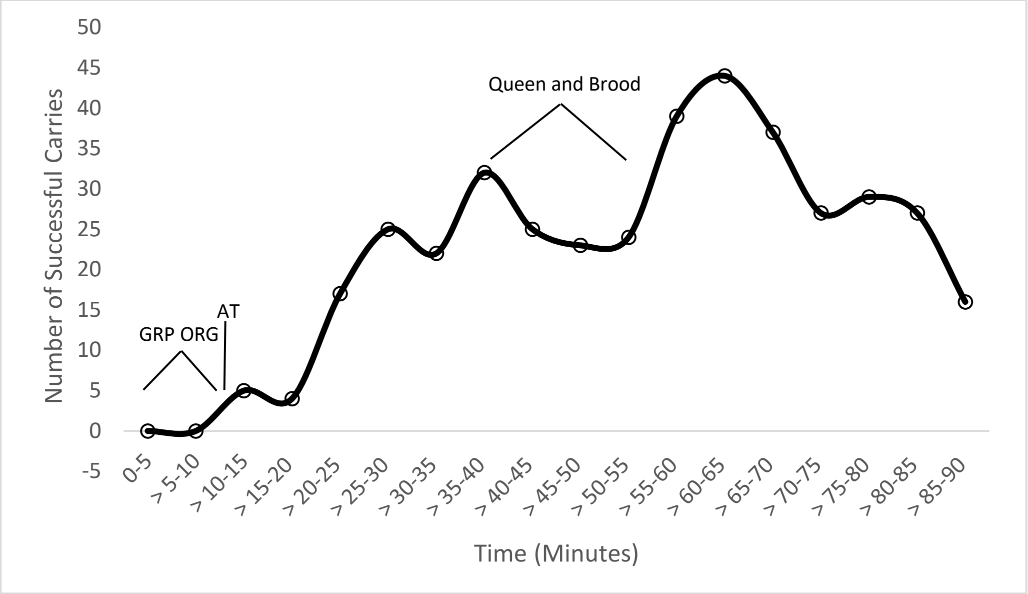

Figure 5.4. Pooled total number of successful carries by *Brachyponera chinensis* (Emery) workers occurring at 5-minute intervals during treatment nest emigration trials (n = 10 colonies). Worker ants organized into groups (GRP ORG) from 0-13 min. Adult transport (AT) began at the 12 min and continued for 90 min. Queens and brood were transported from 35 to 55 min. The number of successful transports performed by *B. chinensis* workers decreased during queen and brood movement. Transport activities increased after the queen and brood were moved to the new harborage.

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CHAPTER SIX

SUMMARY AND RECOMMENDATIONS

As humans continue to encroach upon the natural habitats of organisms and spread into new environments the potential to spread non-indigenous species geographically will remain. Human-assisted transport is responsible for the spread of many organisms throughout the world (Floerl et al. 2009). In fact, human-assisted transport is responsible for the spread of the majority of the 250 non-native ant species throughout the world (Lach et al. 2010). Outside their natural environment, invasive ants can cause public health concerns (Breed 2016), cost municipalities millions in management efforts, and cause ecological damage (Holway et al. 2002). The Asian needle ant, *Brachyponera chinensis* (Emery) an invasive ant species, is a public health and ecological pest in the U.S. The biology of this ant is poorly understood and the need for biological and ecological information on this pest are vital to creating specific management programs for this insect.

Oftentimes, the biology and behavior of invasive ants in new environments differs from the behavior of the same species in their native environment (Holway et al. 2002). Because the biology of Asian needle ant is not well documented, I first wanted to identify the seasonal life cycle of the ants in the U.S. Results from the seasonal life cycle study suggest that Asian needle ant populations follow a seasonal cycle of nest member production. I was able to document the seasonal change in the production of worker ants and reproductives. These results differ from the information presented by Murata et al. (2017) who reported that there was not a seasonal change in the number of workers and queens in native Asian needle ant populations in Japan. However, although my results add to the current knowledge, the sociometry of *B. chinensis* is still incomplete. I collected four nests a month during the study but additional nests should be censused to determine if population sizes can change from year to year, if nest sizes change with age, and to determine the seasonal reproductive output of queens. An ant colony is a cooperative functional unit and the unified characteristics of a colony will lead to further insights and understanding of the ecology and evolution of a species.

The objective of the second study was to determine if *B. chinensis* displayed a polydomous social nesting structure by analyzing spatial nesting patterns and agonistic behavior of workers originating from the same nest, different nests, and separate locations. Results from the spatial nesting study indicated that three of the four, *B. chinensis* display a uniform nesting pattern. However, one of the nests displayed a clumped nesting pattern indicative of a polydomous nesting structure. Results from the second objective suggest that *B. chinensis* has a polydomous nesting structure because aggression was not observed in any of the nestmate recognition assays. Results of the spatial study should be viewed with skepticism. Uniformity and the clustering of ant colonies may be the product of unknown ecological factors and a matter of scale. The plots used in the study were 10 m^2. Larger plots need to be studied to determine if clustering occurs at a larger scale. These results are counter to what was observed during the recognition assays. Polydomy can be inferred from non-aggressive ants originating from separate locations but these results also come with limitations. Aggression comes with costs and ants must make a decision of whether to attack non-nestmates (Ellis et al.

2017). I performed 3 x 3 nestmate recognition assays but a higher number of ants may be needed to elicit aggressive behavior. However, Murata et al. (2017) reported that *B. chinensis* workers in Japan did not display aggression towards non-nestmates. Therefore, *B. chinensis* may be a species that simply does not show aggression towards non-nestmates so nestmate recognition studies may not be viable measure of social nesting structure.

The third objective was to determine if *B. chinensis* workers used adult transport during nest emigrations. I successfully reported that *B. chinensis* uses adult transport during nest emigrations caused by physical disturbance. I also documented the social organization of *B. chinensis* during emigration. Before transport began, ants organized into brood tending, scouting, queen tending, and transport groups. These studies should be further used to explore resource sharing in the form of workers in between nests and to determine the extent of social carrying during emigrations. These results may provide insight into polydomy and further characterize the colony establishment patterns of this invasive ant species.

Because biological and ecological information for *B. chinensis* are lacking, the opportunity to study the organism and create management options before populations spread outside of the southeastern U.S. are great. Mo (2013) and Spicer-Rice et al. (2012) studied the impact of gel and granular baits on Asian needle ants but because we don’t fully understand the emigration processes, nesting patterns, and colony life cycle of *B. chinensis* many of these research endeavors may not fully capture the requirements necessary for efficient and optimal control. In addition, further studies are needed to determine the extent of infestation and to document the true long-term impacts of *B. chinensis* in the U.S.

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Appendix A

Chapter Three Supplementary Data

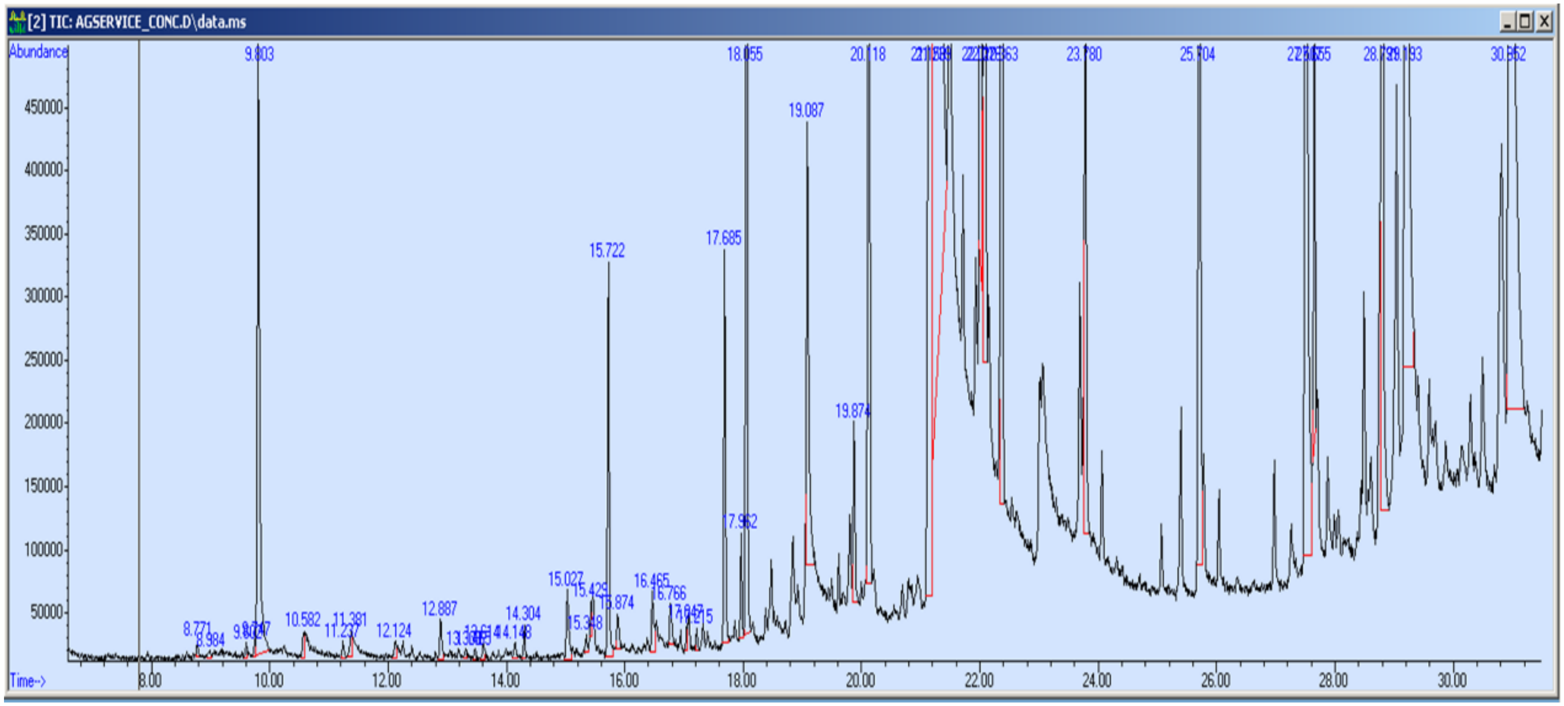

Figure A.1. Chromatogram of cuticular hexane extracts of *Brachyponera chinensis* (Emery) workers collected from Agricultural Service Station (34° 39’ 29.219” N, 82°49’56.241” W; Pickens County, SC). See Table 3.5 for the peak identities.

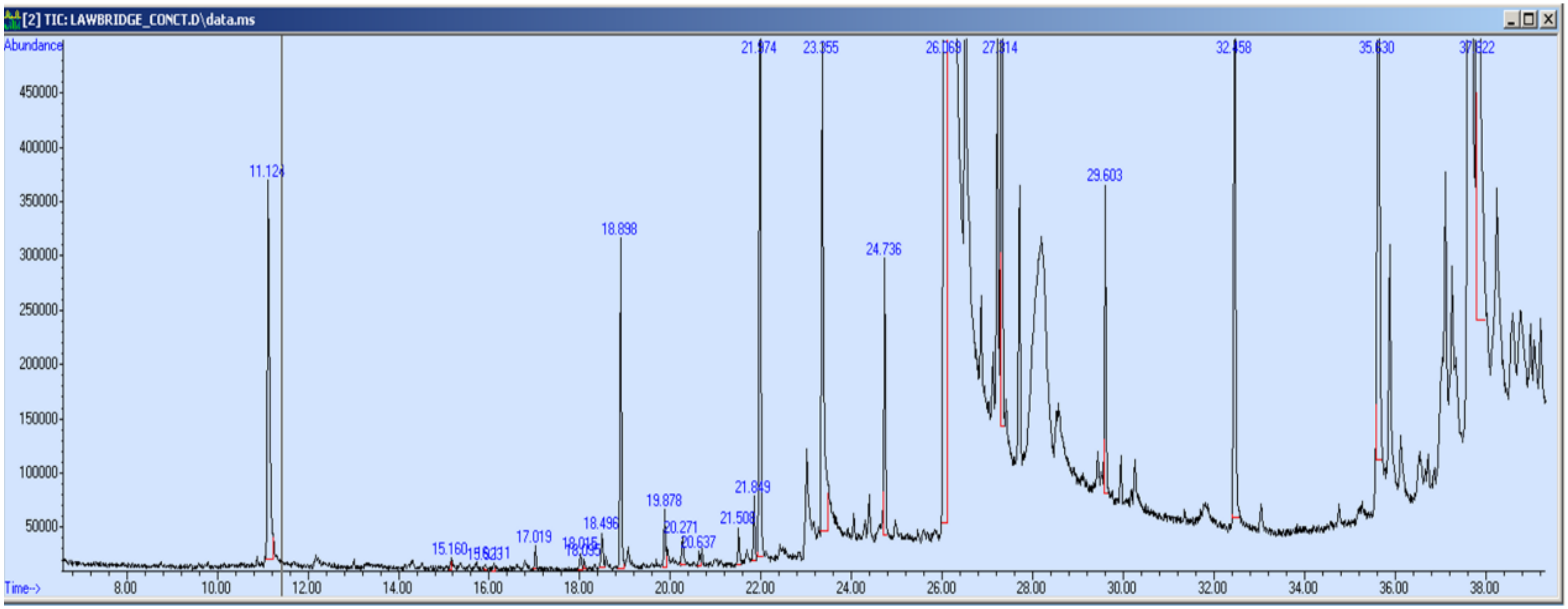

Figure A.2. Chromatogram of cuticular hexane extracts of *Brachyponera chinensis* (Emery) workers collected from Lawrence Bridge (34°44’1.608” N, 82°52’25.895” W; Oconee County, SC). See Table 3.5 for the peak identities.

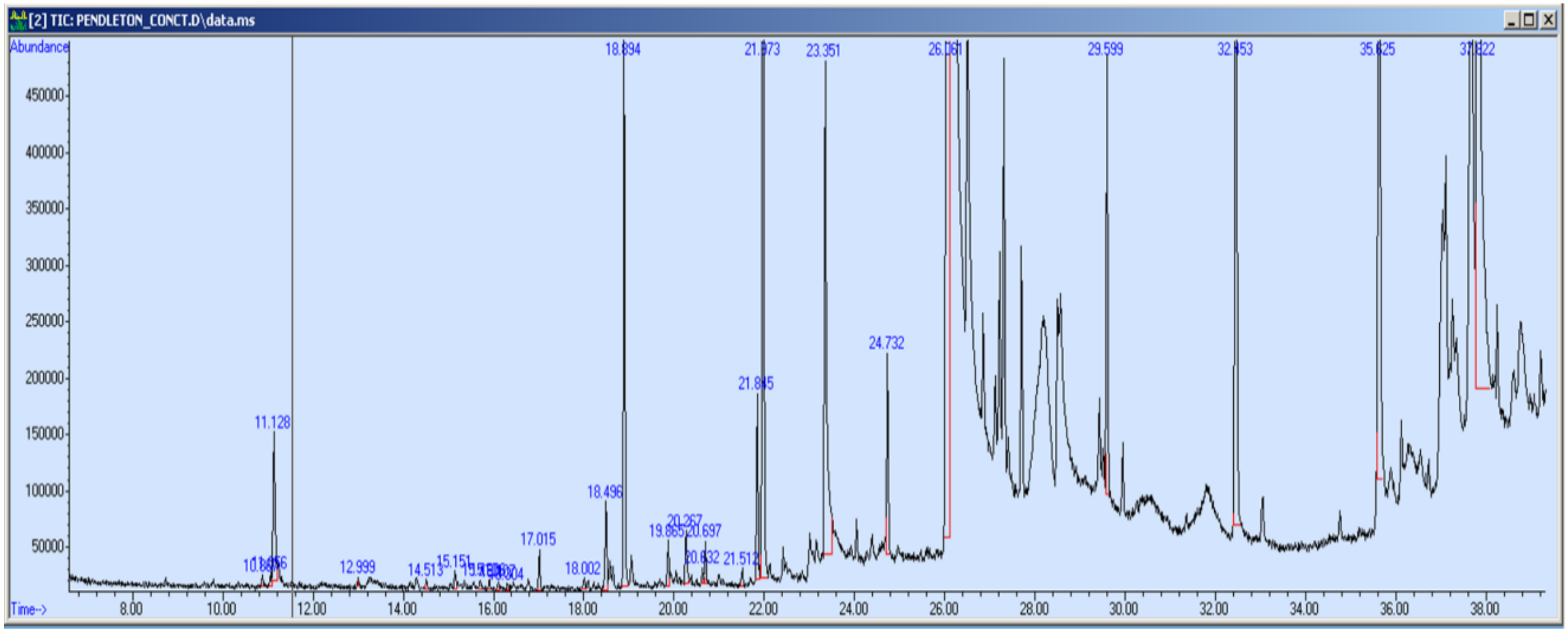

Figure A.3. Chromatogram of cuticular hexane extracts of *Brachyponera chinensis* (Emery) workers collected from Pendleton (34° 40’21.247” N, 82° 48’ 38.75” W; Anderson County, SC). See Table 3.5 for the peak identities.

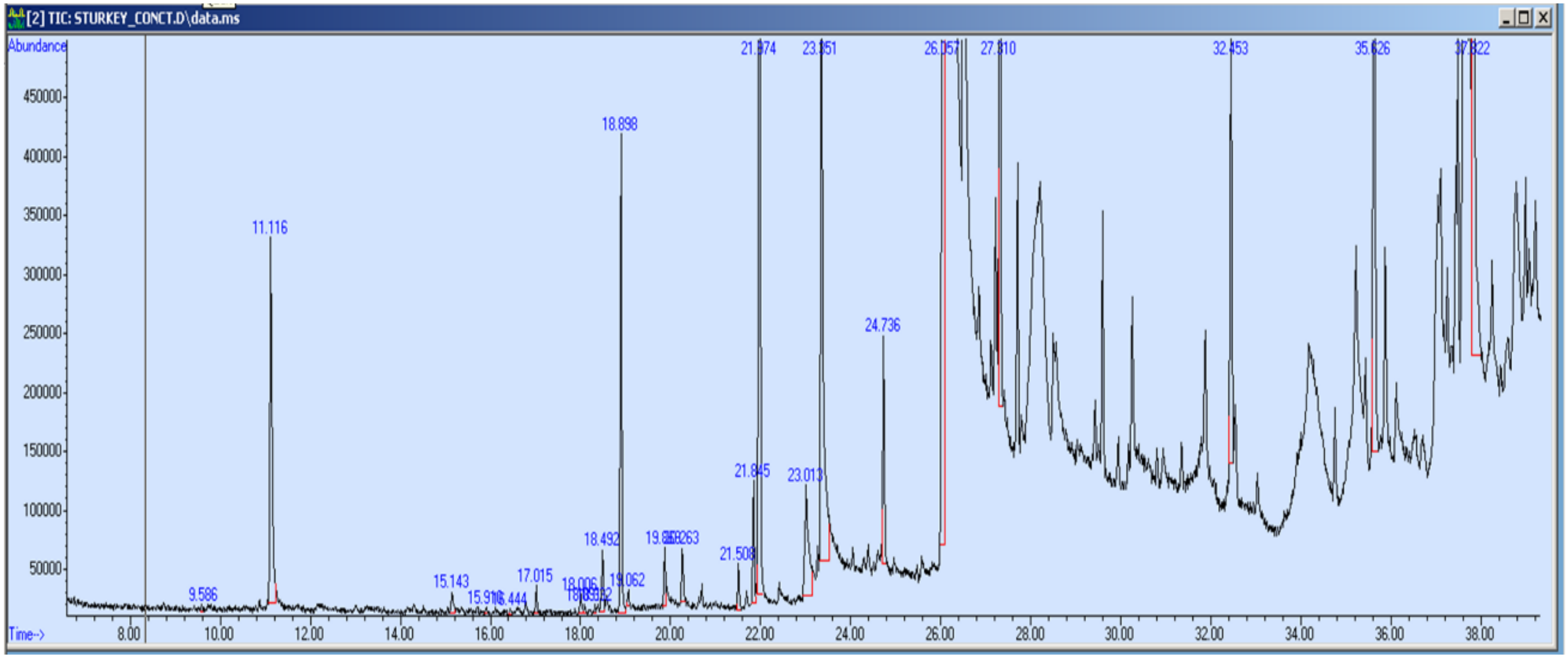

Figure A.4. Chromatogram of cuticular hexane extracts of *Brachyponera chinensis* (Emery) workers collected from Sturkey (34° 38’ 42.78” N, 82°48’46.799” W Pickens County, SC). See Table 3.5 for the peak identities.