POPULATION GENETICS AND COLONY BREEDING STRUCTURE OF THE INVASIVE TAWNY CRAZY ANT, *NYLANDERIA FULVA*

A Thesis

by

BRYANT ANDREW MCDOWELL

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Edward L. Vargo
Committee Members,	Robert T. Puckett
	Jessica E. Light
Department Head,	Pete Teel

May 2019

Major Subject: Entomology

ABSTRACT

Insects that have evolved to live socially are some of the most invasive species on the planet. Of these invaders, many are ant species whose overwhelming success in non-native habitats is possible due to several favorable attributes. Unicoloniality, a social structure adopted by many invasive ant species, is defined by the ability to form supercolonies (a group of individuals throughout a geographic area, where direct interaction of individuals from distant nests does not occur) which may span many kilometers. High queen densities within supercolonies affords an overpowering concentration of worker ants which intermix with members of adjacent colonies without intraspecific aggression, seemingly working together resulting in exponential colony growth. The tawny crazy ant, *Nylanderia fulva* (Mayr), was recently introduced into the southeastern United States from South America. These ants were first discovered in Texas in 2002 and were possibly introduced into Florida as early as the 1950s. Since then, this invasive ant pest has spread to all southeastern Gulf States where it outcompetes native species, reduces arthropod species diversity, and infests urban, agricultural, and natural areas. Colonies of this ant are believed to be unicolonial in invasive populations, but to date this has not been explicitly investigated. Through behavioral assays and genetic analyses, I tested the hypothesis that the tawny crazy ant is unicolonial in its invasive range and estimated the number of possible introductions into the United States.

In this study, I conclude that the tawny crazy ant is, in fact, unicolonial throughout its invasive range. I found no evidence of population differentiation, even

among populations hundreds of kilometers apart. I also found low levels of relatedness ($r = 0$) among nestmates, lack of inbreeding, and an absence of aggression between non-nestmates separated over hundreds of km. I also discovered evidence of weak isolation by distance across the Gulf States. These results show that *N. fulva* forms a single supercolony throughout the entire invasive range of the southeastern United States that is most likely the result of a single introduction, which then spread via human mediated dispersal and colony budding.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Vargo, along with my committee members, Dr. Puckett and Dr. Light for their advice and support during the course of this research project.

I would also like to thank my friends and colleagues for their assistance and suggestions pertaining to my project. I started this project with zero experience working in a molecular lab and the most basic understanding of microsatellites and how they are applied. I have learned so much by working in the Rollins Urban and Structural Entomology Laboratory. I would also like to thank all of my professors who supported me for the past two years. I have the upmost respect for you all.

I am so grateful to be a member of the Department of Entomology at Texas A&M University. Finally, I would like to extend my gratitude to the faculty and staff members who have helped me continuously through the duration of my stay here.

CONTRIBUTORS AND FUNDING SOURCES

Contributors:

This work was supervised by a thesis committee consisting of Professors Edward Vargo and Robert Puckett of the Department of Entomology and Professor Jessica Light of the Department of Wildlife and Fisheries Sciences.

Student/collaborator contributions -

Dr. Dewayne Shoemaker provided the primer sets required to complete this project. These were built from a transcriptome study published by Valles et al. (2012).

All other work conducted for the thesis was completed by the student independently.

Funding Sources:

This work was made possible in part by the Invasive Ant Research and Management Seed Grant Program FY16 & FY17 to ELV and RTP and by the Urban Entomology endowment fund at Texas A&M.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Ants (Hymenoptera: Formicidae) are a key component of terrestrial ecosystems occupying many trophic levels (Holway et al. 2002). In general, ants are beneficial insects providing many important ecosystem services. However, several species of ants are invasive where they cause widespread ecological damage and inflict both direct and indirect negative effects on the natural ecosystems they invade (Holway et al. 2002, Silverman and Brightwell 2008). These invasions are typically the result of human mediated migration into foreign habitats (McGlynn 1999, Suarez et al. 2001, Foucaud et al. 2010).

When a species is introduced into a non-native habitat, it must overcome certain abiotic and biotic factors in order for it to persist and thrive in the new region before it becomes established (Human et al. 1998). Abiotic conditions in the introduced range need to be suitable for the invasive species to spread and thrive (Human et al. 1998). For example, in the United States, two major invasive ant species (the Argentine ant, *Linepithema humile,* and the red imported fire ant, *Solenopsis invicta*), are native to South American regions that more or less share similar abiotic conditions found in parts of the United States (McDonald 2012). Therefore, these invaders have an evolutionary advantage that has helped allow them to establish in those regions.

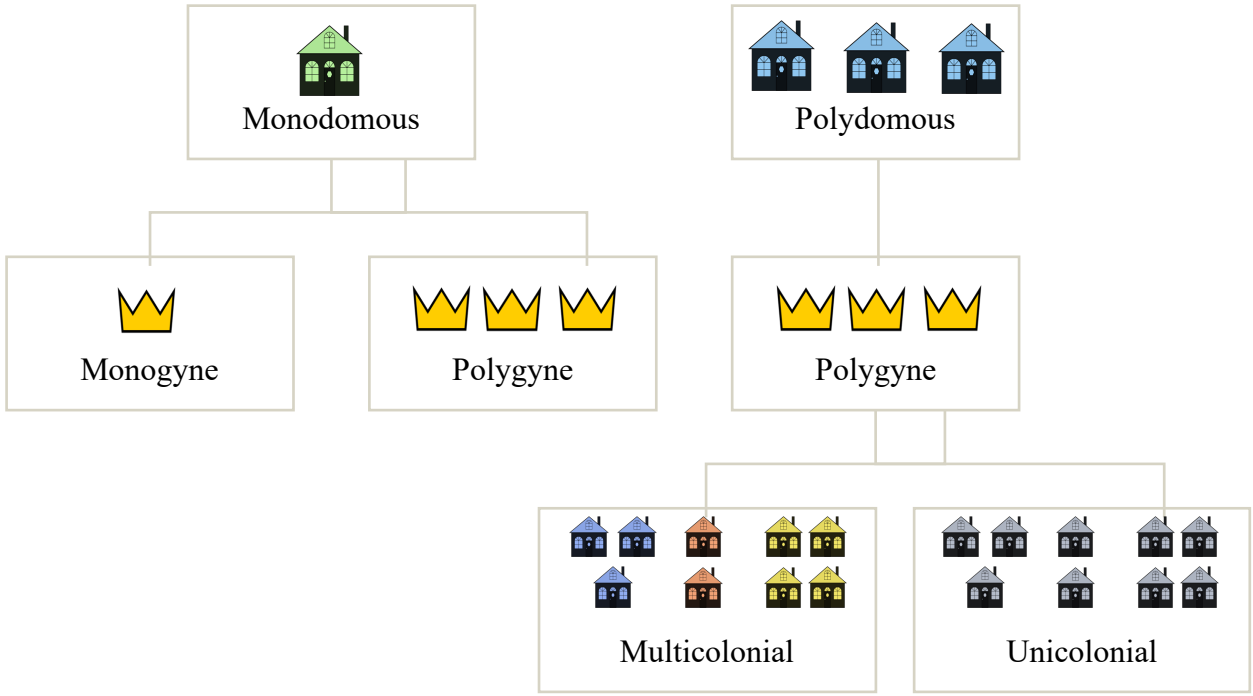
Furthermore, the 'biotic resistance hypothesis' first proposed by Elton (1958) states that the spread of an introduced species may be controlled by the biotic interactions of the native (or established invasive) species that already occupy each niche

in the environment. For example, a species rich environment is more resistant to an introduced species than a species poor environment because those species existing in a diverse environment are more able to utilize their resources than the latter. Introduced species are limited by factors such as the initial number of introduced individuals, the ability of those individuals to locate food, water, and shelter, and the potential for the invading members to reproduce and persist in the new environment (Moller 1996).

Social insects, especially introduced ant species, have attributes which allow colonies to counter these limiting factors in non-native ranges. These groups are more dominant than the native species found in the introduced range and can therefore compete against such species and disrupt those native populations due to their dispersal abilities, large colony size with numerous reproductive queens, omnivorous diets, and the ability of the colony to function as a single entity to more effectively forage and defend (Moller 1996). Social insects such as ants (McGlynn 1999, Holway et al. 2002, Tsutsui and Suarez 2003, Calcaterra et al. 2016) and termites (Evans et al. 2013) are well documented to become successful invaders. Moller (1996) proposed that social insects have advantages that promote invasiveness due to their individual and colony level responses that allow them to withstand biotic pressures in the introduced ranges. Fournier et al. (2009) identified characteristics of invasive ants to include their close association with humans, omnivory, general nesting requirements, polygyny, no intraspecific aggression, and genetic homogenization due to colony reproduction by budding. Invasion success is attributed to not only the social and colony organization of the species, but also the absence of competitors, predators, parasites, or diseases that regulate native populations (Suarez et al. 1999).

Social structures vary among ant species and in some cases, such as the red imported fire ant, within species (Ross and Fletcher 1985, Ross et al. 1996). Monodomous ant species occupy a single nest, represented as one colony, headed by one (monogyne) or more (polygyne) queens. Polydomous ant species occupy two or more nests that are spatially separated yet socially connected. Multiple colonies of polydomous ants can occupy a given area (see Figure 1) and aggression can be observed between such colonies (Suarez et al. 2008, Robinson 2014). Colony boundaries in a given area are formed by nestmate recognition of the individuals within each colony. Colonies are commonly identified by their aggression towards surrounding nests of the same species as well as their genetic distinctions between populations; however, a colony is truly defined by its spatial connectivity, i.e., whether individuals share nest chambers and foraging tunnels.

Figure 1. Pictorial describing various forms of colony organization. Social structures can vary both among and within species. Monodomous ant colonies occupy one nest which is headed by either one (monogyne) or multiple (polygyne) queens. Polydomous ant colonies occupy many nesting sites and are headed by many queens (polygyne). These colonies can be described as multicolonial or unicolonial based on their behavior towards one another and genetic data.



Polydomous ant species are either multicolonial or unicolonial, depending on the size and number of related nests in a given area. Many invasive ants, e.g., the Argentine ant, *L. humile* (Mayr) (Tsutsui and Case 2001, Pedersen et al. 2006), the red ant, *Myrmica rubra* (Seppä and Walin 1996), and the polygyne form of the red imported fire ant *S. invicta* (Morel et al. 1990, Ross 1992), are described as unicolonial, with the ability to form supercolonies (Helanterä et al. 2009), which is how invasive ants become ecologically dominant.

The term "supercolony" is used to distinguish populations that are too large to allow for the direct interaction of individuals from distant nests (Drescher et al. 2010). The size and number of supercolonies present in an area varies among ant species and such differences are even reported within a species. For example, the Argentine ant, *L. humile,* forms one supercolony throughout New Zealand (expanding 700 km) (Corin et al. 2007), at least five supercolonies throughout southern California (one of which expanding 900 km) (Tsutsui et al. 2003), four supercolonies in Japan (Hirata et al. 2008, Sunamura et al. 2009), and two supercolonies throughout southern Europe (one of which expanding >4000 km) (Giraud et al. 2002). Another example is the African big-headed ant, *Pheidole megacephala,* which forms four populations in its introduced range in northeastern Australia, all of which belong to a single genetically distinct supercolony spanning 3000 km (Fournier et al. 2009). In its native range, *P. megacephala* forms at least 8 distinct supercolonies throughout Africa, all of which are much smaller than that found in the invasive range of Australia (Fournier et al. 2012). Supercolonies consist of multiple nests, each of which are polygynous (contain multiple reproductive queens) and nestmates are capable of free movement between nests without any observed

intraspecific aggression within the supercolony. Members of one supercolony are more genetically similar to each other than to members of another supercolony. As a result, intraspecific aggression between workers of neighboring supercolonies can be observed at the colony boundary.

In a unicolonial ant species, individuals collected from different nest locations within the same supercolony do not exhibit aggressive behavior toward one another. These nests are not socially connected and there is no clear observation of colony boundaries among the entire population. With a lack of such boundaries, individuals from separate nest clusters mix between sites without any intraspecific aggression. Throughout large areas, individuals belonging to a unicolonial population seemingly work as one social unit, rather than competing for space and resources between separated nests. In invasive populations, this effectively diminishes foraging territories between nests and the supercolony is then capable of expanding across large geographic areas, in some cases, hundreds of kilometers (Tsutsui and Case 2001, Helanterä et al. 2009, Aguillard et al. 2011). As mentioned above, multiple supercolonies may be present in an invasive range and can be identified based on the genetic differences and intraspecific aggression that occurs between them at the colony boundary. The lack of aggression between spatially separated nests is proposed by many scientists to be the result of reduced diversity at genetic loci influencing nestmate recognition (Giraud et al. 2002), which eliminates the aggressive behavior typically observed between native colonies of the same species (Suarez et al. 2008).

Unicolonial ant species pose a problem for kin-selection (Helanterä et al. 2009). From an evolutionary standpoint, unicoloniality enables short-term success for the

population as a whole, but for the species, it seems to be an evolutionary dead end. Unicolonial species work as one unit, so, the lack of aggression between colonies is thought to be advantageous in that worker ants are capable of allocating more time to the growth and reproduction of the colony (more aggression towards competitors, rapid monopolization of food resources). Polydomous species with polygyne colonies give rise to many workers that are capable of free movement among nests, which results in low relatedness among nestmates and workers in neighboring colonies. Furthermore, many species lack mating flights, so, mating occurs within the nest and new nests are produced by budding (Silverman and Brightwell 2008, Helanterä et al. 2009). Therefore, worker ants will provide resources and care for individuals which are unrelated to them, promoting the survival of the offspring of unrelated reproductives. Investing in the survival and growth of unrelated individuals results in a fitness cost and is a direct contradiction of kinship theory as there is no inclusive fitness benefit to workers. Therefore, the long-term stability of unicolonial supercolonies is very much in question (Helanterä et al. 2009).

Identifying an invasive species can be difficult, and their introduction into non-native regions often goes unnoticed until they have become a serious pest. This has been the case for the tawny crazy ant, *Nylanderia fulva* (Mayr), in the southeastern United States. Native to South America, the tawny crazy ant has invaded the southeastern United States where it has become a resiliently successful pest. In its introduced range, tawny crazy ants outcompete native species, reduce arthropod species diversity (LeBrun et al. 2013), and infest urban, agricultural, and natural areas (Gotzek et al. 2012, Horn et al. 2013). An example of this impact can be seen at Estero Llano Grande State Park in

Hidalgo County, Texas, which reports a decline in multiple arthropod species as well as a distinct negative impact on vertebrate populations such as birds, reptiles, and small mammals (unpublished data/personal observation).

The tawny crazy ant has most likely been in the southeastern United States, specifically Florida, as early as the 1950s (Deyrup et al. 2000, Gotzek et al. 2012). Since then, it has managed to spread throughout the Gulf States (Hooper-Bùi 2010, MacGown and Layton 2010, Valles et al. 2012). It was not until 2002 that this invader was documented in Texas, where it is now present in more than 42 counties and shows no sign of halting its spread.

Interestingly, female reproductives of this species have not been observed to take part in mating flights, suggesting that mating occurs inside the natal nest and long distance dispersal is likely due to human transport (Kumar et al. 2015). Wang et al. (2016) found nuptial flight activity of *N. fulva* males throughout the year, with peak activity during the summer and suggests that queens within the nest attract nearby males via pheromones, and that nests reproduce by budding.

Nylanderia fulva colonies are found in several habitats in their introduced range. In both rural and urban sites, dense numbers of individuals invade structures and are a serious nuisance pest, in addition to causing damage to electrical equipment and outcompeting native species (Meyers and Gold 2008, LeBrun et al. 2013). This can potentially alter ecosystem processes, as observed in other invasive species such as *L. humile* (Silverman and Brightwell 2008, Suarez et al. 2008), *Anoplolepis gracilipes* (O'Dowd et al. 2003, Abbott 2005), and *Wasmannia auropunctata* (Le Breton et al. 2004, Foucaud et al. 2010).

Pest ant species are controlled using multiple approaches. It is common to use bait-insecticides as a method of control as they can be applied over large areas. It is important that control efforts limit the non-target effects in the environment. Baits are formulated to negatively impact the target species and contain low amounts of toxins which are slow acting. Baiting studies have shown that there is a reduction in *N. fulva* infestations immediately following treatment of a given area. One study showed that this was followed by a rapid reinfestation (3-4 weeks after initial baiting) of neighboring ants into already treated areas due to the lack of residual control (McDonald 2012). In this study, the treatment areas consisted of patchily distributed sites within a larger infested region which stretched along the Brazos river. Ant densities within the treatment areas returned to pre-treatment levels 3-4 weeks after treatment. McDonald (2012) gives two possible reasons to explain this reinfestation. It is possible that neighboring nests along the treatment boundaries repopulate the treated area by budding. Another possibility is that queen densities in the surrounding nests were so high that they were simply able to produce enough offspring within the 3-4 weeks post treatment to achieve population densities compared to pre-treatment counts. Therefore, McDonald (2012) suggests an area-wide treatment over entire populations in order to gain control of *N. fulva*. Similarly, Calibeo et al. (2017) found that *N. fulva* populations were not repelled by any insecticide they tested. This study reported that lab colonies of tawny crazy ants were rapidly controlled with Termidor® and Temprid® (at the highest concentration allowed for exterior application) with 100% mortality in 13.4 and 19.0 days, respectively. Calibeo et al. (2017) also performed choice tests using 15 commercially available insecticide baits, of which, three were concluded to provide possible control. This study

found that tawny crazy ants accept carbohydrate-based ant baits over others tested, and that the highest mortality was achieved with the active ingredients hydramethylnon and fipronil. The authors note that this study did not account for environmental conditions, alternative food/water resources, worker avoidance, and queen relocation/adoption into neighboring nests which are all factors that can affect overall suppression of *N. fulva* infestations.

As previously mentioned, *N. fulva* infestations can be extremely dense – covering several km^2 and containing dozens of nests (MacGown and Layton 2010, McDonald 2012, Wang et al. 2016). This observation has led several authors to assume this species is unicolonial (MacGown and Layton 2010, Horn et al. 2013, LeBrun et al. 2013). Horn et al. (2013), found no intraspecific aggression between *N. fulva* workers collected from different nests of a single field in Houston, Texas, after diet manipulation, but did in fact observe interspecific aggression against *S. invicta* workers. The findings of Horn et al. (2013) support unicoloniality in *N. fulva*, but these results are based on a single site and distances between the different nests tested were not reported. In addition, genetic data supporting supercolony formation are lacking. Therefore, more thorough studies of behavior and genetic relationships among nests in the invasive range of *N. fulva* populations are needed to determine if this species is unicolonial.

There are no published studies of the genetic relationships among *N. fulva* nests in a given area, nor is there documentation of the foraging expansiveness of a colony. Unicolonial ants pose a unique problem for traditional control methods due to the density of their nests, resulting in sites becoming quickly reinfested after treatment. Spot treatments are useless because nearby nest sites quickly invade the treated areas within

weeks of control efforts. It is necessary for more aggressive control methods when regulating a unicolonial species (Silverman and Brightwell 2008). Without these studies, we cannot definitively conclude this species is unicolonial. There is an undeniable need to develop effective integrated pest management strategies for invasive ants such as the tawny crazy ant, due to their extremely destructive tendencies.

I hypothesize that this species is, in fact, unicolonial in its invasive range throughout the southeastern United States. To determine this, I investigated if intraspecific aggression occurred between individuals at various spatial scales, ranging from different nests located in a given site, to nests that are separated by >100 km. In combination with these data, I determined the genetic variation among nests by microsatellite analysis. I investigated whether tawny crazy ants are unicolonial in their introduced range by observing the levels of intraspecific aggression between ants collected at various spatial scales, as well as determining the level of genetic differentiation between sites. This research study adds to our understanding of the foraging behavior, breeding structure, nesting strategies, and genetic structure of *N. fulva* colonies, as well the genetic relationships among colonies within its introduced range in the United States. These results will ultimately contribute to the development and implementation of effective control strategies against this serious pest.

CHAPTER II

POPULATION GENETICS AND COLONY BREEDING STRUCTURE OF THE TAWNY CRAZY ANT, *NYLANDERIA FULVA* (MAYR), IN TEXAS

Introduction

Invasive ant species thrive in their introduced ranges due to factors such as the absence of natural enemies or diseases that would normally regulate the populations in the native range (Suarez et al. 1999, Kumar et al. 2015). Invasive ants share characteristics such as a general nesting habit, omnivory, polygyny, reproduction by budding, and a lack of intraspecific aggression within established supercolonies (Tsutsui and Suarez 2003). Furthermore, many invasive ants, for example, the Argentine ant, *Linepithema humile* (Mayr), are capable of producing supercolonies that expand over large geographic distances (Tsutsui and Case 2001, Pedersen et al. 2006) consisting of multiple reproductive queens (termed unicolonial). Other examples of ant species that form unicolonial populations include the big-headed ant *Pheidole megacephala* (Fournier et al. 2009), the red ant *Myrmica rubra* (Seppä and Walin 1996, Hammen et al. 2002), the polygyne form of the red imported fire ant *Solenopsis invicta* (Morel et al. 1990, Ross 1992), and the long-legged ant *Anoplolepis gracilipes* (Silverman and Brightwell 2008). These unicolonial species have no intraspecific aggression among genetically similar populations, which can span several km^2 (Tsutsui and Case 2001, Pedersen et al. 2006).

Unicoloniality allows for the free exchange of individuals between different nest sites with the absence of intraspecific aggression. This phenomenon is most likely the

consequence of decreased genetic diversity due to a genetic bottleneck in the introduced range that ultimately results in a low level of relatedness between individuals in a colony (Tsutsui and Case 2001, Corin et al. 2007). For these populations, relatedness estimates between nestmates which are effectively equal to zero indicates that those nestmates are no more genetically similar to each other than compared to individuals chosen at random within the entire reference population (invasive population) (Helanterä 2009). Invasive unicolonial ants exhibit a lack of aggression between unrelated individuals, affording them the ability to reach much higher densities than that of populations in their native range (Tsutsui and Case 2001, Hammen et al. 2002, Corin et al. 2007). However, the cause of decreased aggression (i.e. mechanisms underlying nestmate recognition) is debated among researchers (Giraud et al. 2002, Helanterä et al. 2009). Invasive unicolonial ants are difficult to control due to the size and abundance of their populations as well as the lack of natural enemies in the introduced range (Suarez et al. 1999, Silverman and Brightwell 2008, LeBrun et al. 2013, Calibeo et al. 2017).

The tawny crazy ant, *Nylanderia fulva* (Mayr), has become an invasive pest species in the southeastern United States. This species was first discovered in Texas in 2002, but has possibly been in the United States (Florida) as early as the 1950's (Deyrup et al. 2000, Gotzek et al. 2012). Since their discovery in Texas, tawny crazy ants have been documented in 42 counties and show no sign of stopping their invasion. *Nylanderia fulva* colonies are found in many habitats. In undisturbed sites, *N. fulva* populations reach extremely high densities and outcompete many different species, potentially disrupting ecosystems (Corin et al. 2007, Silverman and Brightwell 2008, LeBrun et al. 2013). Because these densities are so extreme, there is a huge impact on the natural

fauna including ecological effects, such as a decrease in the diversity of native ant species and other arthropods (LeBrun et al. 2013, Wang et al. 2016), and economic damage for homeowners, public land owners, and farmers (Wang et al. 2016). Tawny crazy ants are also documented to have killed chickens by asphyxiation and have been seen to aggregate around the soft tissues (eyes, nose, and hooves) of larger bodied vertebrates such as cattle (McDonald 2012).

Workers in a heavily infested area reach high densities; *N. fulva* workers basically cover the landscape (personal observation). Unlike *S. invicta,* tawny crazy ants do not tunnel extensively (Bentley et al. 2015) and tend to nest in leaf litter, mulch, or near the roots of shrubs and trees (McDonald 2012). In a major infestation, they are capable of nesting in any cracks or crevasses (Zenner de Polania 1990). Despite having originated in the same geographic region as *S. invicta,* tawny crazy ants do not raft in pools of water (McDonald 2012). It is common for tawny crazy ants to climb trees to escape unfavorable conditions or to tend honeydew-producing insects, such as aphids. In the pecan industry, this has become a problem for producers who shake trees to harvest pecans and inevitably become covered in ants that fall from these trees (unpublished data). Furthermore, in urban landscapes, *N. fulva* has been documented as a serious nuisance pest where they aggregate in electrical equipment either chewing through insulation and wiring, or accumulating dead piles of ants that eventually cause electrical systems to short (Meyers and Gold 2008).

The observed densities of *N. fulva* infestations leads many researchers to believe this species is unicolonial in their invasive range (MacGown and Layton 2010, LeBrun et al. 2013). Horn et al. (2013) reported a lack of intraspecific aggression between tawny

crazy ant workers from different nests within a single field in Houston, Texas, but there is no other behavioral data and no genetic data available to conclude whether or not *N. fulva* is unicolonial in its introduced range. There are no published studies of the associations among *N. fulva* nests in a given area, nor is there documentation of the foraging expansiveness of a colony. Without these studies, we cannot definitively conclude this species is unicolonial. I hypothesize that tawny crazy ants are unicolonial in their invasive range throughout Texas. In this study, I combine behavioral assays as well as genetic data to draw conclusions about the population genetics and colony breeding structure of *N. fulva*.

Methods

Sampling methods

Tawny crazy ant workers were collected from a total of 21 nests in Texas (see Figure 2.). Nests were located in seven counties (Bastrop, Bexar, Brazoria, Hardin, Hays, Hidalgo, and Travis Counties) to represent much of the known tawny crazy ant distribution in Texas (see Figure 2.). I sampled one collection site in each of the seven chosen counties. At each site, I sampled three spatially separated nests separated by a minimum distance of 30 m. In this study, a nest is defined as a collection point that has an aggregation of workers combined with brood and often the presence of queens and males. Nests were typically located at the base of trees and shrubs, in dense leaf litter, or under piles of scrap wood and/or debris. Workers were collected from each nest by using either hotdog baits, an aspirator, or both. Hotdog baits were primarily used for collecting live worker ants for behavioral studies. This was done to avoid any mortality caused by

aspirating or violently shaking ants into collection containers. A small piece of hotdog was attached to a stake flag and placed into the ground where workers were observed. Once the hotdog was covered in workers, the stake flag was removed, and the workers were shaken off into a Fluon coated medium square Ziploc container. Furthermore, a minimum of 30 workers were aspirated from each nest and placed into 90% ethanol to be used for genetic analysis. In some cases, a nest was excavated and placed into a drip bucket to extract the colony (multiple castes) based on the methods of McDonald (2012). Additionally, I was sent worker ants in 90% ethanol from Georgia (17), Louisiana (20), and Mississippi (20) from collaborators in each state. I also received extracted DNA from 59 individuals from various sites in Florida, sent by the Shoemaker lab. Out of state sample results are located in Chapter Three of this thesis. All worker ants collected throughout the southeastern United States were genotyped and analyzed to test the microsatellite markers used in this thesis (see below: basic descriptive statistics). Locality information of each nest was recorded at the time of collection and is shown in Table 1 and Figures 2 and 3.

Table 1. Location of nests of *N. fulva* sampled in Texas.						
Nest Name:	Locality:	Longitude:	Latitude:	Date Collected:	Workers:	Queens:
SM01	USA: TX: Bastrop County: Smithville	30.043671	-97.162376	09.VIII.2016	18	
SM02		30.043972	-97.161941		20	
SM03		30.044522	-97.161156		20	
SA01	USA: TX: Bexar County: San Antonio	29.439968	-98.643049	09.VIII.2016	19	10
SA02		29.440150	-98.642901		19	1
SA03		29.440452	-98.642813		19	
IC01	USA: TX: Brazoria County: Iowa Colony	29.435462	-95.435458	08.VI.2016	20	
IC02		29.435525	-95.433013		20	
IC03		29.435567	-95.431278		20	
SI01	USA: TX: Hardin County: Silsbee	30.353172	-94.125907	09.VIII.2016	20	
SI02		30.353716	-94.126499		20	
SI03		30.354355	-94.126801		20	
BU01	USA: TX: Hays County: Buda	30.076033	-97.845120	09.VIII.2016	20	
BU02		30.075818	-97.845145		20	1
BU03		30.075772	-97.845519		20	
WE01	USA: TX: Hidalgo County: Weslaco	26.126063	-97.957693	19.XI.2016	20	9
WE02		26.135516	-97.982209		19	
WE03		26.124471	-97.958996		20	1
AU01	USA: TX: Travis County: Austin	30.202873	-97.696219	09.VIII.2016	20	
AU02		30.202562	-97.695691		20	
AU03		30.202017	-97.694992		20	

Figure 2. Locality map of individuals located in Texas. Black/yellow triangle = collection site. Blue dot = nest site. Three nests per collection site = 20 individuals per nest = 60 individuals per site. Map developed by The University of Georgia - Center for Invasive Species and Ecosystem Health as part of the Southern IPM Center with funding provided by USDA NIFA, under Agreement No. 2014-70006-22485 via Southern IPM Center Working Group Program (Project 9894994).

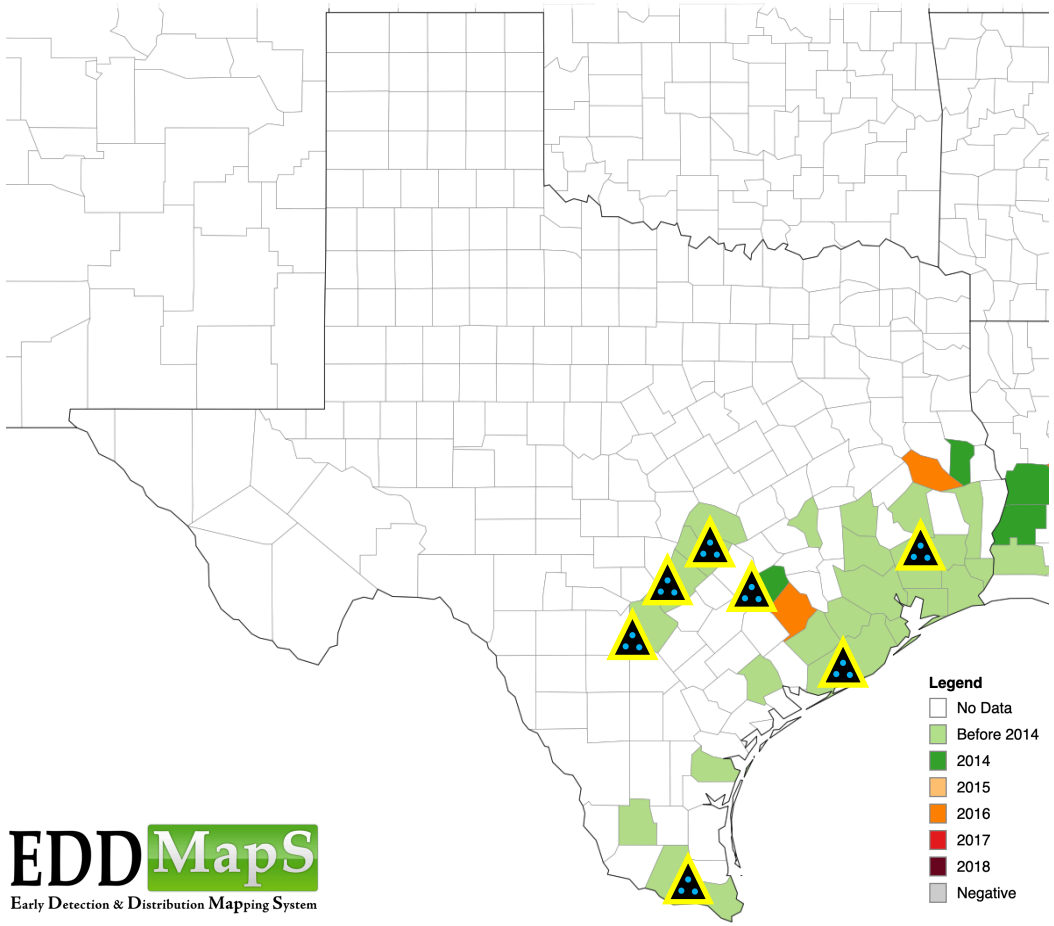
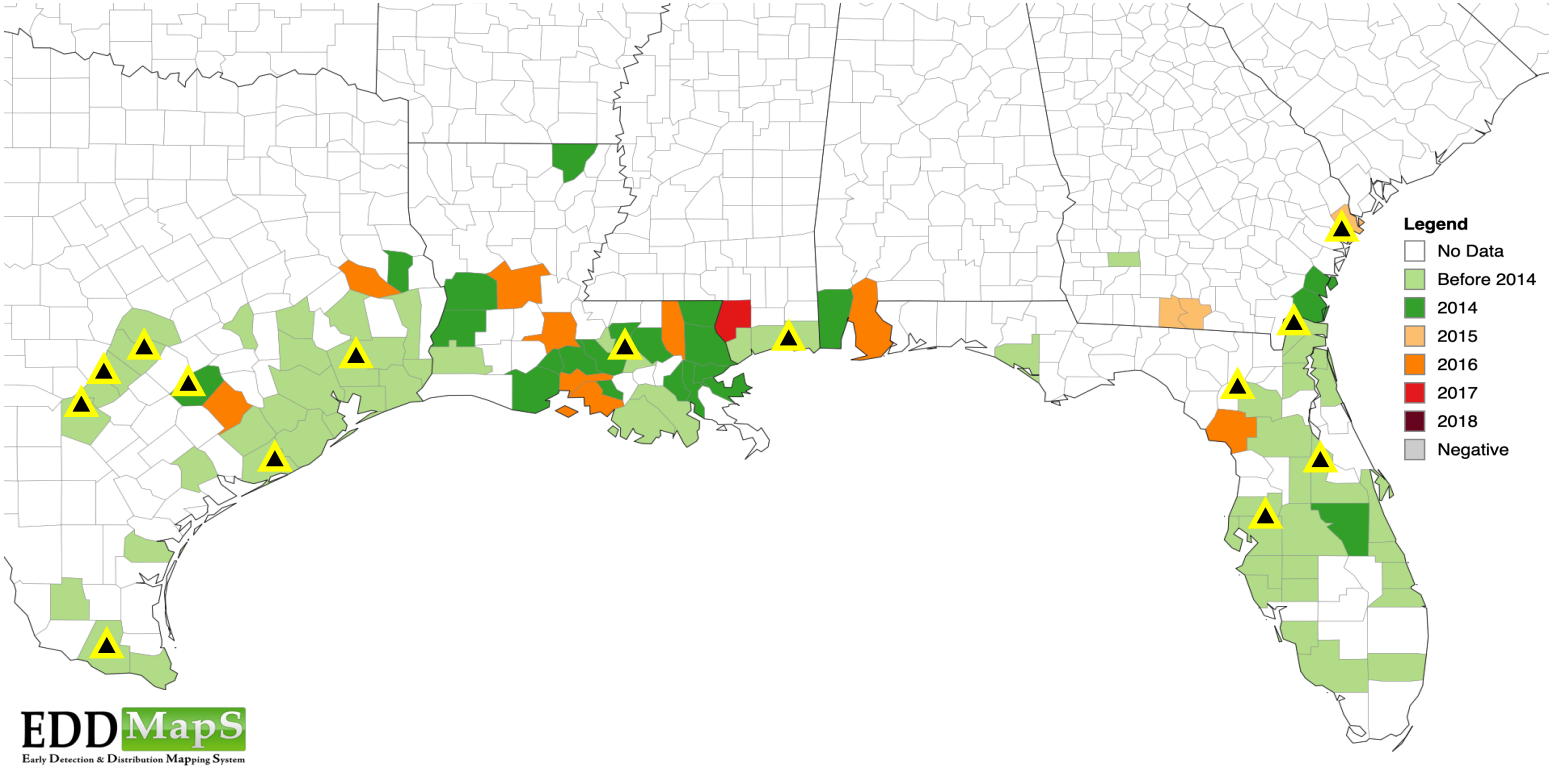


Figure 3. Locality map of individuals collected in the invasive range of the southeastern United States. Black/yellow triangles = one collection site. Map developed by The University of Georgia - Center for Invasive Species and Ecosystem Health as part of the Southern IPM Center with funding provided by USDA NIFA, under Agreement No. 2014-70006-22485 via Southern IPM Center Working Group Program (Project 9894994).



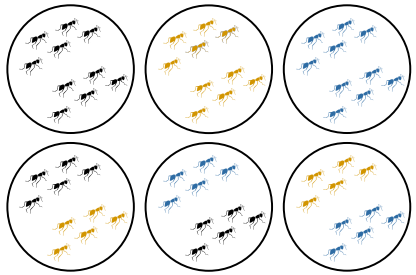
Behavioral interactions

Intraspecific and interspecific aggression analysis. A total of 12 *N. fulva* nests from four sites (Bastrop, Bexar, Hays, and Travis Counties) were used for behavioral experiments. After collection, specimens were brought to the Rollins Urban and Structural Entomology Facility at Texas A&M University, located in College Station, Texas. Behavioral studies were performed the day after the collection event took place to allow for acclimation. Two behavioral bioassays were performed to observe the intraspecific aggressive behavior of tawny crazy ants at two spatial scales: 1) among nests within a site, 2) among nests from different sites. In addition, I tested for 3) interspecific aggression between tawny crazy ants and fire ants. At the smallest spatial scale tested, among nests within a site, five workers from each of the three spatially separated nests (>30 m apart) within a site were paired against each other. At the largest spatial scale, among nests located in different sites, five workers from one site were paired against five workers from another site. In each case nestmate workers were chosen from a single nest at each site and individuals were only used in experiments once. I used a single nest per site because I did not find any aggressive interactions between workers from different nests within a site (i.e., at the smaller spatial scale; see Results). For the interspecific aggression, five *N. fulva* workers collected from one randomly chosen nest within a site were paired against five red imported fire ant workers (*S. invicta*). The interspecific aggression tests were included in this study to ensure that the tawny crazy ants could exhibit aggressive behavior. Each of these experiments was replicated three times. Aggression levels were observed and scored using a modified protocol adapted from Suarez et al. (1999). All behavioral bioassays were scored using a

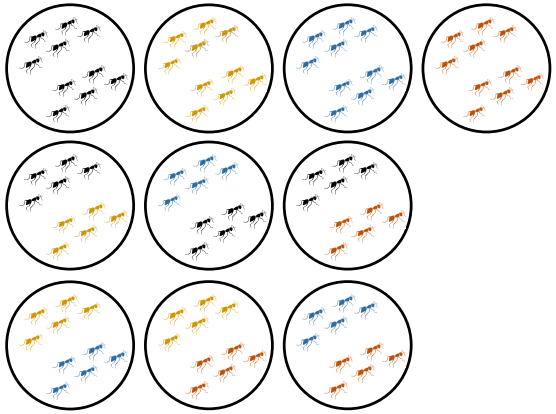
1-5 aggression scale: 1 = no interactions, ants are still or huddled together; 2 = 3 s or more of antennation, allogrooming, or trophallaxis; 3 = biting and quickly releasing; 4 = prolonged biting (>3 s); and 5 = balling, fighting/dragging, or spraying formic acid. It is important to mention that in the interspecific encounters the aggression score was based on the behavior exhibited by tawny crazy ants and not fire ants. Score values of 1 and 2 were interpreted to be non-aggressive behaviors, while scores of 3, 4 and 5, were taken to represent low, medium, and high aggression, respectively. Figure 4 shows the experimental set up. Ants were introduced into a Fluon coated Petri dish with a sterile cotton swab and allowed to acclimate for 2 min. At 2 min, the first score was recorded for each pair. Aggression scores were then recorded every minute for 5 min. The highest value observed by any ant at any observation period was the value assigned for that replicate. If there was a group of ants in the Petri dish that showed zero interaction, while others were biting/dragging one another, the more aggressive behavior was recorded.

Figure 4. Experimental set-up of each aggression assay. Control experiments are uniformly colored (black, yellow, and blue) and treatments are mixed colors. A) Within site comparisons. Three separated nests in one site. B) Among site comparisons. Each color represents *N. fulva* from one site. C) Interspecific aggression of *N. fulva* in the presence of red imported fire ants (green).

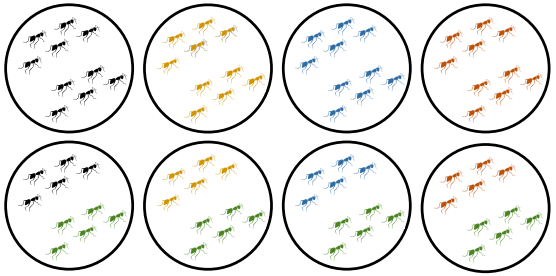
A)



B)



C)



Statistical methods for intraspecific and interspecific aggression. For all experiments, aggression scores were analyzed using an ANOVA. For Experiment 1, I aimed to determine if there was aggression between ants from different nests within a single infested site. Within each of the four sites in Texas, I tested all pairwise interactions between nests (nest 1/nest 2, nest 1/nest 3, nest 2/nest 3). For controls I tested groups of 10 nestmate workers (nest 1/nest 1, nest 2/nest 2, nest 3/nest 3). For example, the control group used for interactions between nest 1/nest 2 (located in the same site) were the combined results of nest 1/nest 1 and nest 2/nest 2. In Experiment 2, I sought to determine if ants from the different study sites would exhibit aggression toward one another. I performed all pairwise interactions and compared these to controls comprised of ants from the same nest as in Experiment 1. For example, the control group used for interactions between (site 1-nest 2) and (site 3-nest 1) were the combined results of nest 2/nest 2 from site 1 and nest 1/nest 1 from site 3. Lastly, in Experiment 3, I determined the level of aggression exhibited by *N. fulva* workers toward *S. invicta* workers. Because no significant aggression was observed between any tawny crazy ant nests located within a site, controls consisted of the combined data from each of the among nest pairwise aggression tests. For example, the controls used for the interaction between (5) red imported fire ants and (5) tawny crazy ants collected from site 1 was the combined data collected from the interactions of nest 1/nest 1 + nest 2/nest 2 + nest 3/nest 3 within site 1.

Genetic analysis

Microsatellite primer design. Thirteen microsatellite primers for *N. fulva* were designed by the Shoemaker laboratory, USDA ARS, Gainesville, FL (Eyer et al. in press). Based on the transcriptome study by Valles et al. (2012), the *Nylanderia pubens* fasta files were obtained from GenBank. Formerly known as *Paratrechina pubens* (Forel) and then *Nylanderia pubens*, this species, previously identified as the Caribbean crazy ant, has been shown to be the same species as the tawny crazy ant in Texas (Zhao et al. 2012) based on molecular data. The fasta files were run in MSATCOMMANDER (Faircloth 2008) to obtain sequences with tri- and tetranucleotide repeats and to design primers. Additionally, a pig-tail sequence GCTTCT was added to the 5' end of each reverse primer to aid in scoring consistency (Ballard et al. 2002). Table 2 shows the list of primers developed and used in this study.

Table 2. Primer sequences and characteristics of thirteen microsatellite loci. (*)Locus = not used in genetic analysis, (N) = number of individual workers successfully genotyped, (N_a) = number of alleles, (H_O) = Observed heterozygosity, (H_E) = Expected heterozygosity.

Locus:	Primer name:	Dye:	Repeat:	Size: (bp)	Injection:	N:	N_a:	H_O	H_E	Primers: (5'-3')	GenBank accession number:
**Nf_L02*	Nf_L_02-F_PET	PET	ACG	172	1	n/a	2	n/a	n/a	F: CGTAATCGCGACTAGGTTAGAG	JP777248
	Nf_L_02-R									R: GCTTCTCAACTGTCATTGATGTGCCAAG	
Nf_L04	Nf_L_04-F_NED	NED	ACT	282	1	480	6	0.64	0.70	F: GATGTGAGATCAAAGGTCGGAG	JP779555
	Nf_L_04-R									R: GCTTCTCTATTACCACTCGATCGTCACG	
**Nf_L06*	Nf_L_06-F_FAM	FAM	ACG	100	1	n/a	3	n/a	n/a	F: CCTATACTCCTATCCTCCCATCG	JP808791
	Nf_L_06-R									R: GCTTCTTGAAGTAGCAGCTAGAGGAGG	
**Nf_L12*	Nf_L_12-F_FAM	FAM	ATC	310	1	n/a	1	n/a	n/a	F: TCTCTCAAAGCATCCTCAGAAC	JP809404
	Nf_L_12-R									R: GCTTCTCCAGGTGATAGATGAGCATGC	
Nf_L14	Nf_L_14-F_FAM	FAM	ATC	205	1	521	7	0.74	0.77	F: GCTGGTGTGTATCGATCCCTC	JP791639
	Nf_L_14-R									R: GCTTCTATAACTGGATTCTCTTGTCGGC	
Nf_L16	Nf_L_16-F_VIC	VIC	ACG	279	1	498	6	0.78	0.76	F: GTGAATCCTCGATACTTGGCTG	JP788023
	Nf_L_16-R									R: GCTTCTGAGGAAGAGGTCGAAGGAGTC	
Nf_L17	Nf_L_17-F_VIC	VIC	ACG	177	1	519	4	0.58	0.61	F: GAAGTGGATGGAACGAGGAATC	JP815225
	Nf_L_17-R									R: GCTTCTCATATATATGTTTGCAAGCGAGC	
Nf_L18	Nf_L_18-F_PET	PET	AGAT	271	1	416	4	0.59	0.66	F: GAGTAGGTACGTGAAAGAGGAC	JP786260
	Nf_L_18-R									R: GCTTCTCGATAAAGCTACACCGTCTCTC	
Nf_L03	Nf_L_03-F_NED	NED	ACG	254	2	499	9	0.81	0.85	F: AAGTTTCCTTAATATCCCGCGG	JP812964
	Nf_L_03-R									R: GCTTCTTATACGGTGCCTTAACGTTGTC	
Nf_L07	Nf_L_07-F_PET	PET	ACAT	240	2	453	3	0.93	0.62	F: TTGACGAATGAGATGAGAAGGC	JP784770
	Nf_L_07-R									R: GCTTCTTAGTGTGGCAGGATAGAAGGAG	
Nf_L08	Nf_L_08-F_FAM	FAM	ACAT	289	2	461	4	0.65	0.68	F: TCTCTCTCTGTTCCGCAAATTC	JP794100
	Nf_L_08-R									R: GCTTCTAGATCGAATTCAATGCACAATC	
Nf_L10	Nf_L_10-F_FAM	FAM	AAGC	218	2	513	6	0.69	0.71	F: GAATACGTCGAGACTTACTGGC	JP790059
	Nf_L_10-R									R: GCTTCTTTTGTCTGTCTGCCTGCTTATC	
Nf_L13	Nf_L_13-F_VIC	VIC	ACGC	247	2	512	8	0.79	0.82	F: CCGCAATTACATGGCTTTGAAC	JP815034
	Nf_L_13-R									R: GCTTCTTAGATACAGGACGTTACACAGC	

DNA extraction. Genomic DNA was individually extracted from a total of 414 tawny crazy ant workers and 22 queens in Texas using a modified PureGene extraction protocol and resuspended in 1x TE buffer. All individuals were collected across Texas from seven sites. Each site contained three spatially separated nests from which 20 workers were collected and used for extraction. Information on queen locality can be found in Table 1.

Microsatellite genotyping. The genetic analysis of tawny crazy ants in Texas was performed using the above-mentioned 13 microsatellite primer pairs. Polymerase chain reaction (PCR) amplifications were performed in a 12 μL reaction mixture that contained 2x Taq-Pro COMPLETE $MgCl_2$ Solution (Denville Scientific INC), 5U/μL Platinum® Taq Antibody (Denville Scientific INC), and the specific primers at 10 pM/μL. Five multiplexes were used pre-PCR: M1-1, M1-2, M1-3, M2-1, and M2-2 (Table 3). Microsatellite loci were amplified using a BIO-RAD T100™ Thermal Cycler using a touchdown program. The initial denaturing step was 94 °C (1 min), followed by 10 cycles of 94 °C (30 s), 60 °C (45 s), and 72 °C (1 min), decreasing the annealing temperature ½ °C per cycle, and then 25 cycles at 94 °C (30 s), 55 °C (45 s), and 72 °C (1 min), followed by the final extension step at 72 °C (30 s). After PCR, samples were diluted as shown in Table 3 and multiplexes were combined into one of two injections: *Injection 1:* (M1-1, M1-2, M1-3) and *Injection 2:* (M2-1 and M2-2) (Table 4). Microsatellite loci were analyzed using a 3500 Genetic Analyzer 8ch RUO (Applied Biosystems). Microsatellite scoring and genotyping was performed using Geneious software v9.1.6 (Biomatters Ltd).

Table 3. Multiplexes used for PCR. Polymerase chain reaction (PCR) amplifications were performed in a 12 µL reaction mixture that contained 2x Taq-Pro COMPLETE $MgCl_2$ Solution (Denville Scientific INC), 5U/µL Platinum® Taq Antibody (Denville Scientific INC), and the specific primers at 10 pM/µL.

Multiplex 1-1	Multiplex 1-2	Multiplex 1-3
Nf_L06_F_FAM	Nf_L02_F_PET	Nf_L16_F_VIC
Nf_L06_rp	Nf_L02_rp	Nf_L16_rp
Nf_L12_F_FAM	Nf_L18_F_PET	Nf_L17_F_VIC
Nf_L12_rp	Nf_L18_rp	Nf_L17_rp
Nf_L14_F_FAM	Nf_L04_F_NED	
Nf_L14_rp	Nf_L04_rp	

Multiplex 2-1	Multiplex 2-2
Nf_L10_F_FAM	Nf_L03_F_NED
Nf_L10_rp	Nf_L03_rp
Nf_L08_F_FAM	Nf_L07_F_PET
Nf_L08_rp	Nf_L07_rp
Nf_L13_F_VIC	
Nf_L13_rp	

Table 4. Dilution ratio of multiplexes after PCR used for injections.

Injection 1	Dilution Ratio
Multiplex 1-1	200:1
Multiplex 1-2	200:1
Multiplex 1-3	300:1

Injection 2	Dilution Ratio
Multiplex 2-1	300:1
Multiplex 2-2	150:1

Statistical methods of genetic analysis

Basic descriptive statistics of microsatellite markers. Using all 530 *N. fulva* workers collected in the invasive regions in the southeastern United States (414 samples from Texas and 116 samples from other states), basic descriptive statistics, including the number of alleles and their frequencies as well as the expected and observed heterozygosity were calculated using FSTAT (Goudet 1995, 2001). I also performed exact tests to identify deviations from Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium (LD) using GenePop on the web (Raymond and Rousset 1995, Rousset 2008). Tests were run using all worker samples collected throughout the southeastern United States in order to ensure the selected microsatellite loci were suitable for this study.

Genetic differentiation within and among sites. I estimated multiple measures to determine whether the markers met the basic assumptions of Hardy Weinberg equilibrium. I determined the relatedness coefficients (r) of individual workers within each nest in Texas, as well as the relatedness among queens and between queens and workers found in a given nest using the program Coancestry (Queller and Goodnight 1989, Wang 2010). I tested to see if nests located within a site were genotypically differentiated by calculating log-likelihood G-statistics in the program FSTAT (Goudet 1995, 2001). I also estimated pairwise F_{ST} values between each nest using the program FSTAT (Goudet 1995, 2001). If nests within a site were not found to be statistically differentiated from one another, I then grouped those nests together into one colony (delineated by a single site). This was the case for each site sampled. I estimated F_{IS} values to determine the levels of inbreeding as well as the degree of relatedness (r)

between worker ants in separate nests within a site (average value between three nests in a site). In addition, I determined the proportion of shared alleles between nests located within a site. I also calculated the proportion of shared alleles between the various collection sites for a more precise measure of the genetic similarity between them. Relatedness (*r*) values are calculated by using a reference population and can become misleading when analyzing only data collected in introduced ranges. Giraud et al. (2002) notes that in the case of an invasive population which arose from a single introduction, individuals are genetically homogenous resulting in low relatedness values if such estimates are calculated using only data from the introduced range (relatedness coefficients are effectively zero). In other words, the genetic variation among nestmates is similar to that between colonies throughout the entire reference population (throughout the introduced range) and no genetic differentiation is observed, which, if present, would indicate workers within colonies are more genetically similar to each other than they are to workers from other colonies and therefore, more related. I took this into consideration and therefore used other additional measures to determine the genetic relationships of *N. fulva* in their introduced range. Following the protocol of Giraud et al. (2002), I divided the number of alleles shared by the two populations by the sum of alleles found in both populations. I then determined the genetic structure within a colony and the genetic differentiation among colonies using the same procedures as described above. In this study, I also tested for isolation by distance by correlating F_{ST} and geographic distance among all samples by means of a Mantel test in the program GenePop on the web (Raymond and Rousset 1995, Rousset 2008).

Results

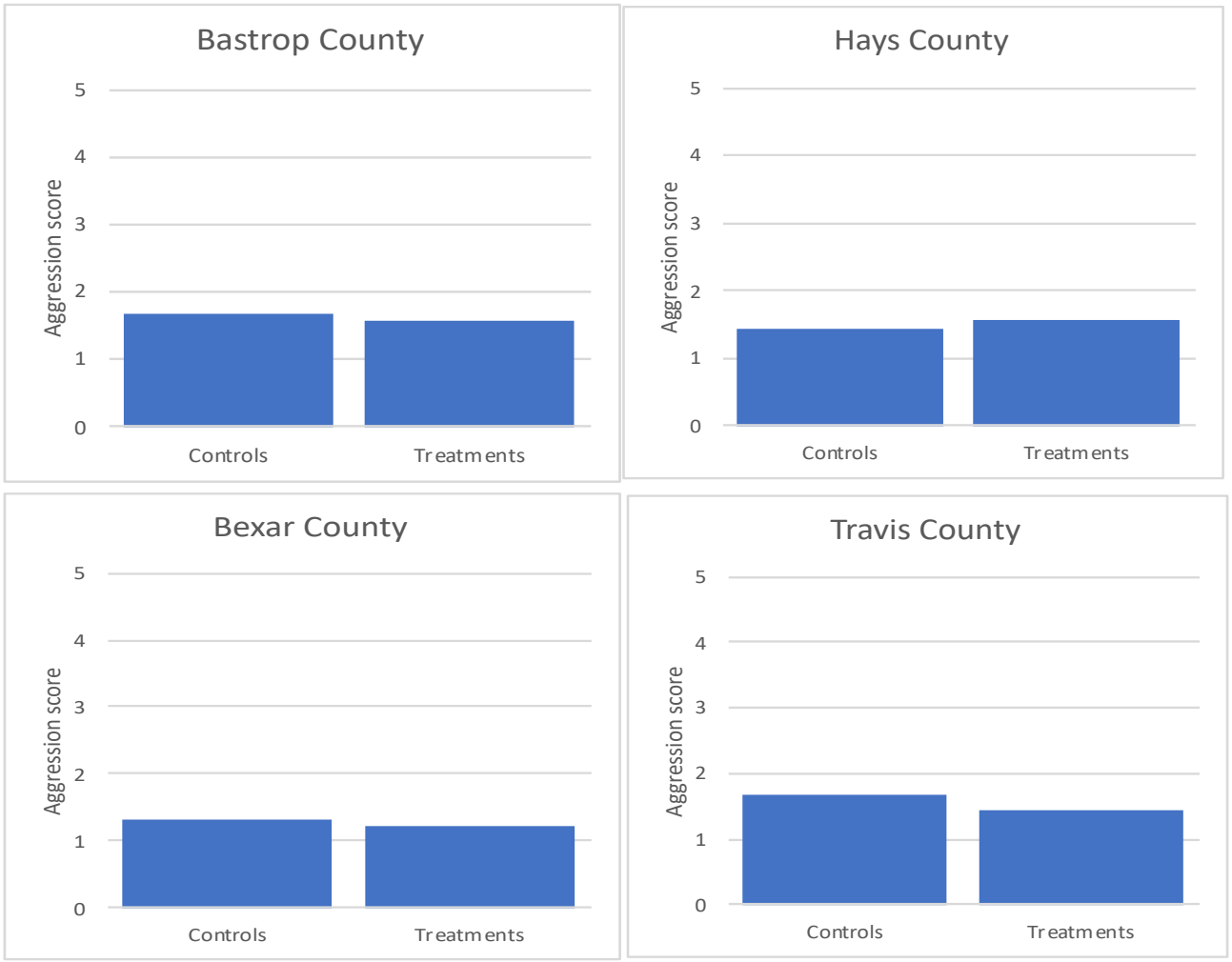
Behavioral interactions

Intraspecific aggression within a site. I found no aggression between any pairs of worker ants collected from separate nests originating from the same site (ANOVA, $P > 0.05$; Table 5 and Figure 5). This was observed in each of the four sites I sampled for this study. All score values assigned were ≤ 2, indicating no sign of aggression between individuals collected at various spatial scales within a site. When paired against each other, workers from separate nests either remained still for the duration of the experiment, or participated in antennation, allogrooming, or trophallaxis for longer than 3 s, but did not exhibit any aggressive behavior beyond this level.

Table 5. Within site ANOVA. I found no aggression between any pairs of worker ants collected from separate nests originating from the same site (ANOVA, $P > 0.05$). All score values assigned were ≤ 2, indicating no sign of aggression between individuals collected at various spatial scales within a site. Results graphed in Figure 5.

Site:	F-statistic:	DF:	p-value:
Bastrop	0.6800	12	>0.05
Bexar	0.8500	12	>0.05
Hays	0.2667	12	>0.05
Travis	0.8000	12	>0.05
Total	1.6510	68	>0.05

Figure 5. Levels of aggression of worker ants collected from different nests within a site: Three nests were tested per site. No aggressive interactions observed. (all $P > 0.05$)

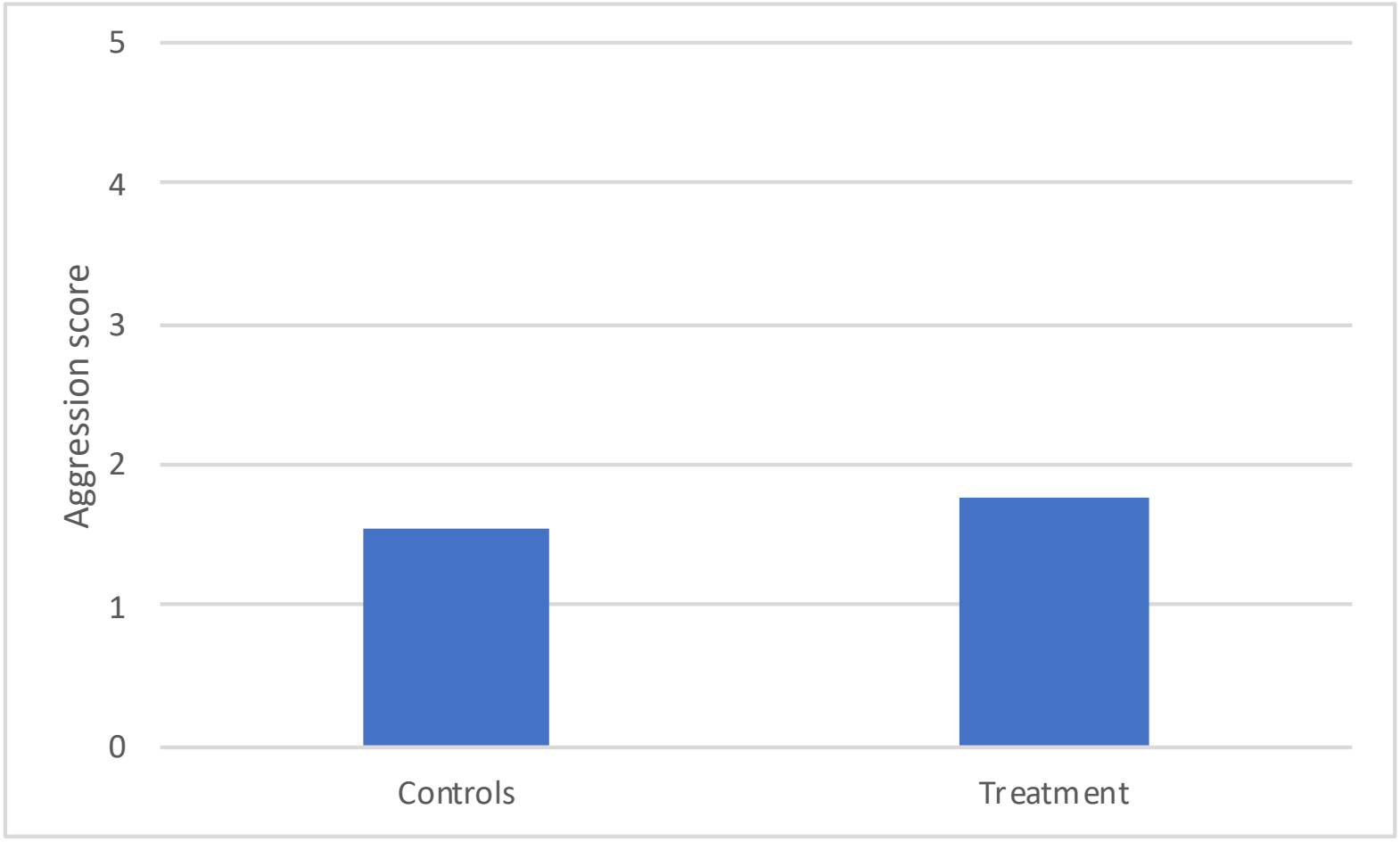


Intraspecific aggression among sites. I found no aggression between any *N. fulva* workers collected from nests between separate sites. There were no significant differences between the average aggression scores of nestmates in a given site when compared to non-nestmates from another site. All score values assigned were ≤ 2, indicating no sign of aggression between individuals collected from nests located in different sites (ANOVA, $F_{9, 44} = 0.7649$, $P > 0.05$; Table 6 and Figure 6).

Table 6. Among site ANOVA. I found no aggression between any *N. fulva* workers collected from nests between separate sites. There were no significant differences between the average aggression scores of nestmates in a given site when compared to non-nestmates from another site. All score values assigned were ≤ 2, indicating no sign of aggression between individuals collected from nests located in different sites (ANOVA, $F_{9, 44} = 0.7649$, $P > 0.05$) Results graphed in Figure 6.

Site:	F-statistic:	DF:	p-value:
BastropxBexar	1.1020	18	>0.05
HaysxBexar	0.6429	18	>0.05
TravisxBexar	1.1020	18	>0.05
BastropxHays	0.1169	18	>0.05
BastropxTravis	0.6429	18	>0.05
HaysxTravis	0.9474	18	>0.05

Figure 6. Levels of aggression of worker ants collected among different sites: No aggressive interactions observed ($P > 0.05$)

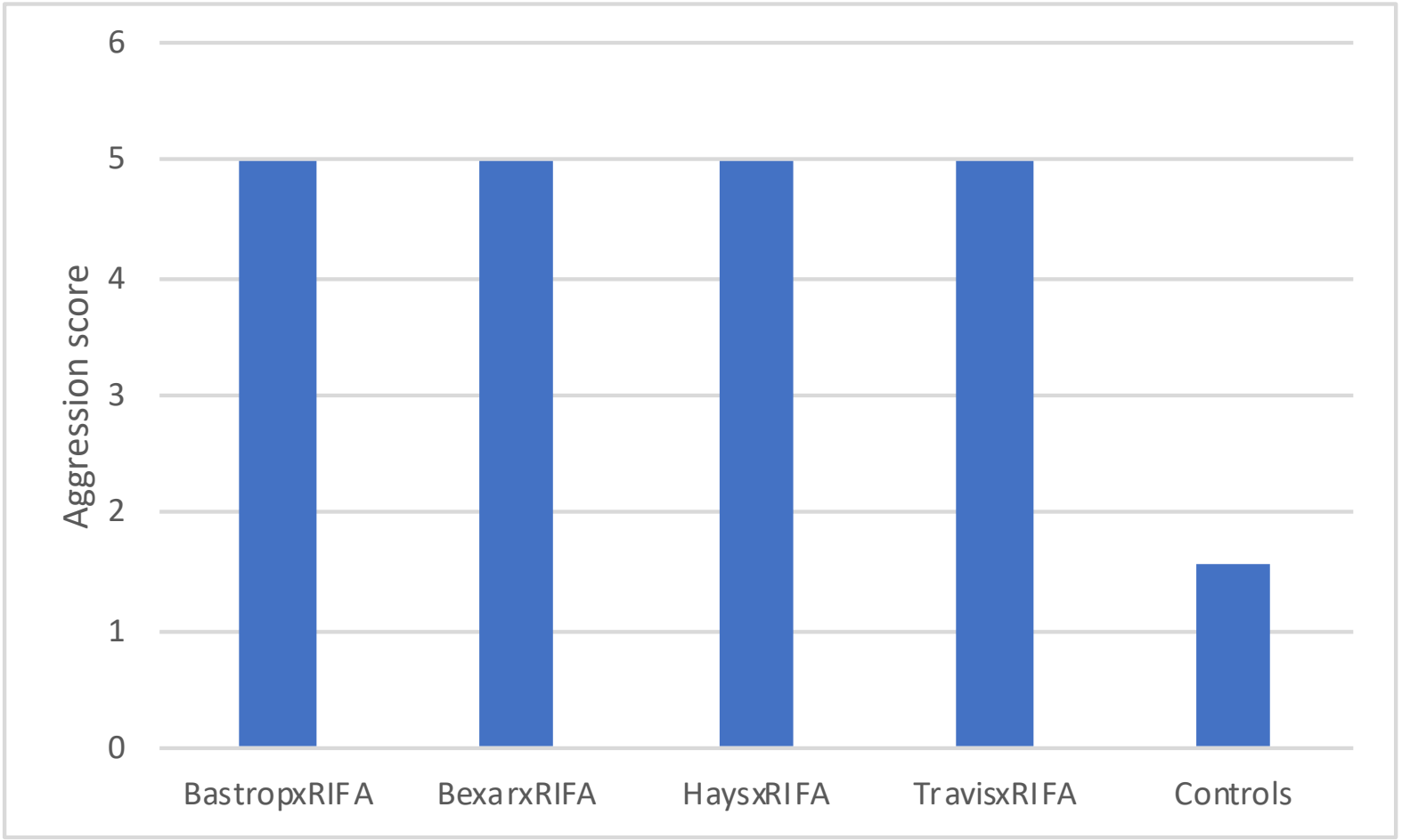


Interspecific aggression of N. fulva *vs.* S. invicta. I found highly aggressive behavior displayed by *N. fulva* workers taken from a given nest and placed in the presence of red imported fire ant workers (ANOVA, $F_{7, 40} = 74.67$, $P < 0.001$; Table 7 and Figure 7). Furthermore, aggressive behavior was observed by *N. fulva* workers collected from each site (total of 4 sites, each site represented by 1 nest) when paired against *S. invicta* workers. Tawny crazy ant workers continually displayed aggressive behavior including both quick and prolonged biting, balling, dragging of *S. invicta* workers, and spraying formic acid.

Table 7. *Nylanderia fulva* vs. *Solenopsis invicta* ANOVA. I found highly aggressive behavior displayed by *N. fulva* workers taken from a given nest and placed in the presence of red imported fire ant workers (ANOVA, $F_{7, 40} = 74.67$, $P < 0.001$). Results graphed in Figure 7.

Site:	F-statistic:	DF:	p-value:
Bastrop vs. RIFA	125.00	10	<0.001
Bexar vs. RIFA	151.30	10	<0.001
Hays vs. RIFA	120.10	10	<0.001
Travis vs. RIFA	125.00	10	<0.001

Figure 7. Levels of aggression between *N. fulva* workers collected in different sites (Bastrop, Bexar, Hays, and Travis) and *S. invicta* (RIFA) workers. Controls consisted of the combined data from each of the "among nest" pairwise aggression tests. All *N. fulva/S. invicta* pairs show aggressive behavior ($P < 0.005$).



Genetic analysis

Basic descriptive statistics of microsatellite markers. Before running my analysis on ants collected exclusively in Texas, I needed to determine the suitability of the 13 microsatellite loci that were developed for this study concerning ants collected throughout the invasive range of the United States. Of the 13 microsatellite loci developed for this study, 12 were polymorphic among all ants collected in the invasive range. *Nf_L12* was monomorphic across all genotyped individuals from the invasive range and was therefore excluded from this study. As seen in Table 2, I found 2 to 9 alleles per locus, with an average of 5.17 alleles per locus over the 12 polymorphic loci. I found evidence of significant linkage disequilibrium (LD, log likelihood-ratio, $P <$ 0.007 with Bonferroni correction) between *Nf_L06* and *Nf_L02* and thus excluded these two loci from this study. Once removed, no evidence of significant linkage disequilibrium was detected (Table 8), leaving 10 microsatellite loci to be used in this study. Basic descriptive statistics can be found in Table 2.

Table 8. Genotypic linkage disequilibrium for each pair of loci using a log likelihood-ratio statistic (significance: $P < 0.001111$, after Bonferroni correction).

Survey population		
Locus pair	*P*-Value	S.E.
Nf_L14xNf_L17	0.32408	0.030038
Nf_L14xNf_L16	0.59093	0.042001
Nf_L17xNf_L16	0.01004	0.003087
Nf_L14xNf_L04	0.78973	0.030069
Nf_L17xNf_L04	0.22569	0.026459
Nf_L16xNf_L04	0.02065	0.009217
Nf_L14xNf_L18	0.9485	0.012158
Nf_L17xNf_L18	0.87151	0.01738
Nf_L16xNf_L18	0.41435	0.030182
Nf_L04xNf_L18	0.85411	0.019531
Nf_L14xNf_L10	0.44688	0.038035
Nf_L17xNf_L10	0.10075	0.017392
Nf_L16xNf_L10	0.48368	0.035513
Nf_L04xNf_L10	0.00522	0.004366
Nf_L18xNf_L10	0.42672	0.030929
Nf_L14xNf_L08	0.4488	0.03057
Nf_L17xNf_L08	0.00315	0.001532
Nf_L16xNf_L08	0.82191	0.020195
Nf_L04xNf_L08	0.12166	0.01708
Nf_L18xNf_L08	0.1639	0.017366
Nf_L10xNf_L08	0.81137	0.023069
Nf_L14xNf_L13	0.45859	0.040972
Nf_L17xNf_L13	0.21574	0.026816
Nf_L16xNf_L13	0.20911	0.028012
Nf_L04xNf_L13	0.08333	0.021502
Nf_L18xNf_L13	0.18389	0.025466
Nf_L10xNf_L13	0.3377	0.038807
Nf_L08xNf_L13	0.93247	0.016617
Nf_L14xNf_L03	0.12069	0.026018
Nf_L17xNf_L03	0.23651	0.031896
Nf_L16xNf_L03	0.29232	0.037117
Nf_L04xNf_L03	0.01479	0.008037
Nf_L18xNf_L03	0.54826	0.036908
Nf_L10xNf_L03	0.40174	0.040525
Nf_L08xNf_L03	0.26863	0.031302

Table 8. (continued) Genotypic linkage disequilibrium for each pair of loci using a log likelihood-ratio statistic (significance: $P < 0.001111$, after Bonferroni correction).

Survey population		
Locus pair	*P*-Value	S.E.
Nf_L13xNf_L03	0.57949	0.042306
Nf_L14xNf_L07	0.13031	0.015701
Nf_L17xNf_L07	0.21904	0.014884
Nf_L16xNf_L07	0.67287	0.019414
Nf_L04xNf_L07	0.41891	0.019923
Nf_L18xNf_L07	0.71491	0.014848
Nf_L10xNf_L07	0.48694	0.022863
Nf_L08xNf_L07	0.14431	0.012593
Nf_L13xNf_L07	0.64114	0.022324
Nf_L03xNf_L07	0.65639	0.026944

Each of the 10 microsatellite loci were tested using 530 worker ants from 14 geographically separated colonies for a population survey. Four-hundred fourteen individuals were collected among seven colonies in Texas, while 116 individuals were submitted by collaborators from Mississippi (17), Georgia (20), Louisiana (20), and Florida (59). Expected heterozygosity for the 10 loci used ranged from 0.61 to 0.85 (Table 2). The observed heterozygosity for each locus was found to be close to the expected heterozygosity in most cases. None of the 10 loci deviated from values expected under Hardy-Weinberg equilibrium. Allele frequencies for each locus from the survey population can be found in Table 9. These results confirm that the 10 microsatellite markers used in this study are suitable, in that they are independent polymorphic loci that are in HWE.

Table 9. Allele frequencies for all genotyped workers.

Locus:	Allele:	Frequency:
Nf_L04	274	0.02
	277	0.05
	280	0.41
	283	0.15
	286	0.33
	289	0.04
Nf_L14	199	0.08
	202	0.29
	205	0.34
	208	0.06
	211	0.09
	214	0.01
	217	0.13
Nf_L16	251	0.18
	257	0.18
	263	0.05
	269	0.22
	272	0.35
	281	0.02
Nf_L17	161	0.05
	164	0.24
	179	0.55
	182	0.16
Nf_L18	255	0.03
	271	0.46
	275	0.24
	279	0.27
Nf_L03	237	0.00
	239	0.04
	242	0.23
	245	0.15
	254	0.19
	257	0.12
	263	0.06
	266	0.09
	274	0.12
Nf_L07	215	0.52
	225	0.26
	244	0.22
Nf_L08	272	0.39
	276	0.20
	284	0.35
	292	0.06
Nf_L10	209	0.10
	213	0.18
	217	0.29
	221	0.40
	224	0.01
	225	0.02
Nf_L13	222	0.06
	230	0.21
	231	0.01
	235	0.01
	239	0.12
	242	0.19
	243	0.20
	254	0.19

Genetic differentiation within and among sites. I found a total of 54 alleles over the 10 loci from the 414 individuals sampled throughout Texas. I tested for population differentiation between each of the three nests located in each site. Genotypic differentiation was not found between any nests located in a given site (Table 10). All pairwise F_{ST} values between nests located in a site were low (-0.0002 to 0.0220) and not significantly different from zero (Table 11). I calculated the relatedness coefficient (r) between individual workers, between queens, and between workers and queens collected in a single nest. Relatedness values between workers in a given nest were zero (-0.0027 – 0.0997; Table 12a). The relatedness value between queens in a given nest (SA01 = 0.0329 and WE01 = -0.0233; Table 12b), and also between worker ants and queens found in the same nest were again not significantly different from zero (-0.0317 – 0.1893; Table 12c). I also determined the proportion of shared alleles between nests located in a site (Table 13). Nests within sites located in Texas showed an average of 89.35% shared alleles between each other.

Table 10. Genotypic differentiation between three nests located in one site over all loci. Adjusted significance $P < 0.0167$ after Bonferroni correction.

Site	Chi2	df	*P* - value
Bastrop County	26.6937	20	0.1441
Bexar County	9.0214	20	0.9827
Brazoria County	20.953	20	0.3999
Hardin County	13.458	20	0.8569
Hays County	19.0615	20	0.5178
Hidalgo County	31.9328	20	0.0440
Travis County	27.7434	20	0.1156

Table 11. Pairwise F_{ST} for each Texas site. Adjusted significance $P < 0.016667$ after Bonferroni correction. (Bastrop County = SM01, SM02, and SM03; Bexar County = SA01, SA02, and SA03; Brazoria County = IC01, IC02, and IC03; Hardin County = SI01, SI02, and SI03; Hays County = BU01, BU02, and BU03; Hidalgo County = WE01, WE02, and WE03; Travis County = AU01, AU02, and AU03)

Bastrop County		
Nest:	SM01	SM02
SM02	-0.0011	
SM03	0.0121	0.0049

Bexar County		
Nest:	SA01	SA02
SA02	-0.0158	
SA03	0.0020	-0.0065

Brazoria County		
Nest:	IC01	IC02
IC02	-0.0012	
IC03	0.0134	0.0058

Hardin County		
Nest:	SI01	SI02
SI02	-0.0044	
SI03	-0.0066	-0.0010

Hays County		
Nest:	BU01	BU02
BU02	-0.0035	
BU03	-0.0002	0.0062

Hidalgo County		
Nest:	WE01	WE02
WE02	0.0220	
WE03	0.0020	0.0106

Travis County		
Nest:	AU01	AU02
AU02	0.0058	
AU03	0.0019	0.0075

Table 12a. Average coefficient of relatedness between workers located in a single nest.

Bastrop County		
Nest	r	variance
SM01	0.0552	0.0477
SM02	0.0181	0.0441
SM03	0.0309	0.0428

Bexar County		
Nest	r	variance
SA01	0.05565	0.04392
SA02	0.07574	0.03791
SA03	0.11673	0.06213

Brazoria County		
Nest	r	variance
IC01	-0.0027	0.0514
IC02	0.0234	0.0441
IC03	0.0220	0.0358

Hardin County		
Nest	r	variance
SI01	0.0258	0.0420
SI02	0.0013	0.0403
SI03	0.0439	0.0405

Hays County		
Nest	r	variance
BU01	0.0694	0.0533
BU02	0.0754	0.0468
BU03	0.0349	0.0395

Hidalgo County		
Nest	r	variance
WE01	0.0790	0.0584
WE02	0.0215	0.0459
WE03	0.0094	0.0436

Travis County		
Nest	r	variance
AU01	0.0003	0.0373
AU02	0.0997	0.0504
AU03	-0.0152	0.0436

Table 12b. Average coefficient of relatedness between queens located in a single nest.

Bexar County		
Nest	r	variance
SA01	0.0329	0.0538

Hidalgo County		
Nest	r	variance
WE01	-0.0233	0.0630

Table 12c. Average coefficient of relatedness between workers and queens located in a single nest.

Bexar County		
Nest	r	variance
SA01	0.0213	0.0400
SA02	0.0492	0.0413

Hays County		
Nest	r	variance
BU02	0.1893	0.0545

Hidalgo County		
Nest	r	variance
WE01	0.0020	0.0485
WE03	-0.0317	0.0439

Table 13. Percent of shared alleles between nests located within Texas sites.

Population Pair			**% Shared Alleles**
Bastrop County			
SM01	&	SM02	84.62
SM01	&	SM03	87.04
SM02	&	SM03	86.54
Bexar County			
SA01	&	SA02	93.75
SA01	&	SA03	89.58
SA02	&	SA03	91.49
Brazoria County			
IC01	&	IC02	92.15
IC01	&	IC03	88.24
IC02	&	IC03	88.24
Hardin County			
SI01	&	SI02	86.54
SI01	&	SI03	91.67
SI02	&	SI03	86.27
Hays County			
BU01	&	BU02	95.92
BU01	&	BU03	93.88
BU02	&	BU03	94.00
Hidalgo County			
WE01	&	WE02	93.88
WE01	&	WE03	90.20
WE02	&	WE03	88.24
Travis County			
AU01	&	AU02	84.31
AU01	&	AU03	90.20
AU02	&	AU03	79.63
		Average:	89.35

Once I determined that nests within a site were not genetically differentiated, and that they did not show aggression towards each other, I grouped nests within a site together and treated each site as a single colony (number of workers = ~ 60). I estimated the average F_{IS} value for each colony in Texas; these ranged from 0.0800 – 0.0750 (Table 14). Additionally, I found the average relatedness values between nests in a site to be near zero (r = -0.016 to 0.033; Table 15). Again, I determined the proportion of shared alleles, this time between each colony located in Texas. I found the average proportion of shared alleles between colonies in Texas was 93.9%, ranging between 88.9% to 100% (Table 16). I then calculated pairwise F_{ST} values between all colonies and found a low degree of genetic differentiation which ranged from -0.0016 to 0.0330 with few pairs being significant (Table 17). Furthermore, I estimated the average F_{ST} (0.015, se = 0.003) and F_{IS} (-0.020, se = 0.049) values among the entire Texas population. I also found no evidence of isolation by distance in Texas (Mantel test, P = 0.1670; Figure 8).

Table 14. F_{IS} values for each colony over all loci. Adjusted significance: $P < 0.00071$.

Bastrop County	-0.0280	$P > 0.05$
Bexar County	-0.0770	$P > 0.05$
Brazoria County	0.0750	$P > 0.05$
Hardin County	-0.0270	$P > 0.05$
Hays County	-0.0800	$P > 0.05$
Hidalgo County	0.0180	$P > 0.05$
Travis County	-0.0260	$P > 0.05$

Table 15. Coefficient of relatedness among workers in three nests located within a site. (Jackknifing over all loci)

Site:	Relatedness:	95% CI
Bastrop	0.013	-0.019 - 0.055
Bexar	-0.015	-0.030 - -0.000
Brazoria	0.013	-0.010 - 0.041
Hardin	-0.009	-0.023 - 0.006
Hays	0.003	-0.015 - 0.024
Hidalgo	0.019	-0.002 - 0.037
Travis	0.01	-0.005 - 0.023

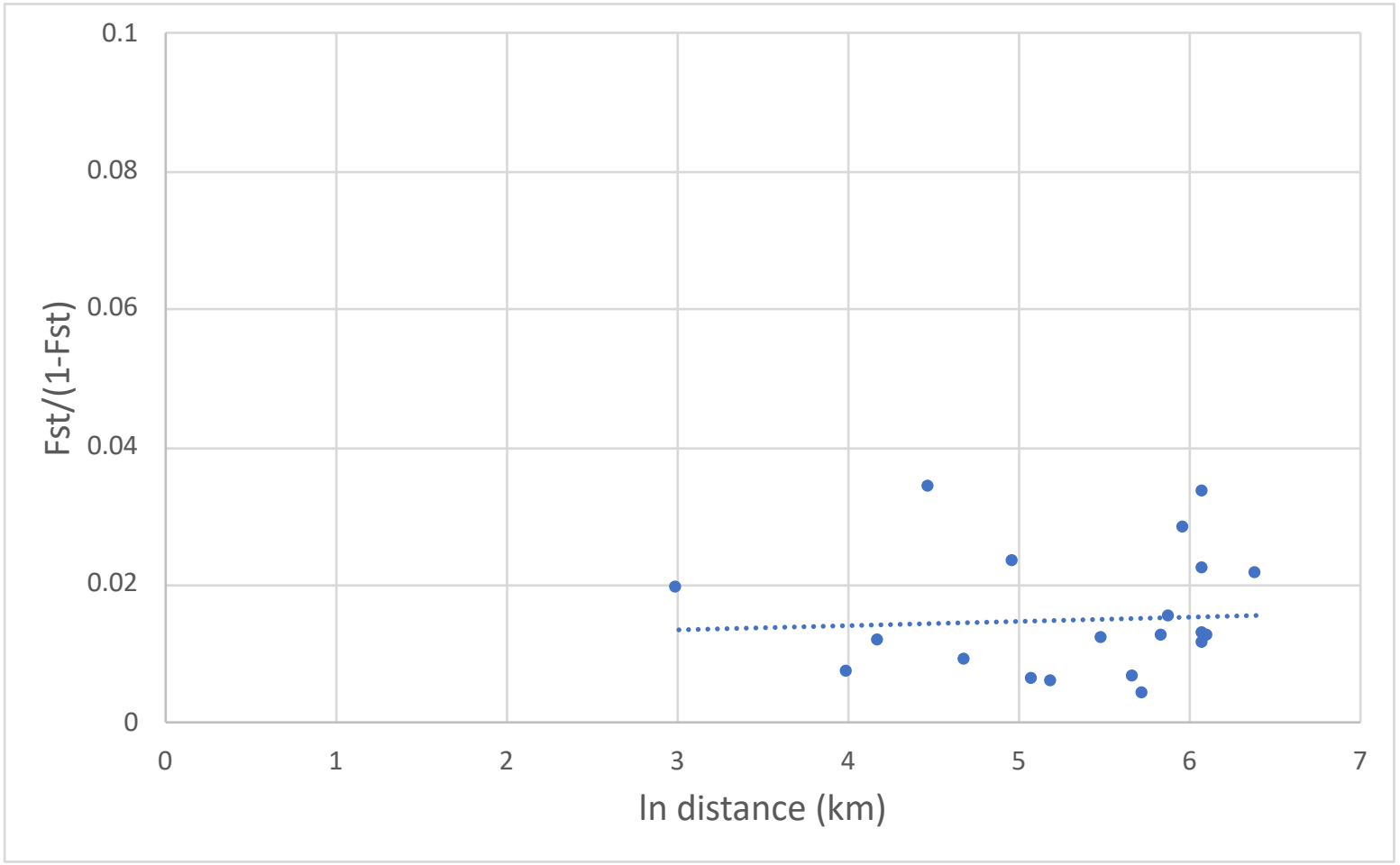
Table 16. Percent of shared alleles between sites located in Texas.

Population Pair			% Shared Alleles
Brazoria Co.	&	Travis Co.	96.30
Brazoria Co.	&	Bastrop Co.	96.30
Brazoria Co.	&	Bexar Co.	94.23
Brazoria Co.	&	Hardin Co.	96.23
Brazoria Co.	&	Hays Co.	92.45
Brazoria Co.	&	Hidalgo Co.	92.59
Travis Co.	&	Bastrop Co.	100.00
Travis Co.	&	Bexar Co.	90.74
Travis Co.	&	Hardin Co.	96.30
Travis Co.	&	Hays Co.	92.59
Travis Co.	&	Hidalgo Co.	96.30
Bastrop Co.	&	Bexar Co.	90.74
Bastrop Co.	&	Hardin Co.	96.30
Bastrop Co.	&	Hays Co.	92.59
Bastrop Co.	&	Hidalgo Co.	96.30
Bexar Co.	&	Hardin Co.	90.57
Bexar Co.	&	Hays Co.	94.12
Bexar Co.	&	Hidalgo Co.	90.57
Hardin Co.	&	Hays Co.	88.89
Hardin Co.	&	Hidalgo Co.	96.23
Hays Co.	&	Hidalgo Co.	92.45
		Average:	93.94

Table 17. Pairwise F_{ST}'s across all Texas sites and all loci. Adjusted significance $P < 0.002381$ after Bonferroni correction.

	Brazoria Co.	Travis Co.	Hays Co.	Bexar Co.	Hardin Co.	Bastrop Co.
Travis Co.	-0.0016					
Hays Co.	0.0123	0.0192				
Bexar Co.	0.0044	0.0092	0.0330			
Hardin Co.	0.0064	0.0126	0.0154	0.0326		
Bastrop Co.	0.0062	0.0075	0.0118	0.0230	0.0067	
Hidalgo Co.	0.0129	0.0126	0.0219	0.0274	0.0212	0.0116

Figure 8. Isolation by distance (IBD). Pairwise genetic differentiation ($F_{ST}/(1-F_{ST})$) plotted against the log transformation of geographic distances between sites in Texas. Mantel test, $r = 0.058$, $P = 0.26590$; y = 0.0006x + 0.0115, $R^2 = 0.0034$



Discussion

The research conducted for Chapter 2 of this thesis aimed to determine if the tawny crazy ant, *N. fulva*, is unicolonial throughout the invasive range in Texas. To investigate this hypothesis, I studied sites throughout Texas, combining aggression assays and genetic data from 10 microsatellite markers. I found a lack of aggressive behavior among nests within a site and between sites. I also found a lack of genetic differentiation within and among sites with a high proportion of shared alleles among sites. These results indicate that tawny crazy ants are unicolonial throughout the introduced range of Texas, likely belonging to one genetically distinct supercolony.

Tawny crazy ants exhibit characteristics commonly associated with unicolonial species (Horn et al. 2013), such as extremely high densities of ants in infested areas and high levels of polygyny (Zenner de Polania 1990, Wang et al. 2016). These results are the first to conclusively show ants from spatially separated but interconnected nests of *N. fulva* are non-aggressive toward each other and are genetically homogeneous. Furthermore, my data are the first to demonstrate the lack of aggression between *N. fulva* workers collected between geographically separated non-connected nests in Texas (maximum distance of 600 km) and that colonies sampled throughout Texas are also genetically homogeneous.

Genetic differentiation between populations coupled with observed intraspecific aggression between them indicates the presence of more than one supercolony existing in the introduced range (i.e., large-scale colony boundaries delineate the size and number of supercolonies). This can be observed in introduced populations of the Argentine ant in California, United States, where at least five supercolonies have been identified

(although one is notably larger and dominates most of the invaded area; (Tsutsui et al. 2003, Thomas et al. 2006). Invasive unicolonial ant species are genetically similar and lack intraspecific aggression between spatially separated sites within the supercolony. This results in the absence of colony boundaries among populations within the supercolony. With a lack of such boundaries, unicolonial population densities flourish and overwhelm the native habitat. It is hypothesized that territorial defense against conspecifics is costly, and by eliminating such behaviors, unicolonial ant species are able to allocate more time and energy into foraging efforts and the reproduction of the colony (Holway et al. 1998). The lack of aggression among tawny crazy ants, even between different colonies, is not due to this species being "generally non-aggressive." In their native habitats, tawny crazy ants have been reported being an ecologically non dominant species when compared to other ants such as *L. humile* and *Solenopsis richteri*, which are also invasive outside South America (Calcaterra et al. 2016).

Another study, however, concluded that when competing against *S. invicta* in the Pantanal, where both species are native, *N. fulva* dominated those interactions which suggested the use of a "specialized attack behavior" by *N. fulva* (Feener Jr. et al. 2008). This "specialized attack behavior" was concluded to be a detoxification interaction exhibited by *N. fulva* workers in the presence of *S. invicta* and was hypothesized to have evolved in the shared native range of South America (LeBrun et al. 2014). LeBrun et al. (2014) performed bioassays which observed the number of times *N. fulva* workers engaged in the detoxification behavior described above. This was done by observing tawny crazy ant workers for two minutes after being exposed to one of seven different ant species, including *S. invicta.* LeBrun et al. (2014) recorded the behavior to occur 6.7

times more frequently after interactions with fire ant species than the average response of non-fire ant species. I observed such contacts in the interspecific aggression bioassay as *N. fulva* showed high levels of aggression against the competitor species *S. invicta*. Interestingly, Zhang et al. (2015) found that tawny crazy ants utilize a synergistic attack behavior via volatiles released by the Dufour's gland in combination with their venom secretions which allows *N. fulva* workers to outcompete native arthropods by quickly attracting nearby workers (Zhang et al. 2015). Observations of introduced populations of *N. fulva* have led many scientists to report their ability to displace *S. invicta* populations in Texas (Meyers and Gold 2008, McDonald 2012), in some cases, even eliminating populations of *S. invicta* from areas previously infested by them (LeBrun et al. 2013).

Horn et al. (2013) found that tawny crazy ants fed on a low sugar diet were significantly more aggressive toward fire ant workers. Additionally, no effect was found of fire ant worker size on aggression score, or on the number of *N. fulva* workers engaging in aggressive behaviors, although, the number of small fire ant workers engaging in aggressive behavior towards *N. fulva* were 51.6% more than large workers (Horn et al. 2013).

These results are comparable with findings in the literature concerning the interactions between *N. fulva* and *S. invicta*. While collecting ants for this project, I observed *N. fulva* workers dominating hotdog baits. During the aggression assays, I found *N. fulva* workers would quickly react to the presence of *S. invicta* workers (aggression often observed during two-minute acclimation time). The detoxification interaction mentioned above could be seen during the experiments as *N. fulva* workers would curl their abdomens under their bodies and secrete formic acid which was used to

denature the venom of *S. invicta* workers. Tawny crazy ant workers would also bite and drag *S. invicta* workers during the experiments.

This study only includes genetic data obtained from individuals collected in the introduced range. Eyer et al. (in press) reports the population genetics and colony breeding structure of native tawny crazy ants from South America, as well as an analysis of introduced (southeastern United States) ants. STRUCTURE analyses performed by Eyer et al. (in press) revealed 13 genetic groups (optimal k = 13) throughout the native range and uncovered a lack of genetic structure among introduced ants in the United States, which clustered together into a single group (k = 1). These authors showed a 60% decrease in the number of alleles between native (153 alleles) and introduced (61 alleles) populations of tawny crazy ants. Eyer et al. (in press) also found significant population structure and significant population differentiation among nests in the native range of *N. fulva* and a positive relationship between pairwise F_{ST} and geographic distance among native populations of tawny crazy ants, which suggests limited gene flow among those populations. This indicates that native populations of *N. fulva* are at equilibrium under a stepping-stone model of population structure in their native range. A stepping-stone model of population structure is used to describe the level of migration, or movement of individuals between sub-populations. If the movement of individuals is restricted to neighboring populations, this forms a stepping-stone pattern.

This is a consistent observation in studies concerning other invasive ants. For example, Tsutsui and Case (2001) report *L. humile* to have a decrease in alleles (about 54%) between ants collected in the native range (63 alleles) compared to those collected in the introduced range of California (29 alleles). With the exception of two alleles,

which were noted to occur at very low frequencies, all other alleles found in the introduced range were also found in the native range. Tsutsui and Case (2001) also found a high degree of population differentiation among native nests, in addition to significant positive relationships between F_{ST} and geographic distance at both the nest and colony level of native *L. humile* workers.

In another example, Fournier et al. (2008) found introduced populations of *Pheidole megacephala* in Australia to have a decrease in the number of alleles per locus than compared to native workers collected in Africa. This was further explored by Fournier et al. (2009) who concluded that four invasive populations of *P. megacephala* belonged to the same supercolony. As for native populations of *P. megacephala*, Fournier et al. (2012) uncovered a unicolonial social structure within eight native African supercolonies. Fournier et al. (2012) note the size of these native supercolonies to be much smaller than those of invasive unicolonial populations found in Australia, and that there is significant population structure and genetic differentiation between these native populations.

I report that individuals collected from spatially separated nests within a site show no evidence of inbreeding or genetic differentiation from one another. I observed high percentages of shared alleles between nests in a site (89.4%) and between sites within Texas (93.9%), which are consistent with findings in other unicolonial ant populations (Suarez et al. 1999, Tsutsui and Case 2001, Corin et al. 2007). Notably, Tsutsui et al. (2000) found that invasive Argentine ants in the introduced range (United States, California) who shared an average of about 75% or more of their alleles between nest pairs did not exhibit aggression towards each other. Tsutsui et al. (2000) found low

intraspecific aggression between nest pairs sampled in the native range, as well as nest pairs sampled in the introduced range, which both had high percentages of shared alleles between them. This is comparable to my findings of tawny crazy ants and suggests the low intraspecific aggression in unicolonial ants may be explained by a decrease in genetic variability, especially in introduced populations that suffer genetic bottlenecks. However, there are five known supercolonies of Argentine ants throughout California that are delineated by their aggressive behavior toward conspecifics along colony boundaries as well as genetic similarities within each population (Thomas et al. 2006). This is not what I observe in tawny crazy ants in Texas as the results suggest that ants belong to a single non-aggressive, genetically homogeneous supercolony.

I found that colonies distributed throughout Texas do not differ greatly in the composition of alleles present in each colony. This genetic homogeneity found in the introduced range is likely explained by the widespread human-mediated dispersal pattern found in many unicolonial species. High population densities in both rural and urban landscapes increase the possibility of the unintentional transfer of sub-populations of ants (queens, workers, males, and/or brood), which may be hidden in mulch or soil, wood debris, and even motor vehicles. Based on the findings of a lack of genetic differentiation coupled with a homogenization of alleles across the entire introduced range, it is highly likely that large groups of ants are transferred over great distances via human mediated dispersal, where they can successfully reproduce and invade new areas quickly. Laboratory studies of the Argentine ant have shown that as few as 10 workers with one queen can survive, successfully produce brood, and effectively increase their population size (Hee et al. 2000).

Arcila et al. (2002) shows that small propagules of *N. fulva* can establish and survive, but based on my findings, this does not seem to be how this species is spreading in Texas, as we would see greater genetic differentiation among locations in the introduced range if that were the case. Tawny crazy ant colonies observed throughout Texas had a high number of queens and males, in addition to brood being present and dense worker populations which covered the landscape. The free mixing of workers over large spatial areas and close proximity of mature nesting sites suggests that new colonies are produced via budding. Queens have not been observed in mating flights, and it is hypothesized that they mate within the nest and soon drop their wings, whereas male tawny crazy ants have been observed in short distance mating flights, and are most likely attracted to pheromones released by queens (Wang et al. 2016). I showed that there is a lack of aggression, even at the largest spatial scale, between workers collected throughout Texas, combined with a lack of genetic diversity among those individuals. These findings provide evidence of colony expansion by budding, in addition to the overwhelming numbers of worker ants in a given area which intermingle and, in some cases, do not seem to belong to one specific nest site. Therefore, large sub-populations of ants are most likely transferred over long distances via human mediated jump dispersal, which then have a potential to establish and then rapidly spread in the new location (via nest budding). Human mediated jump dispersal patterns exacerbate the spread of ants over great distances where they quickly reproduce to dominate the local invaded area.

This is the first study to investigate the behavioral and genetic relationships of tawny crazy ants, both within, and between populations in the introduced range. This study documents the lack of intraspecific aggression between geographically separated

colonies within the invasive range of Texas. I combine this finding with an analysis of genetic data which demonstrates a lack of genetic variability and high percentages of shared alleles between populations of tawny crazy ants throughout Texas. I also found that estimates of relatedness values within and between groups of introduced ants in Texas to be effectively zero. The lack of behavioral aggression and genetic differentiation among these colonies in Texas tells us that a single introduction containing large numbs of unrelated queens likely occurred in the invasive range of Texas, which spread human mediated jump dispersal throughout much of the state.

I conclude that the tawny crazy ant is a unicolonial invader throughout Texas. It continues to spread in Texas where it causes numerous ecological and economic problems. We can expect the problem to get worse until effective control measures are implemented.

CHAPTER III

POPULATION GENETICS AND COLONY BREEDING STRUCTURE OF THE TAWNY CRAZY ANT, *NYLANDERIA FULVA* (MAYR), IN THE SOUTHEASTERN UNITED STATES

Introduction

The tawny crazy ant, *Nylanderia fulva,* has invaded the southeastern United States, possibly as early as the mid 1950s, and they are extremely successful in their introduced range especially throughout the state of Texas. In Chapter two of this thesis, I determined if this species was unicolonial throughout its introduced range in Texas. I reported that the tawny crazy ant does not display aggression toward non-nestmates collected from separate colonies at various spatial scales throughout Texas. Additionally, I found limited genetic variability between individuals and found a high percentage of shared alleles between each of these geographically separated colonies in Texas. This led us to conclude that in Texas there are multiple populations of genetically similar, geographically separated colonies of tawny crazy ants that show no aggression towards each other when paired. I determined *N. fulva* to be a unicolonial species that was most likely the result of a single introduction into Texas. This initial introduced population would have successfully established and via nest budding, spread at a local level. Once this population was large enough, sub-populations of ants would more easily be transported via human mediated jump dispersal throughout the state. The aggressive invasion success of the tawny crazy ant is proposed to be a combination of factors such as the lack of natural enemies in the introduced habitat, no competitive behaviors

between separate colonies of the same species, highly polygynous nesting sites, and a lack of genetic variability among each of these populations. In this chapter, I test the hypothesis that *N. fulva* is unicolonial throughout the entire introduced range of the southeastern United States.

Mentioned above, the tawny crazy ant was most likely introduced into Florida as early as the 1950's (Deyrup et al. 2000, Gotzek et al. 2012). It is uncertain exactly when this species was introduced to the United States and this is largely the result of inconsistencies and misidentifications concerning what species is presently invading. *Nylanderia* (formerly *Paratrechina*) *pubens* was first reported in Florida in 1953. Despite identification and taxonomic discrepancies, Zhao et al. (2012) showed that *N. pubens* and *N.* sp. nr. *pubens* (now known as *N. fulva*) were in fact the same species using two methods. First, by comparing five genes between the two possible species which showed 99-100% identity and also by comparing four partial genomic DNA sequences which yielded the same result. Furthermore, Gotzek et al. (2012) identified ants collected from Texas, Louisiana, Mississippi, and north Florida as *N. fulva* and determined that the suspected populations in south Florida (initially thought to be *N. pubens*) were most likely misidentified populations of *N. fulva* because *N. pubens* is not characteristically invasive. Therefore, the first reported case in 1953 were most likely *N. fulva*, tawny crazy ants.

Since their first introduction, tawny crazy ants have invaded throughout the southeastern United States into Florida, Georgia, Alabama, Mississippi, Louisiana, and Texas. In Chapter two, I concluded that this species is unicolonial throughout Texas and forms one genetically distinct supercolony. In Chapter three, I investigate the breeding

structure and genetic relationships among geographically separated colonies across the southeastern United States.

Methods

Sampling methods

Data collected for Chapter 2 of this thesis (for Texas populations) were also used in this chapter. Sampling methods for individuals collected in Texas were exactly the same for Chapter 3 of this thesis (see Chapter 2, Sampling Methods). In addition to the individuals collected within Texas, I received out of state samples from multiple contributors. I received 17-20 whole worker ants collected and stored in 90% ethanol from a single site located in each of the following states; Louisiana , Mississippi , and Georgia. Additionally, the Shoemaker laboratory at the Center for Agricultural, Medical and Veterinary Entomology, USDA ARS, provided us with extracted DNA from 59 workers from four collection sites in Florida. Locality information of each nest was recorded at the time of collection and is shown in Table 18.

Table 18. Location of nests of *N. fulva* sampled in southeastern United States.

State:	Nest Name:	Locality:	Longitude:	Latitude:	Date Collected:	Workers:	Queens:
TX	SM01	USA: TX: Bastrop County: Smithville	30.043671	-97.162376	09.VIII.2016	18	
	SM02		30.043972	-97.161941		20	
	SM03		30.044522	-97.161156		20	
	SA01	USA: TX: Bexar County: San Antonio	29.439968	-98.643049	09.VIII.2016	19	10
	SA02		29.440150	-98.642901		19	1
	SA03		29.440452	-98.642813		19	
	IC01	USA: TX: Brazoria County: Iowa Colony	29.435462	-95.435458	08.VI.2016	20	
	IC02		29.435525	-95.433013		20	
	IC03		29.435567	-95.431278		20	
	SI01	USA: TX: Hardin County: Silsbee	30.353172	-94.125907	09.VIII.2016	20	
	SI02		30.353716	-94.126499		20	
	SI03		30.354355	-94.126801		20	
	BU01	USA: TX: Hays County: Buda	30.076033	-97.845120	09.VIII.2016	20	
	BU02		30.075818	-97.845145		20	1
	BU03		30.075772	-97.845519		20	
	WE01	USA: TX: Hidalgo County: Weslaco	26.126063	-97.957693	19.XI.2016	20	9
	WE02		26.135516	-97.982209		19	
	WE03		26.124471	-97.958996		20	1
	AU01	USA: TX: Travis County: Austin	30.202873	-97.696219	09.VIII.2016	20	
	AU02		30.202562	-97.695691		20	
	AU03		30.202017	-97.694992		20	
LA	LA01	USA: LA: East Baton Rouge Parish: Baton Rouge	30.407768	-91.174509	Fall 2016	20	
GA	GA01	USA: GA: Chatham County: Savannah	32.030746	-81.134671	01.VII.2015	20	
MS	MS01	USA: MS: Jackson County: Ocean Springs	30.440000	-88.843330	06.VII.2011	17	
FL	FL01	USA: FL: Callahan	30.574417	-81.828283	09.VII.2015	10	
	FL02	USA: FL: Winter Garden	28.490583	-81.669117	27.VIII.2015	18	
	FL03	USA: FL: Gainesville	29.631333	-82.471517	05.X.2015	12	
	FL04	USA: FL: Lithia	27.876417	-82.181000	09.X.2015	19	

Genetic analysis

Microsatellite primer design. The 10 microsatellite loci used in Chapter 2 of this thesis were used in this Chapter for genetic analysis of individuals collected throughout the southeastern United States.

DNA extraction. All genomic DNA was extracted as part of Chapter 2 for basic descriptive statistics analysis.

Microsatellite genotyping. The genetic analysis of tawny crazy ants in the southeastern United States was performed using the above-mentioned 10 microsatellite primers designed by the Shoemaker lab. Polymerase chain reaction (PCR) conditions, along with multiplex and dilution information, are described in Chapter 2. Microsatellite loci were analyzed using a 3500 Genetic Analyzer 8ch RUO (Applied Biosystems). Microsatellite scoring and genotyping was done using Geneious software v9.1.6 (Biomatters Ltd).

Statistical methods of genetic analysis. I sought to determine if tawny crazy ants throughout the invasive range of the United States are genetically differentiated by calculating pairwise F_{ST} values between each pair of populations using the program FSTAT (Goudet 1995, 2001). For this analysis, a total of 14 populations were used (seven from Texas, four from Florida, and one each from Louisiana, Mississippi and Georgia). I recorded the percent of shared alleles among the 14 populations sampled in the United States. I report F_{IS} values to determine the levels of inbreeding as well as the degree of relatedness (r) among worker ants within colonies in the southeastern United States. In this study, I also test for isolation by distance by correlating F_{ST} and

geographic distance among all samples by means of a Mantel test in the program GenePop on the web (Raymond and Rousset 1995, Rousset 2008).

Results

Genetic analysis

Genetic differentiation across the southeastern United States. In the southeastern United States, I determined there to be low F_{ST} values between all population pairs of *N. fulva* (Table 19) (all *P* were non-significant after Bonferroni corrections). Furthermore, I calculated the percent of shared alleles among all individuals collected from the invasive range in the southeastern United States to be 84.7% (Tables 20). The colonies that shared the fewest alleles in common were Hays County, Texas ,and Florida 1 (73.2%), Louisiana and Florida 1 (74.1%), and Mississippi and Florida 1 (74.5%). I interpret these results as showing low levels of genetic differentiation between ants collected from several sites across the introduced range in the United States. F_{IS} values for each population ranged from -0.0920 to 0.0750 (Table 21) and were not significant. Individuals genotyped from Mississippi had the fewest number of alleles detected (43) while colonies sampled from Travis County and Bastrop County in Texas had all 54 alleles present. Of the sites located in Texas, Bexar County had the fewest number of alleles detected (49). The number of alleles in each colony is shown in Table 22. I observed a slight decrease in the percent of shared alleles when I compared individuals genotyped among Texas to those genotyped from other states. As expected, relatedness values within the 14 populations in the invasive range were effectively zero. Finally, I

found significant isolation by distance across the southeastern United States (Mantel test, $r = .42$, $P = 0.0003$; Figure 9).

Table 19. Pairwise F_{ST} values across all states and all loci. Adjusted significance: $P < 0.000549$ after Bonferroni correction.

pop	Brazoria Co.	Travis Co.	Hays Co.	Bexar Co.	Hardin Co.	Bastrop Co.	Hidalgo Co.	Georgia	Louisiana	Mississippi	Florida 1	Florida 2	Florida 3
Travis Co.	-0.0016												
Hays Co.	0.0123	0.0192											
Bexar Co.	0.0044	0.0092	0.0330										
Hardin Co.	0.0064	0.0126	0.0154	0.0326									
Bastrop Co.	0.0062	0.0075	0.0118	0.0230	0.0067								
Hidalgo Co.	0.0129	0.0126	0.0219	0.0274	0.0212	0.0116							
Georgia	0.0328	0.0406	0.0295	0.0597	0.0297	0.0218	0.0117						
Louisiana	0.0204	0.0224	0.0273	0.0466	0.0246	0.0080	0.0104	0.0175					
Mississippi	0.0460	0.0543	0.0691	0.0447	0.0559	0.0436	0.0309	0.0370	0.0475				
Florida 1	0.0288	0.0432	0.0390	0.0456	0.0346	0.0371	0.0296	0.0415	0.0336	0.0380			
Florida 2	0.0353	0.0366	0.0446	0.0397	0.0516	0.0456	0.0261	0.0425	0.0360	0.0449	-0.0032		
Florida 3	0.0206	0.0314	0.0196	0.0381	0.0190	0.0145	0.0291	0.0286	0.0230	0.0450	-0.0169	0.0087	
Florida 4	0.0587	0.0635	0.0799	0.0706	0.0645	0.0764	0.0703	0.1050	0.0759	0.1021	0.0267	0.0406	0.0306

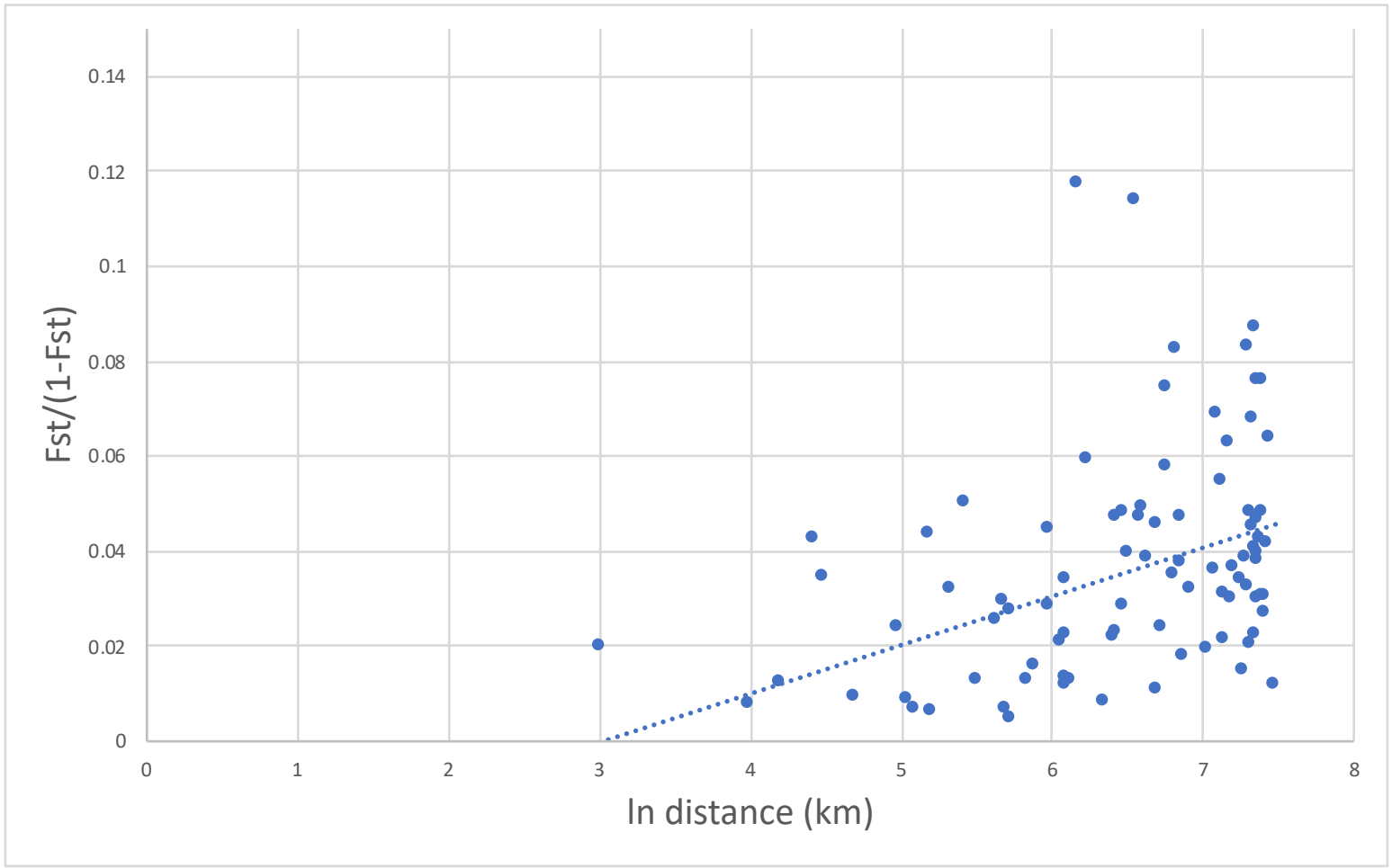
Table 20. Percent of shared alleles between sites located across the southeastern United States.

Population Pair			% Shared Alleles	Population Pair			% Shared Alleles
Brazoria Co.	&	Travis Co.	96.30	Hardin Co.	&	Hays Co.	88.89
Brazoria Co.	&	Bastrop Co.	96.30	Hardin Co.	&	Hidalgo Co.	96.23
Brazoria Co.	&	Bexar Co.	94.23	Hardin Co.	&	Louisiana	87.04
Brazoria Co.	&	Hardin Co.	94.44	Hardin Co.	&	Georgia	88.68
Brazoria Co.	&	Hays Co.	94.23	Hardin Co.	&	Mississippi	81.13
Brazoria Co.	&	Hidalgo Co.	92.59	Hardin Co.	&	Florida 1	78.18
Brazoria Co.	&	Louisiana	85.19	Hardin Co.	&	Florida 2	82.14
Brazoria Co.	&	Georgia	85.19	Hardin Co.	&	Florida 3	78.57
Brazoria Co.	&	Mississippi	79.63	Hardin Co.	&	Florida 4	80.00
Brazoria Co.	&	Florida 1	78.18	Hays Co.	&	Hidalgo Co.	92.45
Brazoria Co.	&	Florida 2	85.19	Hays Co.	&	Louisiana	86.54
Brazoria Co.	&	Florida 3	77.19	Hays Co.	&	Georgia	86.79
Brazoria Co.	&	Florida 4	83.33	Hays Co.	&	Mississippi	80.77
Travis Co.	&	Bastrop Co.	100.00	Hays Co.	&	Florida 1	73.21
Travis Co.	&	Bexar Co.	94.23	Hays Co.	&	Florida 2	81.82
Travis Co.	&	Hardin Co.	96.30	Hays Co.	&	Florida 3	78.18
Travis Co.	&	Hays Co.	92.59	Hays Co.	&	Florida 4	81.48
Travis Co.	&	Hidalgo Co.	96.30	Hidalgo Co.	&	Louisiana	92.31
Travis Co.	&	Louisiana	88.89	Hidalgo Co.	&	Georgia	88.68
Travis Co.	&	Georgia	88.89	Hidalgo Co.	&	Mississippi	80.77
Travis Co.	&	Mississippi	77.19	Hidalgo Co.	&	Florida 1	75.00
Travis Co.	&	Florida 1	77.19	Hidalgo Co.	&	Florida 2	82.14
Travis Co.	&	Florida 2	87.72	Hidalgo Co.	&	Florida 3	81.82
Travis Co.	&	Florida 3	78.95	Hidalgo Co.	&	Florida 4	80.00
Travis Co.	&	Florida 4	80.36	Louisiana	&	Georgia	88.24
Bastrop Co.	&	Bexar Co.	94.23	Louisiana	&	Mississippi	85.71
Bastrop Co.	&	Hardin Co.	96.30	Louisiana	&	Florida 1	74.07
Bastrop Co.	&	Hays Co.	92.59	Louisiana	&	Florida 2	81.48
Bastrop Co.	&	Hidalgo Co.	96.30	Louisiana	&	Florida 3	79.63
Bastrop Co.	&	Louisiana	88.89	Louisiana	&	Florida 4	82.69
Bastrop Co.	&	Georgia	88.89	Georgia	&	Mississippi	78.43
Bastrop Co.	&	Mississippi	77.19	Georgia	&	Florida 1	77.36
Bastrop Co.	&	Florida 1	83.33	Georgia	&	Florida 2	81.48
Bastrop Co.	&	Florida 2	82.46	Georgia	&	Florida 3	79.63
Bastrop Co.	&	Florida 3	78.95	Georgia	&	Florida 4	79.25
Bastrop Co.	&	Florida 4	83.33	Mississippi	&	Florida 1	74.51
Bexar Co.	&	Hardin Co.	88.89	Mississippi	&	Florida 2	77.36
Bexar Co.	&	Hays Co.	94.23	Mississippi	&	Florida 3	75.00
Bexar Co.	&	Hidalgo Co.	90.57	Mississippi	&	Florida 4	80.39
Bexar Co.	&	Louisiana	84.62	Florida 1	&	Florida 2	88.24
Bexar Co.	&	Georgia	86.54	Florida 1	&	Florida 3	78.85
Bexar Co.	&	Mississippi	84.00	Florida 1	&	Florida 4	80.77
Bexar Co.	&	Florida 1	75.93	Florida 2	&	Florida 3	88.46
Bexar Co.	&	Florida 2	80.00	Florida 2	&	Florida 4	81.13
Bexar Co.	&	Florida 3	79.63	Florida 3	&	Florida 4	82.69
Bexar Co.	&	Florida 4	84.62				

Table 21. F_{IS} values over all loci per population. Adjusted significance: $P < 0.00045$.		
Bastrop County	-0.0280	$P > 0.05$
Bexar County	-0.0770	$P > 0.05$
Brazoria County	0.0750	$P > 0.05$
Hardin County	-0.0270	$P > 0.05$
Hays County	-0.0800	$P > 0.05$
Hidalgo County	0.0180	$P > 0.05$
Travis County	-0.0260	$P > 0.05$
Georgia	-0.0870	$P > 0.05$
Louisiana	-0.0920	$P > 0.05$
Mississippi	-0.0780	$P > 0.05$
Florida 1	0.0050	$P > 0.05$
Florida 2	0.0270	$P > 0.05$
Florida 3	0.0080	$P > 0.05$
Florida 4	-0.0780	$P > 0.05$

Table 22. The number of alleles present in each of 14 populations.	
Population:	Number of Alleles:
Brazoria Co.	52
Travis Co.	54
Hays Co.	50
Bexar Co.	49
Hardin Co.	52
Bastrop Co.	54
Hidalgo Co.	52
Georgia	48
Louisiana	48
Mississippi	43
Florida 1	46
Florida 2	50
Florida 3	48
Florida 4	47
Total alleles:	57

Figure 9. Isolation by distance (IBD). Pairwise F_{ST} values ($F_{ST}/(1-F_{ST})$) plotted against the log transformation of geographic distances between sites throughout the southeastern United States. Mantel test, r = .42, P = 0.0003; y = 0.0104x - 0.0315, R^2 = 0.1725.



Discussion

The research conducted for Chapter 3 of this thesis aimed to determine if the tawny crazy ant, *N. fulva*, is unicolonial throughout their invasive range of the southeastern United States and to determine the genetic relationships among colonies across the invasive range. I used behavioral and genetic data collected for Chapter 2 in combination with genetic data from individuals collected in states other than Texas to draw my conclusions. I cannot exclude the possibility that more than one supercolony of *N. fulva* is present throughout its invasive range. However, based on the addition of data collected in Chapter 3, my results indicate that the tawny crazy ant is a unicolonial species which likely forms a single supercolony throughout the invaded region of the southeastern United States.

I determined the relatedness coefficients of individuals within and between each colony to be close to zero, consistent with the findings of other unicolonial species such as *Linepithema humile* and *P. megacephala* (Tsutsui et al. 2000, Fournier et al. 2009). Tsutsui et al. (2000) found that introduced populations of *L. humile* were less genetically diverse than native populations. This finding was also uncovered in tawny crazy ants (Eyer et al. in press), which were observed to have a 60% decrease in the genetic diversity found in the introduced range of the southeastern United States compared to native South American ants. Despite undergoing a decrease in genetic variability, introduced populations of *L. humile* are still capable of exhibiting intraspecific aggression between populations which are, to an extent, genetically similar, but presumably retain some variation in loci underlying intraspecific recognition mechanisms (Thomas et al. 2006). Because intraspecific aggression is maintained

between distinct colony boundaries, it was concluded that *L. humile* forms at least five distinct supercolonies throughout the introduced range in California. I did not observe any colony boundaries delineated by intraspecific aggressive interactions among tawny crazy ants in the introduced range.

Similar to what I observe in introduced *N. fulva* populations, Fournier et al. (2009) determined that four introduced populations of *P. megacephala* located throughout Australia did not show any aggressive behaviors among one another. In some cases, these populations were geographically separated by as much as 3000 km. These *P. megacephala* populations also revealed low genetic diversity among them as well as no genetic differentiation among nests located within a population. Fournier et al. (2009) report some differentiation between populations; however, this is described as weak and differentiation is attributed to private alleles found in 3 of the 4 populations. These authors concluded that these ants form one unicolonial supercolony throughout the invaded range.

Travis and Bastrop County sites both had the highest number of alleles (54 out of 57 total alleles) present. I observed fewer allele totals found from individuals collected from Mississippi (n = 43), Florida 1 (n = 46), and Florida 2 (n = 47). This lower allelic diversity could be the result of the small sample size of individuals collected and successfully genotyped from these additional states outside of Texas. In Texas, I genotyped a total of 480 individual worker ants from seven different sites, and each site was represented by 60 individuals. The number of genotyped individuals from other states was much lower (see Table 18). Another explanation for the lower allelic diversity outside of Texas could be the result of a series of small genetic bottlenecks that occurred

from multiple consecutive human mediated jump dispersals. Notably, I did not detect any alleles that were exclusively found in only one site (i.e., no private alleles).

I determined the average proportion of shared alleles between all individuals collected in the United Stated to be 84.7%, which is expected of a unicolonial population. Tsutsui et al. (2000) compared the proportions of shared alleles between native and introduced populations of *L. humile* and found that introduced populations of ants belonging to the same supercolony shared at least 75% of their alleles and were unicolonial, while most native populations shared between ~17-63% of their alleles. That same study also found that in both native and introduced populations, the average intraspecific aggression decreased as genetic similarity increased. Therefore, introduced populations did exhibit intraspecific aggression between populations that were less genetically similar, indicating the presence of multiple supercolonies throughout California. Additionally, Tsutsui and Case (2001) reported that native *L. humile* nests from across colony boundaries were found to be more genetically different than neighboring nests of the same colony. Thus, ants found in the same colony are more genetically similar to one another than to individuals found in other distant colonies. Similar to the findings of invasive Argentine ants, the yellow crazy ant or long-legged ant, *Anoplolepis gracilipes* forms multiple supercolonies in its invasive range in Borneo (Drescher et al. 2010). Drescher et al. (2010) found that colonies which share less than half of their alleles between each other were also aggressive towards one another, while non-aggressive populations shared 86–100% of their alleles. Abbott (2005) also reported low levels of aggression among most of the population pairs of *A. gracilipes* tested on Christmas Island, Indian Ocean, suggesting they form a supercolony, although genetic

analyses were not conducted in this case. In introduced ranges, colonies often carry a subset of alleles relative to the native range and therefore are more genetically homogenized throughout the introduced range. Comparisons of shared alleles will then be much higher in the introduced range. The results from Chapter 3 of this study conclude that *N. fulva* workers throughout the invasive range of the United States share a minimum of 73% of their alleles, further demonstrating unicoloniality in this species.

Both native and introduced populations of ants can form supercolonies which may also be described as unicolonial. In fact, the formation of supercolonies is a fairly common occurrence of invasive ants (Ross et al. 1996, Helanterä et al. 2009). Native supercolonies tend to be much smaller in size compared to introduced invasive ants, as seen in the Argentine ant (Pedersen et al. 2006) and the African big-headed ant (Fournier et al. 2012). Unlike invasive unicolonial populations, native supercolonies of those same species are genetically distinct from one another and intraspecific aggression occurs at the colony boundaries (Suarez et al. 1999, Fournier et al. 2012). Supercolonies of the Argentine ant in the native range show reduced nestmate relatedness within supercolonies, despite being more genetically similar to each other than to members belonging to different native supercolonies (Tsutsui and Case 2001, Pedersen et al. 2006). This was also the case of introduced populations of the Argentine ant in California, where genetic differentiation and intraspecific aggression occurs between separate supercolonies (Wilgenburg et al. 2009) and the within supercolony relatedness is zero (Tsutsui et al. 2000, Tsutsui and Case 2001). I conclude that *N. fulva* forms one supercolony throughout the United States due to the lack of any population differentiation among all populations sampled, in combination with the behavioral data

recorded across Texas. Texas ants showed no signs of aggression towards each other over all spatial scales, and populations did not differ genetically throughout the entire state. Furthermore, ants collected in Texas were no different from each other than to workers collected from any site in the United States. From these data, I predict that intraspecific aggression would not occur between these populations of ants, therefore, colony boundaries do not exist, and the United States population is unicolonial, forming one supercolony. However, additional studies are needed to confirm that ants from throughout the southeastern United States are not aggressive toward each other.

Eyer et al. (in press) found that native *N. fulva* populations are multicolonial and genetically distinct. Significant population structure was found among different localities (11.3% genetic diversity among localities) as well as significant differentiation among nests within localities (average $F_{ST} = 0.36$, ± SD = 0.14; with 25.9% variation among nests within localities). Additionally, native populations of *N. fulva* were found to have a high degree of isolation by distance between them, indicating a lack of gene flow between populations which are at equilibrium under a stepping-stone model of population structure. These results suggest that colonies in the native range are multicolonial, forming distinct family units with clear boundaries between them and that dispersal is limited restricting gene flow between distant locations.

Although I found low F_{ST} values (average = 0.0333) and high percentages of shared alleles between population pairs in the introduced range, I did observe evidence of isolation by distance throughout the southeastern United States. In comparison to the strong genetic differentiation found among localities in the native range (Eyer et al. (in press), their results bring attention to the weak genetic differentiation found throughout

the introduced population (2.0% genetic diversity among United States localities with 98.1% genetic variation within nests), demonstrating that each nest contains almost the complete genetic diversity found throughout the entire invasive range. There are a few factors that could be affecting the significant positive IBD result in the invasive range. First, there was a much smaller sample size of genotyped individuals located in states other than Texas, which may not accurately represent the entire population of ants in those areas. As mentioned in Chapter 2, I did not observe evidence of isolation by distance between ants collected in Texas (seven populations), which was not surprising given the close proximity of each of the collection sites and the high percentages of shared alleles between them, especially if the entire population of individuals in Texas were sourced from one single introduction. Furthermore, the lack of nuptial flights of mated queens and colony expansion by budding limits the dispersal of *N. fulva* colonies across greater distances. Limited dispersal resulting in limited gene flow, over time, may contribute to eventual population differentiation in the introduced range. However, despite these limitations, introduced populations of tawny crazy ants seem to be spreading over large distances primarily via human mediated jump dispersal, which increases the genetic homogeneity among geographically separated populations. Finally, this could also be the result of serial introductions through human mediated dispersal which started from one source population in Florida and was carried throughout the Gulf States all the way into Texas, resulting in small-scale genetic differences between them. Isolation by distance may have been undetectable throughout Texas due to the smaller invasion area as well as the fact that Texas introductions are fairly recent (within the past two decades).

Unicolonial ants exhibit specific behavior, nesting habits, and population structure which creates a problem for successful control efforts (Silverman and Brightwell 2008). Control efforts of unicolonial ant populations documented in the literature can help shed light on effective methods of control. Differences in the biology of each species must be taken into consideration when attempting to control unicolonial populations. For example, *Solenopsis germinata* (Mcinnes and Tschinkel 1995), *S. invicta* (Silverman and Brightwell 2008), and *A. gracilipes* (Abbott 2006) produce colonies that utilize short distance dispersal via nuptial flights of mated queens which allows ants to escape treated areas and establish elsewhere. Fortunately, *N. fulva* queens have not been observed to participate in mating flights throughout the invasive range. This makes it less likely for queens to relocate themselves into untreated areas while treatment is taking place. Population sizes are also something to consider while aiming to control ant infestations. Research shows that control efforts of unicolonial populations, such as *S. invicta*, are best when the population is localized and just beginning its invasion (Frank 1988).

Understanding the toxicological effects of insecticides is also important for proper integrated pest management practices. It is in our best interest to limit the amount of pesticide used during treatments to decrease the exposure of toxic chemicals to non-target species and to avoid prolonged exposure of these chemicals to humans (Josens et al. 2014). Insecticides are widely used around buildings as a perimeter control, creating a toxic barrier against any invading species. There are problems with this "barrier method" when trying to achieve control over a unicolonial ant invader, which has been observed in treatment attempts of *N. fulva* infestations. The population density of unicolonial

species in an area create a problem for standard control methods, as neighboring colonies can expand via budding into previously treated areas. Studies show *N. fulva* workers are not completely repelled by many commercially available insecticides (Calibeo et al. 2017). Additionally, toxic bait formulas have been documented to provide a temporary reduction in ant densities immediately following treatment, but this is short lived as rapid reinfestation occurs just 3-4 weeks after treatment (McDonald 2012). Because reinfestation occurs so rapidly, repeated use of toxic baits at rates much higher than the recommended dose would be necessary to maintain temporary control, which is not a sustainable treatment method.

Unicolonial ant populations overwhelm and outcompete native species as they are able to more efficiently recruit members of the colony to food and water resources. The type of bait (liquid, gel, solid) and composition (carbohydrate, protein) accepted can differ depending on the species of ant, and the time of year the bait is applied. For example, Argentine ants have been observed to consume solid granules with high protein content during the spring and summer (Silverman and Brightwell 2008), while laboratory studies show that tawny crazy ants more readily accept baits with a high carbohydrate content (Calibeo et al. 2017). Successful control of *A. gracilipes* on Christmas Island, Indian Ocean, was achieved by first using toxic baits in easily accessible areas, and then, utilization of a helicopter to distribute baits over dense infestations mapped with GIS technology (Green et al. 2004), with the goal of re-establishing native fauna by providing alternative lures outside of the treatment areas. It is important that any control method utilized aims to limit negative effects on non-target species. Another suggested method of control in unicolonial species (Argentine ants) is

to increase the intraspecific aggression within supercolonies by either introducing genetic variability or altering the cuticular hydrocarbon profiles which aid in colony recognition cues (Silverman and Brightwell 2008). Interestingly, Tsutsui et al. (2003) shows that the loss of genetic diversity after a population bottleneck in Argentine ants results in an increased intraspecific aggression toward genetically different groups, which may create a problem for this specific control method as the already present supercolony is likely to outcompete subsequent introductions of genetically dissimilar ants.

As mentioned, tawny crazy ant infestations are dense and often occupy large areas of land. This has caused problems with successful management because the entire population is not treated at a given time. Possible approaches for successful control of tawny crazy ants should include a plan for area-wide treatment across the entire infested areas using carbohydrate-based baits that are preferred by these ants.

The data suggest that *N. fulva* in the introduced range forms one supercolony that expands across all of the Gulf States, over 1700 km. Based on my results, which reflect high proportions of shared alleles, relatedness estimates effectively equal to zero, and an absence of intraspecific aggression, the supercolony is almost certainly the result of a single introduction into the United States (most likely into Florida in 1953) from South America, which continues to spread throughout the southeastern United States via human mediated dispersal. From the single introduction into Florida, ants would have established locally, spreading via colony budding and increasing their colony numbers. Eventually, sub-populations of this initial introduction began to spread via human mediated dispersal. This is just the beginning of a chain reaction as every small sub-

population would grow on a local level, they become more likely to be picked up and transported by humans which results in many geographically separated colonies which are genetically homogeneous and are not aggressive towards one another.

CHAPTER IV

CONCLUSION

In conclusion, this research confirms that the tawny crazy ant, *Nylanderia fulva*, is unicolonial in its invasive range throughout the southeastern United States. Colonies throughout the introduced range show no evidence of genetic differentiation and have relatedness estimates between each other effectively equal to zero. I also observed no evidence of competitive aggression in the introduced range between ants collected at various spatial scales. Based on the results, I conclude that the southeastern United States is home to an individual supercolony that is patchily distributed from Florida and Georgia to Texas. This was most likely the consequence of one main introduction event into the United States and the spread of this infestation is the result of multiple human mediated dispersal events across the entire invaded range.

My findings suggest this species forms a single supercolony across its invaded range, likely the result of one introduction event. First, there is no evidence of intraspecific aggression among worker ants collected throughout the invasive range of Texas. In addition to the lack of intraspecific aggression, ants collected throughout Texas are not genetically distinct from one another. Furthermore, ants collected throughout Texas are not genetically distinct from any of the other populations sampled throughout the United States. It is likely that if one were to collect individuals in a given area and then release them in an already infested area in the introduced range, those ants would not show any aggressive interactions with one another and would essentially mesh as one group, although this should be tested in future studies. Based on research

findings in other ant species, I am able to confirm unicoloniality in this species and provide further evidence of another species of ant adopting a unicolonial structure in their introduced range. Further studies on *N. fulva* are needed to determine any possible driving forces of developing unicoloniality in their introduced habitat, which in theory, should not be a sustainable social structure due to the inclusive fitness costs of promoting the survival of unrelated offspring from unrelated reproductives, which directly contradicts kin theory (Helanterä et al. 2009).

Successful control of unicolonial ant populations is possible with proper funding for such relief efforts, in addition to the combined efforts and communication between federal, state, and local governments, researchers, and the general public (Silverman and Brightwell 2008). In the case of the tawny crazy ant, we know population sizes increase rapidly in a given area via colony budding, and that sub-populations of ants are transported via human mediated dispersal, which further promotes unicoloniality by increasing the genetic homogeneity among geographically separated populations. Nuptial flights of mated queens have not been observed in *N. fulva*, which may aid in control success as ants are less likely to disperse away from treated areas. Control of large populations have been unsuccessful for the most part, as nearby workers re-infest areas treated with residual insecticides. Successful control of the tawny crazy ant should start early, as soon as an introduction occurs. In already established populations, more successful control may be achieved by implementing an area-wide treatment across entire infested areas using baits designed for tawny crazy ants. More research is needed on the types of baits accepted by the tawny crazy ant in addition to the practicality of applying baits across heavily infested areas, especially those in undisturbed habitats.

REFERENCES

Abbott, K. L. 2005. Supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on an oceanic island: Forager activity patterns, density and biomass. *Insectes Sociaux* **52**:266-273.

Abbott, K. L. 2006. Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. *Diversity and Distributions* **12**(1):101-110.

Aguillard, D., R. M. Strecker, and L. M. Hooper-Bùi. 2011. Extraction of super colonies of crazy ants from soil and wood. *Midsouth Entomologist* **4**:53-56.

Arcila, A. M., L. A. Gómez, and P. Ulloa-Chacón. 2002. Immature development and colony growth of crazy ant *Paratrechina fulva* under laboratory conditions (Hymenoptera: Formicidae). *Sociobiology* **39**(2):307-321.

Ballard, L. W., P. S. Adams, Y. Bao, D. Bartley, D. Bintzler, L. Kasch, L. Petukhova, and C. Rosatog. 2002. Strategies for genotyping: effectiveness of tailing primers to increase accuracy in short tandem repeat determinations. *Journal of Biomolecular Techniques* **13**(1):20-29.

Bentley, M. T., F. M. Oi, S. A. Gezan, and D. A. Hahn. 2015. Tunneling performance increases at lower temperatures for *Solenopsis invicta* (Buren) but not for *Nylanderia fulva* (Mayr). *Insects* **6**:686-695.

Calcaterra, L., S. Cabrera, and J. Briano. 2016. Local co-occurrence of several highly invasive ants in their native range: are they all ecologically dominant species? *Insectes Sociaux* **63**:407-419.

Calibeo, D., F. Oi, D. Oi, and C. Mannion. 2017. Insecticides for suppression of *Nylanderia fulva*. *Insects* **8**(3):15.

Corin, S. E., K. L. Abbott, P. A. Ritchie, and P. J. Lester. 2007. Large scale unicoloniality: the population and colony structure of the invasive Argentine ant (*Linepithema humile*) in New Zealand. *Insectes Sociaux* **54**(3):275-282.

Deyrup, M., L. Davis, and S. Cover. 2000. Exotic ants in Florida. *Transactions of the American Entomological Society* **126**(3):293-326.

Drescher, J., N. Bluthgen, T. Schmitt, J. Buhler, and H. Feldhaar. 2010. Societies drifting apart? Behavioural, genetic and chemical differentiation between supercolonies in the yellow crazy ant *Anoplolepis gracilipes*. *PLoS ONE* **5**(10):8.

Elton, C. S. 1958. The ecology of invasions by animals and plants. edition.10.1007/978-1-4899-7214-9

Evans, T. A., B. T. Forschler, and J. K. Grace. 2013. Biology of invasive termites: A worldwide review. *Annual Review of Entomology* **58**:455-474.

Eyer, P.-A., B. McDowell, L. N. L. Johnson, L. A. Calcaterra, M. B. Fernandez, D. Shoemaker, R. T. Puckett, and E. L. Vargo. in press. Supercolonial structure of invasive populations of the tawny crazy ant *Nylanderia fulva* in the US. *BMC Evolutionary Biology*.

Faircloth, B. C. 2008. MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* **8**(1):92-94.

Feener Jr., D. H., M. R. Orr, K. M. Wackford, J. M. Longo, W. W. Benson, and L. E. Gilbert. 2008. Geographic variation in resource dominance-discovery in Brazilian ant communities. *Ecology* **89**(7):1824-1836.

Foucaud, J., J. Orivel, A. Loiseau, J. H. C. Delabie, H. Jourdan, D. l. Konghouleux, M. Vonshak, M. Tindo, J.-L. Mercier, D. Fresneau, J.-B. Mikissa, T. McGlynn, A. S. Mikheyev, J. Oettler, and A. Estoup. 2010. Worldwide invasion by the little fire ant: routes of introduction and eco-evolutionary pathways. *Evolutionary Applications* **3**(4):363-374.

Fournier, D., J.-C. d. Biseau, and S. Aron. 2009. Genetics, behaviour and chemical recognition of the invading ant *Pheidole megacephala*. *Molecular Ecology* **18**(2):186-199.

Fournier, D., D. Dubois, and S. Aron. 2008. Isolation and characterization of microsatellite loci from the invasive ant *Pheidole megacephala*. *Molecular Ecology Resources* **8**(4):919-922.

Fournier, D., M. Tindo, M. Kenne, P. S. M. Masse, V. V. Bossche, E. D. Coninck, and S. Aron. 2012. Genetic structure, nestmate recognition and behaviour of two cryptic species of the invasive big-headed ant *Pheidole megacephala*. *PLoS ONE* **7**(2):16.

Frank, W. A. 1988. Report of limited establishment of red imported fire ant, *Solenopsis invicta* Buren in Arizona. *Southwestern Entomologist* **13**:307-308.

Giraud, T., J. S. Pedersen, and L. Keller. 2002. Evolution of supercolonies: The Argentine ants of southern Europe. *PNAS* **99**(9):6075-6079.

Gotzek, D., S. G. Brady, R. J. Kallal, and J. S. LaPolla. 2012. The importance of using multiple approaches for identifying emerging invasive species: the case of the Rasberry crazy ant in the United States. *PLoS ONE* **7**(9):10.

Goudet, J., 1995(56): *FSTAT* (Version 1.2)*:* A computer program to calculate F-statistics Journal of Heredity, p. 485-486.

Goudet, J., 2001(57): *FSTAT* (Version 2.9.3), A program to estimate and test gene diversities and fixation indices,

Green, P., S. Comport, and D. Slip. The management and control of the invasive alien crazy ant (*Anoplolepis gracilipes*) on Christmas Island, Indian Ocean: aerial baiting campaign September 2002. 2004.

Hammen, T. v. d., J. S. Pedersen, and J. J. Boomsma. 2002. Convergent development of low-relatedness supercolonies in Myrmica ants. *Heredity* **89**(2):83-89.

Hee, J. J., D. A. Holway, A. V. Suarez, and T. J. Case. 2000. Role of propagule size in the success of incipient colonies of the invasive Argentine ant. *Conservation Biology* **14**(2):559-563.

Helanterä, H. 2009. Do unicolonial wood ants favor kin? *Journal of Biology* **8**(56).

Helanterä, H., J. E. Strassmann, J. Carrillo, and D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going? *Trends in Ecology and Evolution* **24**(6):341-349.

Hirata, M., O. Hasegawa, T. Toita, and S. Higashi. 2008. Genetic relationships among populations of the Argentine ant, *Linepithema humile,* introduced into Japan. *Ecological Research* **23**(5):883-888.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* **33**(1):181-233.

Holway, D. A., A. V. Suarez, and T. J. Case. 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science* **282**(5390):949-952.

Hooper-Bùi, L. M., Strecker, R. M., Chen, X., Aguillard, D., and Miller, A. 2010. Supercolonies of crazy ants in Louisiana. *Imported Fire Ant and Invasive Ant Conference Proceedings*:13-16.

Horn, K. C., M. D. Eubanks, and E. Siemann. 2013. The effect of diet and opponent size on aggressive interactions involving caribbean crazy ants (*Nylanderia fulva*). *PLoS ONE* **8**(6):7.

Human, K. G., S. Weiss, A. Weiss, B. Sandler, and D. M. Gordon. 1998. Effects of abiotic factors on the distribution and activity of the invasive Argentine ant (Hymenoptera: Formicidae). *Environmental Entomology* **27**(4):822-833.

Josens, R., F. J. Sola, N. Marchisio, M. A. D. Renzo, and A. Giacometti. 2014. Knowing the enemy: ant behavior and control in a pediatric hospital of Buenos Aires. *Springer Plus* **3**(229):13.

Kumar, S., E. G. LeBrun, T. J. Stohlgren, J. A. Stabach, D. L. McDonald, D. H. Oi, and J. S. LaPolla. 2015. Evidence of niche shift and global invasion potential of the tawny crazy ant, *Nylanderia fulva*. *Ecology and Evolution* **5**(20):4628-4641.

Le Breton, J., J. H. C. Delabie, J. Chazeau, A. Dejean, and H. Jourdan. 2004. Experimental evidence of large-scale unicoloniality in the tramp ant *Wasmannia auropunctata* (Roger). *Journal of Insect Behavior* **17**(2):263-271.

LeBrun, E. G., J. Abbott, and L. E. Gilbert. 2013. Imported crazy ant displaces imported fire ant, reduces and homogenizes grassland ant and arthropod assemblages. *Biological Invasions* **15**(11):2429-2442.

LeBrun, E. G., N. T. Jones, and L. E. Gilbert. 2014. Chemical warfare among invaders: A detoxification interaction facilitates an ant invasion. *Science* **343**(6174):1014-1017.

MacGown, J., and B. Layton. 2010. The invasive Rasberry crazy ant, *Nylanderia* sp. nr. *pubens* (Hymenoptera: Formicidae), reported from Mississippi. *Midsouth Entomologist* **3**:44-47.

McDonald, D. E. 2012. Investigation of an invasive ant species: *Nylanderia fulva* colony extraction, management, diet preference, fecundity, and mechanical vector potential. Doctoral dissertation, Texas A&M University

McGlynn, T. P. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. *Journal of Biogeography* **26**(3):535-548.

Mcinnes, D. A., and W. R. Tschinkel. 1995. Queen dimorphism and reproductive strategies in the fire ant *Solenopsís geminata* (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* **36**:367-375.

Meyers, J. M., and R. E. Gold. 2008. Identification of an exotic pest ant, *Paratrechina sp.nr.pubens* (Hymenoptera: Formicidae), in Texas. *Sociobiology* **52**:589-604.

Moller, H. 1996. Lessons for invasion theory from social insects. *Biological Conservation* **78**:125-142.

Morel, L., R. K. V. Meer, and C. S. Lofgren. 1990. Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (Hymenoptera: Formicidae). *Annals of the Entomological Society of America* **83**(3):642-647.

O'Dowd, D. J., P. T. Green, and P. S. Lake. 2003. Invasional 'meltdown ' on an oceanic island. *Ecology Letters* **6**(9):812-817.

Pedersen, J. S., M. J. B. Krieger, V. Vogel, T. Giraud, and L. Keller. 2006. Native supercolonies of unrelated individuals in the invasive Argentine ant. *Evolution* **60**(4):782-791.

Queller, D. C., and K. F. Goodnight. 1989. Estimating Relatedness Using Genetic Markers. *Evolution* **43**(2):258-275.

Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. . *Journal of Heredity* (86):248-249.

Robinson, E. J. 2014. Polydomy: the organisation and adaptive function of complex nest systems in ants. *Current Opinion in Insect Science* **5**:37-43.

Ross, K. G. 1992. The breeding system of the fire ant *Solenopsis invicta:* effects on colony genetic structure. *The American Naturalist* **141**(4):554-576.

Ross, K. G., and D. J. C. Fletcher. 1985. Comparative study of genetic and social structure in two forms of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* **17**(4):349-356.

Ross, K. G., E. L. Vargo, and L. Keller. 1996. Social evolution in a new environment: the case of introduced fire ants. *Proceedings of the National Academy of Sciences of the United States of America (USA)* **93**(7):3021-3025.

Rousset, F. 2008. GENEPOP'007: a complete re-implimentationof the genepop software for Windows and Linux. *Molecular Ecology Resources* (8):103-106.

Seppä, P., and L. Walin. 1996. Sociogenetic organization of the red ant *Myrmica rubra*. *Behavioral Ecology and Sociobiology* **38**(3):207-217.

Silverman, J., and R. J. Brightwell. 2008. The Argentine ant: challenges in managing an invasive unicolonial pest. *Annual Review of Entomology* **53**:231-252.

Suarez, A. V., D. A. Holway, and T. J. Case. 2001. Patterns of spread in biological invasions dominated by long-distance jump dispersal: Insights from Argentine ants. *PNAS* **98**(3):1095-1100.

Suarez, A. V., D. A. Holway, and N. D. Tsutsui. 2008. Genetics and behavior of a colonizing species: The invasive Argentine ant. *The American Naturalist* **172**(1):72-84.

Suarez, A. V., N. D. Tsutsui, D. A. Holway, and T. J. Case. 1999. Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biological Invasions* **1**(1):43-53.

Sunamura, E., S. Hatsumi, S. Karino, K. Nishisue, M. Terayama, O. Kitade, and S. Tatsuki. 2009. Four mutually incompatible Argentine ant supercolonies in Japan: Inferring invasion history of introduced Argentine ants from their social structure. *Biological Invasions* **11**(10):2329-2339.

Thomas, M. L., C. M. Payne-Makrisa, A. V. Suarez, N. D. Tsutsui, and D. A. Holway. 2006. When supercolonies collide: territorial aggression in an invasive and unicolonial social insect. *Molecular Ecology* **15**(14):4303-4315.

Tsutsui, N. D., and T. J. Case. 2001. Population genetics and colony structure of the Argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution* **55**(5):976-985.

Tsutsui, N. D., and A. V. Suarez. 2003. The colony structure and population biology of invasive ants. *Conservation Biology* **17**(1):48-58.

Tsutsui, N. D., A. V. Suarez, and R. K. Grosberg. 2003. Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *PNAS* **100**(3):1078-1083.

Tsutsui, N. D., A. V. Suarez, D. A. Holway, and T. J. Case. 2000. Reduced genetic variation and the success of an invasive species. *PNAS* **97**(11):5948-5953.

Valles, S. M., D. H. Oi, F. H. Yu, X. X. Tan, and E. A. Buss. 2012. Metatranscriptomics and pyrosequencing facilitate discovery of potential viral natural enemies of the invasive Caribbean crazy ant, *Nylanderia pubens*. *PLoS ONE* **7**(2):9.

Wang, J. 2010. Coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources* **11**(1):141-145.

Wang, Z., L. Moshman, E. C. Kraus, B. E. Wilson, N. Acharya, and R. Diaz. 2016. A review of the tawny crazy ant, *Nylanderia fulva*, an emergent ant invader in the southern United States: is biological control a feasible management option? *Insects* **7**(77):10.

Wilgenburg, E. v., C. W. Torres, and N. D. Tsutsui. 2009. The global expansion of a single ant supercolony. *Evolutionary Applications* **3**:136-143.

Zenner de Polania, I. 1990. Biological aspects of the "hormiga loca," *Paratrechina (Nylanderia) fulva* (Mayr), in Colombia. *Applied Myrmecology: A World Perspective*:290-297.

Zhang, Q.-H., D. L. McDonald, D. R. Hoover, J. R. Aldrich, and R. G. Schneidmiller. 2015. North American invasion of the tawny crazy ant (*Nylanderia fulva*) Is enabled by pheromonal synergism from two separate glands. *Chemical Ecology* **41**(9):853-858.

Zhao, L. M., J. Chen, W. A. Jones, D. H. Oi, and B. M. Drees. 2012. Molecular comparisons suggest Caribbean crazy ant from Florida and Rasberry crazy ant from Texas (Hymenoptera: Formicidae: Nylanderia) are the same species. *Environmental Entomology* **41**(4):1008-1018.