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**EARLY INVASION DYNAMICS OF THE JAPANESE PAVEMENT ANT,
TETRAMORIUM TSUSHIMAE, IN THE SAINT LOUIS, MISSOURI
METROPOLITAN AREA**

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Abstract

Invasive species are non-native species that undergo rapid population growth and incur significant biological costs. Worldwide, they are the second leading cause of species extinctions. Invasive ants are particularly destructive, constituting 5 of the world's 100 worst invasive species. A major push in the study of biological invasions has been to understand their incipient stages, because once thoroughly established, invasions are nearly impossible to eradicate. The Japanese pavement ant, *Tetramorium tsushimae*, is a recently discovered invasive species found throughout the St. Louis metropolitan area and one of the few successful early stage ant invasions known. The primary goal of this research is to use this species to understand how early biological invasions proceed by using methods in population genetics, animal behavior, and community ecology. The first chapter is a literature review on what is known about the early stages of ant invasions. The second chapter uses microsatellite variation measured from nests collected throughout the invaded range to quantify population genetic structure. I demonstrate that the invasion is likely the result of a single invasion event originating in St. Louis, MO and subsequently spreading through human-mediated and natural dispersal throughout the present range of Eastern Missouri and Western Illinois. The third chapter quantifies intra-specific aggression in North American *T. tsushimae*. The Japanese pavement ant demonstrates low levels of intra-specific aggression (i.e. unicoloniality) throughout the invaded range. Aggression is correlated to microsatellite genetic distance; aggression increases as nest pairs are less genetically similar. The fourth chapter addresses the ecological effect of the Japanese pavement ant on native ants by comparing community

structure in invaded and uninvaded prairie and turf grass ecosystems. When paired with the results of an artificial introduction competition experiment, this research indicates that the Japanese pavement ant has a significant negative effect on native ant species. The Japanese pavement ant is a successful, unicolonial, and dominant invasive species that is being spread geographically through human activity. As a model system, this research can provide insight into the ecology and evolution of early stage ant invasions; thereby aiding in creating effective and efficient conservation policy.

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Dedication

This is for everyone that has pushed me and guided me to realize my passion, as strange as it may be.

This is for everyone that has kept me sane along the way, including the dogs sleeping by my side as I write.

This is for my loving wife, Marsha, and my loving family.

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CHAPTER 1 – TRANSPORT AND ESTABLISHMENT OF RECENT ANT INVASIONS – A REVIEW

Biological invasions are a leading cause of conservation concern. Ecosystems and human society have been drastically altered by the proliferation of invasive species, particularly in the current age of globalization (Perrings et al. 2005; Huber 2002). Invasive species are a leading cause of extinctions and population declines in native species, second only to habitat loss and alteration (Wilcove et al. 1998). Often, these species comprise a significant proportion of many communities, adversely affecting native species through competition, predation, disease, hybridization, herbivory, parasitism, and alteration of disturbance regimes (Gurevitch and Padilla 2004). Not only do biological invasions contribute to a loss of biodiversity, but also incur an annual cost of \$137 billion in the United States alone, primarily through urban and agricultural eradication efforts and crop damage (Pimentel et al. 2000).

In particular, ants represent some of the most cosmopolitan and destructive invasive species. Five of the 100 most damaging invasive species are ants: the red imported fire ant (*Solenopsis invicta*), the Argentine ant (*Linepithema humile*), the big-headed ant (*Pheidole megacephala*), the little fire ant (*Wasmannia auropunctata*), and the yellow crazy ant (*Anoplolepis gracilipes*; Lowe et al. 2001). Ant invasions cause significant economic costs, alter the environment, and impact native biodiversity (Holway et al. 2002). Two ants native to South America, the Argentine ant and the red fire ant have become some of the most ubiquitous and detrimental invasive species in the United States. The red fire ant can live in densities as high as 250 nests per acre and are

responsible for \$670 million in annual economic damages in Texas alone (Lard et al. 2002).

The effects of ant invasions are even more significant on oceanic islands that have no native ant biota, such as Polynesia. Ant invasions in naïve island ecosystems significantly impact diversity, trophic structure, and functional stability (Krushelnycky and Gillespie 2008). In the case of the invasive yellow crazy ant, an “invasional meltdown” has resulted in the wholesale alteration of a tropical rainforest ecosystem on Christmas Island (O’Dowd et al. 2003). Only 2 years after colonization, this ant significantly reduced populations of red land crab, a keystone omnivore. Island-wide, 10-15 million crabs have been killed. The absence of the red land crab has altered seedling and invertebrate diversity and the rate of seedling recruitment and leaf litter breakdown. Additionally, a sharp increase in the density of honeydew producing insects has led to greater incidences of leaf dieback and sooty molds (O’Dowd et al. 2003). In this system, the invasion of a single ant species has significantly altered entire ecosystems, changing the composition of both plant and animal communities.

The most commonly documented negative effect of successful ant invasions is the loss of native ant diversity (Holway et al. 2002). Through competitive dominance, predation, and nest raiding, native ant diversity can be significantly reduced (Human and Gordon 1997; Porter and Savignano 1990; De Kock 1990). The species most commonly affected are ground-foraging, conspicuous species that occupy similar niche space with the invader (Human and Gordon 1997). Cryptic species that often forage and live entirely under the soil surface may escape eradication and consequently comprise a significantly larger proportion of the ant community (Human and Gordon 1997; Ward 1987).

Due to the extreme costs incurred by invasions, there is a great need for strong and effective conservation efforts aimed at attenuating and eradicating ant invasions. Unfortunately, it has been generally accepted that eliminating large-scale, established invasions is practically impossible (Wilson 1986). Therefore, there has been a recent shift to studying the early stages of the invasion process, in the hope of enabling conservation efforts to effectively prevent the further spread and establishment of incipient invasions (Tsutsui and Suarez 2003; Kolar and Lodge 2001). It is more efficacious and cost-effective to identify and eliminate a potential invader in transit or soon after colonization, then after it has already spread and attained ecological dominance. The crux of the problem studying early invasion dynamics is that model systems are infrequently discovered and poorly studied. The vast majority of our knowledge base comes from the study of a few established, cosmopolitan invasive ants, such as the Argentine ant, red fire ant, and little fire ant (Holway et al. 2002). However, as the effort to intercept exotic species in transit is increasing and more recent invasions are being documented, the nature of invasions in their early stages is rapidly becoming better understood. The focus of this paper is to review what is known about the earliest stages of ant invasions: transport and establishment. The elimination of an invasive species is more tenable during the early stages of an invasion when population sizes are smaller and more manageable; thus comprehensive knowledge of the factors that lead to the successful transport and establishment of invasive species is of utmost importance.

Stages of an ant invasion

Before a potential invader can undergo significant population growth and geographic spread, it must successfully navigate a series of stages inherent to the invasion process. First, individuals must be anthropogenically transported from one location to another. In the event this propagule survives transport, it must then survive in a novel, exotic location. After colonization, the population must become established and eventually grow to the point where range expansion and ecological impact are significant and infiltrate natural ecosystems (Sakai et al. 2001). The characteristics and circumstances necessary to succeed in each of these stages are largely different, as I will demonstrate is the case among exotic ants (Lockwood et al. 2007).

The stochastic nature of the invasion process combined with the differing strategies necessary to survive each step in the invasion process results in only a small fraction of introduced individuals actually becoming successfully invasive populations (Williamson 1996; Lockwood et al. 2007). Williamson (1996) analyzed the mortality of British plant invasions at various stages and determined that approximately 10% of the populations that survived one stage successfully proceeded to the following stage. Analyses of flatworms and marine organisms in invaded ecosystems have supported the “Tens Rule,” which postulates that only 10% of exotic systems that have attained one stage of the invasion process will successfully proceed to the following invasion stage (Rao 2005; Boag and Yeates 2001). While terrestrial vertebrates and ants have been shown to have a success rate an order of magnitude higher than predicted by the “Tens Rule” after being transported, there is little doubt invasive species comprise a small fraction of the organisms being transported worldwide (Jeschke and Strayer 2005; Suarez

et al. 2005). Dissecting invasions and identifying the characteristics associated with successfully passing each stage can help identify stage-specific modes of control and prevention (Williamson 1996).

Stage 1: Transport

Transport – vectors and pathways

Humans have transported species, intentionally and unintentionally, for thousands of years. In recent times, exotic species have been intentionally introduced to novel locations for agricultural, aesthetic, and conservation reasons (Lockwood et al. 2007). While experience has shown that the intentional introduction of species requires detailed scientific inquiry, the ornamental plant and pet trade are responsible for the export and import of thousands of exotic species annually (Mack 2004; Kraus 2004). Although the intentional introduction of ants is rare and primarily associated with their role as potential biocontrol agents, ants commonly hitchhike within cargo (Lim et al. 2008; Carroll and Risch 1990). It is the unintentional, global redistribution of ant species that has exponentially increased as a result of modern increases in international travel and trade volume (Suarez et al. 2005; Ward et al. 2006).

Introduction frequency depends on the association between ants and vectors at the point of origin and the ability of propagules to survive in transport. The prevalence of ants in plant material is likely due to ants being present in soil prior to the collection and transportation of plant material. Also, the ability of ants to homeostatically regulate the environment inside soil allows propagules to survive extended periods of transport (Horstmann and Schmid 1986).

While a large body of research has focused on characteristics of well established ant invasions, little is known about the initial transport stage. Seminal studies by Suarez et al. (2005) and Ward et al. (2006) have shed light on the unintentional movement of ants into the United States and New Zealand, respectively. Both studies have taken advantage of existing custom records documenting the interception of exotic ants and associated transport vectors and pathways. While long-range jump dispersal occurs over a continuum of spatial scales, this section will focus on the continental dispersal of species to novel locations – the first stage of the invasion process.

Suarez et al. (2005) examined port-of-entry (POE) samples collected by the United States Department of Agriculture between 1927 and 1985. This database provides information regarding failed introductions; the most poorly understood aspect of biological invasion (Simberloff 1989). Out of a total of 394 samples collected, 232 species of ants were intercepted entering the U.S. The taxonomic and biogeographical distribution of these ants does not differ significantly from ants in general. A majority of the POE samples originated from the Neotropics, the primary hotspot for ant diversity worldwide. Each subfamily was represented in the POE samples proportional to its known diversity, both worldwide and when the Neotropics are considered separately. The vast majority (94%) of interceptions involved the ant being transported with plant material. Plant material was most often in the form of ornamental plants, fruit, and *Acacia* trees. The taxonomic and biogeographic status of the ants documented in the POE dataset indicates that there is little bias in the transport of ants. The probability an ant subfamily will be unintentionally transported is a function of its prevalence.

The history of ant invasions is perhaps best documented in New Zealand, a nation with some of the most stringent regulations and accurate records regarding the intentional and unintentional import of ants (Harris et al. 2005; Lester 2005; Ward et al. 2006). Ward et al. (2006) examined two datasets of ant species intercepted in-transit by the New Zealand Ministry of Agriculture and Forestry. The first dataset is of historical POE records collected between 1955 and 2003. The second database was collected as part of the All Ants All Pathways (AAAP) initiative, in which all ants intercepted were catalogued and identified between December 2004 and June 2005. Among the POE records, there was a significant difference between the proportion of each subfamily collected and among ants in general, with Myrmicinae underrepresented and Dolichoderinae overrepresented. The difference between these results and the U.S. POE data examined by Suarez et al. (2005) may be due to the New Zealand dataset being almost 11 times larger. Of the New Zealand POE records, 27.7% were of the highly invasive big-headed ant, *P. megacephala*. The taxonomic composition of the New Zealand POE and AAAP datasets were similar.

The smaller AAAP dataset provides detailed information on the transport vectors and pathways responsible for transporting exotic ants into New Zealand (Ward et al. 2006). Similar to the U.S. dataset, ants were most commonly intercepted in plant material, primarily fresh fruit. Most ants were transported via maritime cargo (42.6%) and airline passengers (34.4%). The most prevalent pathways originated from Pacific Islands (64.4%) and Asia (22.2%). Interestingly, overall trade volume from the Pacific Islands to New Zealand represents less than 1%, with much greater levels of trade originating from the Neotropics (Ward et al. 2006).

Overall, studies of custom intercepts of exotic ant propagules indicates that there is no evidence any particular physical, behavioral, or phylogenetic trait increases the likelihood that an ant species will be transported anthropogenically (Suarez et al. 2005; Lester 2005; Ward et al. 2006). A global distribution – common among major invasive ants - is the only characteristic correlated with increased transport frequency. However, species that are intercepted in-transit are not representative of those that become successfully established (Suarez et al. 2005; Ward et al. 2006).

In North America, international trade was responsible for the unintentional introduction of both the red fire ant and the Argentine ant. In the 1930s, the red fire ant first arrived in the port city of Mobile, Alabama, most likely within earth ballast dumped from ships before taking goods back to South America (Lofgren 1986). Genetic analyses indicate that the Argentine ant invasion in North America originated from near the Argentinean port city of Rosario, an international shipping hub in the late nineteenth century (Tsutsui et al. 2001). In 1891, Ed Foster first discovered the Argentine ant in the North American port city of New Orleans, LA (Suarez et al. 2008). Simultaneously, the ant was being spread to other countries that heavily traded with South America, particularly Europe and South Africa (Suarez et al. 2008).

Transport – propagule pressure

Few individuals are necessary to initiate a self-sustaining ant population. In many species, such as the red imported fire ant, virgin queens participate in mating flights and afterward must be able to initiate new colonies without the aid of other individuals (Hölldobler and Wilson 1990). Thus, a single inseminated queen can successfully

establish a colony after transport to a novel location. However, mortality is high among solitary queens of most invasive ant species (Tsutsui and Suarez 2003). Most cosmopolitan invasive ants reproduce through budding – incipient nest fragments often include numerous workers and multiple reproductive gynes that do not have the fat storage necessary to survive alone (Keller and Passera 1989). The addition of as few as 10 sterile workers to a single queen significantly increases propagule survival in the Argentine ant and the big-headed ant, *Pheidole megacephala* (Chang 1985; Hee et al. 2000). In fact, Hee et al. (2000) found that 100% of Argentine ant propagules experienced queen mortality in the absence of workers. Queen mortality occurred in only 6% of propagules with workers present. Increasing the number of workers from 10 to 1000 did not significantly decrease mortality or increase per capita growth rate (Hee et al. 2000). While smaller propagules take longer to increase absolute population size and are more subject to environmental stochasticity, competition, and predation, a very small number of ants are required to initiate a population.

Empirical studies of population genetic structure among Argentine and red fire ant introductions support the likelihood that small propagules have initiated continental-scale invasions (Giraud et al. 2002; Ross and Shoemaker 2008). Ross and Shoemaker (2008) analyzed three classes of nuclear markers and mitochondrial DNA in red fire ants collected from both the native range and throughout North America. Consensus among all markers indicates that as few as 9-20 unrelated queens comprised the initial propagule that successfully colonized Mobile, Alabama in the 1930s. Only a few unrelated queens are necessary to significantly reduce the negative effects of a genetic bottleneck, such as loss of genetic diversity at the sex-determining locus. Homozygosity at the sex-

determination locus produces reproductively sterile diploid males; the population-wide frequency of diploid males often increases after a founder event (Crozier et al. 2002). Among European populations of the Argentine ant, 19-39 unrelated queens likely provided all the initial genetic diversity necessary to produce the multiple supercolonies that presently inhabit the continent (Giraud et al. 2002).

In addition to the size of individual propagules, the number of discrete introduction events to a single region (i.e. propagule number) positively correlates with the probability of successful establishment (Lockwood et al. 2005). Empirical evidence among insects, birds, and mammals demonstrates that the number of independent introduction events explains significant variation in establishment success (Cassey et al. 2004; Forsyth et al. 2004). Mikheyev et al. (2008) found a similar pattern when observing the distribution of the little fire ant, *Wasmannia auropunctata*, in Gabonese oil fields. The likelihood the little fire ant established at any given site increased with the number of drilling events – a proxy for propagule number. A dose-response establishment curve roughly follows a Poisson process with a 34% mean chance of establishment during each drilling event.

Propagule number can affect establishment probability through three potential mechanisms. First, if separate propagules arrive at an identical location and form a cohesive population, then total propagule size increases, which in turn elevate establishment success (Lockwood et al. 2005). This is particularly true among invasive ants, where even a slight increase in effective population size can have a significant impact on establishment success of Argentine ants (Hee et al. 2000). Second, due to the idiosyncratic nature of invasion success, multiple introductions increase the chance of

establishment by sheer chance alone, as was the case for the European starling and the house sparrow (Lockwood et al. 2005; Sakai et al. 2001). Third, multiple propagules that originate from different source populations and combine to form a single invasion can have greater genetic variability than native populations (Lockwood et al. 2005; Ahlroth et al. 2003). Multiple introductions of the lizard, *Anolis sagrei*, have created an introduced population in Florida with greater genetic variability than any single native population (Kolbe et al. 2004). However, an increase in genetic variability from multiple introductions may not be a strong factor promoting success among invasive ants.

Unicoloniality is the primary characteristic associated with invasive success in ants and is associated with significant reductions in genetic diversity (Holway et al. 2002; Suarez et al. 2008). Unicoloniality evolves when genetically derived recognition cues become homogenized after a genetic bottleneck (Suarez et al. 1999). All individuals in a population recognize each other as nestmates; thus, territorial behavior becomes absent and population density is no longer self-limiting. Unicolonial populations often exist at densities orders of magnitude higher than multicolonial conspecifics (Holway et al. 2002). Strong negative selection may act against the addition of new genetic variation to a unicolonial population by introduced propagules, because they harbor distinct recognition cues that identify them as conspecific competitors. Upon introduction, intraspecific aggression is high between incipient propagules and an established unicolonial population (Giraud et al. 2002). New propagules can survive and form separate supercolonies if they settle in uninvaded habitats and either grow rapidly or remain geographically isolated, as is the case in the Argentine ant in North America and Europe (Giraud et al. 2002; Buczowski et al. 2004).

Stage 2: Establishment

Analyses of exotic ants have elucidated specific natural history characteristics associated with establishment success after initial colonization. Ants with generalized nesting and feeding habits are more likely to become established in disturbed habitats (Holway et al. 2002). These habitats are the most likely to be associated with transport vectors, such as railways, airports, and seaports (Richardson and Pysek 2008). Among ants, ground nesting species are more likely to become permanently established than those that are strictly arboreal. Of the ant species intercepted in the U.S., 52% are arboreal, while only 14% of the species that have become established are arboreal (Suarez et al. 2005). Arboreal ants have more specialized requirements than ground-dwelling ants nesting in soil (Hölldobler and Wilson 1990; Lockwood et al. 2007). Additionally, certain common phylogenetic groups have never been found successfully establish outside of their native range. Leaf cutter ants of the genera *Acromyrmex* and *Atta* are common and widespread pests in the Neotropics (Vander Meer et al. 1990). However, the absence of these genera from introduced locations is likely due to climatic restraints, dependence on mutualistic fungi, and the low probability that a large queen from a monogynous nest will be successfully transported (Hölldobler and Wilson 1990; Currie et al. 2003).

Exotic ants tend to be smaller than native congeners (Passera 1994). Of 26 genera examined, exotic ants were smaller than congeners in 22 cases (McGlynn 1999b). Body size is the primary discriminating factor between ants that failed and succeeded to colonize New Zealand (Lester 2005). While the former study excluded species that

exhibit size polymorphism, such as *A. gracilipes*, *S. invicta*, and *S. geminata*, the inclusion of these species by Lester (2005) did not affect the results (Holway et al. 2002). While many hypotheses have been proposed to explain the correlation between establishment success and body size, evidence is generally lacking. Smaller ants may be better at avoiding predators or acquiring resources (Farji-Brener 2004). Smaller exotic ants tend to be more combative; the loss of a small individual is energetically less costly (McGlynn 1999b). A reduction in energy cost per individual can also lead to greater colony sizes, a primary factor in the competitive dominance of invasive ants (Johnson et al. 2007; Holway et al. 2002). Two characteristics, unicoloniality and budding reproduction, are more associated with invasiveness rather than successful establishment alone. Unicoloniality and budding reproduction in invasive ants are reviewed in depth elsewhere (e.g. Holway et al. 2002; Tsutsui and Suarez 2003; Suarez et al. 2008; Keller 1991).

Conclusion

Ants are frequently transported worldwide and become established in novel locations. Preventing invasions from progressing past these early stages is the most efficient strategy to prevent invasive ants from incurring significant ecological and economic damage (Tsutsui and Suarez 2003; Kolar and Lodge 1993). Successful prevention requires data on the processes and patterns that characterize the transport and establishment of exotic ants; however, until recently this data was distinctly lacking in the literature. Currently, in-depth customs interception data in the United States and New Zealand, greater knowledge of cosmopolitan invaders, and the identification of incipient

invasions provides the foundation for comprehensive conservation planning. Further study of known early stage invasions, such as: *Tetramorium tsushimae* in Missouri (Steiner et al. 2006; Steiner et al. 2008), *Myrmica rubra* in Maine (Groden et al. 2005), *Lasius neglectus* in Eastern Europe (Ugelvig et al. 2008), *Linepithema humile* in Japan (Sunamura et al. 2009), and *Myrmica specioides* in Washington (Jansen and Radchenko 2009) will hopefully show common patterns between each early stage in a successful ant invasion. The different characteristics necessary to advance through each stage in the invasion process indicates that separate conservation plans and strategies for preventing transport, colonization, and success are necessary to effectively prevent widespread ant invasions.

CHAPTER 2: POPULATION GENETIC STRUCTURE OF AN EARLY STAGE
INVASION: THE JAPANESE PAVEMENT ANT, *TETRAMORIUM TSUSHIMAE* IN
SAINT LOUIS, MO

Chapter Abstract

Invasive species are a leading cause of extinctions and have significant effects on ecosystems worldwide. Among these species, ants are among the most cosmopolitan and destructive. Nearly impossible to eradicate once widely established, there has been a recent focus on understanding the population genetics and biology characteristics of early stage invasions. The Japanese pavement ant, *Tetramorium tsushimae*, is one of the most recently documented ant invasions in North America. I analyzed microsatellite genotypes at 10 loci from individuals collected from 52 nests in the invaded range and 5 nests from native Japan. Low genetic differentiation at the level of the sub-population and nest suggests gene flow is occurring throughout the introduced population. However, high allelic diversity, significant isolation by distance, and sub-population structure determined by Bayesian methods indicate colonization likely did not occur recently and range expansion may have begun after an extended lag phase. The spatially disjunct nature of one sub-population located in central St. Louis, MO and the periphery of the range in central Missouri indicates that recent range expansion may have been characterized by frequent anthropogenic jump dispersal. This study provides insight into patterns of genetic diversity in an early stage ant invasion and identifies source regions and dispersal patterns within the invaded range.

Introduction

Invasive species undergo rapid range expansion and incur significant environmental, economic, and/or health costs (Lockwood et al. 2007). Invasive species may comprise a large proportion of local communities, adversely affecting native populations through competition, predation, disease, herbivory, parasitism, and the alteration of disturbance regimes (Gurevitch and Padilla 2004; Lockwood et al. 2007). Invasive ant species represent five of the 100 worst invasive species worldwide and have the capacity to inhabit most of the world's ecosystems (Lowe et al. 2001). In North America, the Argentine ant, *Linepithema humile*, and the red imported fire ant, *Solenopsis invicta*, have negatively affected native ant and arthropod communities (Holway et al. 2002). While its native range is restricted to Northern Argentina and Southern Brazil, the Argentine ant has already invaded 15 countries on six continents and numerous islands and has the potential to further spread to parts of Asia and Africa (Roura-Pascual et al. 2004).

Due to high population density and extensive geographic ranges, it is generally accepted that widespread, established biological invasions are essentially impossible to eradicate (Kolar and Lodge 2001; Simberloff 2003). While successful eradication may be achieved at isolated local scales, halting the growth of large invasive populations and their negative effects is rare and logistically complicated (Lockwood et al. 2007).

Recently, a major push in the study of biological invasions has been to understand the transportation, establishment, and early growth of incipient invasive populations (Holway et al. 2002; Kolar and Lodge 2001; Wilson 1986). Understanding the evolution and ecology of incipient invasions helps characterize the conditions that drive their

success, perhaps providing a means to prevent early invasions from establishing permanently. However, model systems of recent invasions are rare (Kolar and Lodge 2001; Holway et al. 2002). By nature, early invasions often escape notice or may be mistaken for innocuous exotic species. Therefore, it is imperative to study the few known recent ant invasions (e.g. *Myrmica rubra* in Maine, *Lasius neglectus* in Eastern Europe) to understand the factors that lead to rapid range expansion (Grodén et al. 2005; Ugelvig et al. 2008).

In the absence of historical records, population-level genetic analysis of known early stage invasions is the most widely applicable and standardized method for inferring past and current population growth and dispersal (Hastings et al. 2005; Peterson and Denno 1998; Ingram and Gordon 2003). Tracking the distribution and evolution of alleles uncovers patterns of gene flow, long-range dispersal, genetic bottlenecks, founder events, and drift (Avice 2004; Suarez et al. 2008). However, increasingly cosmopolitan biological invasions can leave complex signatures that render interpretation of genetic patterns unclear. Admixture, multiple global transport pathways, and high propagule pressure can cloud patterns of evolution in an incipient invasion *in situ*. The ideal model system to study early stage ant invasions would have a known native range and a single, contiguous exotic range.

The Japanese pavement ant, *Tetramorium tsushimae* is one of the most recently documented ant invasion in North America and one of few known early-stage ant invasions in the world (Steiner et al. 2006; Holway et al. 2002; Ugelvig et al. 2008). Native populations of the Japanese pavement ant are found throughout Japan, eastern China, Korea, and Mongolia (Steiner per. comm.; Steiner et al. 2006). Colonies inhabit

primarily open areas, such as grassland and disturbed urban areas where they nest primarily under stones (Imai et al. 2003). The Japanese pavement ant was first discovered outside its native range in 1988 when it occupied an area of approximately 600km² within the city of Saint Louis, Missouri and a few adjacent townships (Steiner et al. 2006; Trager per. comm.). Analysis of mitochondrial DNA markers show that the Japanese pavement ant invasion likely originated from Central Japan (Steiner et al. 2006). Since its discovery, the invaded range has expanded throughout Eastern Missouri and across the Mississippi River into Western Illinois. Where the Japanese pavement ant is abundant, common ant genera such as *Crematogaster*, *Formica*, *Camponotus*, *Myrmica*, *Lasius*, *Tapinoma*, *Prenolepis*, and *Paratrechina* are rare or absent (Steiner et al. 2006; Chapter 4).

With the suitable habitat distributed throughout the Midwest and no other known occurrences outside of the native range, this system is a simple model for studying the ecology and evolution of early biological invasions (Steiner et al. 2008). This study is the first to examine microsatellite variation among Japanese pavement ant nests in North America. I will address the following questions to better understand the historical dynamics of this invasion: i) is there any microsatellite sub-population structure within the invaded range? ii) What do patterns of genetic variation at different scales tell us about gene flow, genetic drift, and patterns of dispersal? iii) Is there any evidence that indicates whether the invasion has undergone a significant latent period between colonization and range expansion? Addressing these questions will provide insight into the origins and spread of the Japanese pavement ant invasion.

Methods

Specimen sampling

I collected 10 adult worker ants from each of 52 Japanese pavement ant nests between 5 May 2006 and 14 July 2007 (N = 52 nests). Nests were sampled throughout the known invaded range; samples were collected at a higher density where Japanese pavement ant abundance is highest (Steiner et al. 2006). I mapped the extent of the range by traveling away from Saint Louis, MO along major transportation corridors and periodically searching for nests of the Japanese pavement ant in preferred habitat until I could not longer locate the Japanese pavement ant (Figure 1; e.g. disturbed open soil or turf grass associated with human activity; Trager pers. comm.; Steiner et al. 2006). For each specimen sample I recorded global geographic position using a Garmin Geko 201 GPS device (Garmin International Inc.). Long distance, human-mediated dispersal drives large-scale spread in the Argentine ant, so the edge of the invaded range likely lies along major transportation corridors (Suarez et al. 2001).

F. Steiner and B. Schlick-Steiner of James Cook University, Townsville, Australia provided 5-8 adult worker ants from each of 5 colonies collected from putative source populations within the native Japanese range (Steiner et al. 2006). We placed all specimens in 95% ethanol and stored at -20°C for later genetic analyses.

Microsatellite analysis

In total 551 adult worker ants from 57 nests were genotyped at 10 microsatellite loci: Tspe51a, Tspe51b, Tspe51c, Tspe51h, Tspe52b, Tspe52k, Tspe53a, Tspe53b, Ttsu56d, and Ttsu54g (Steiner et al. 2008). Genomic DNA was extracted using a Sigma

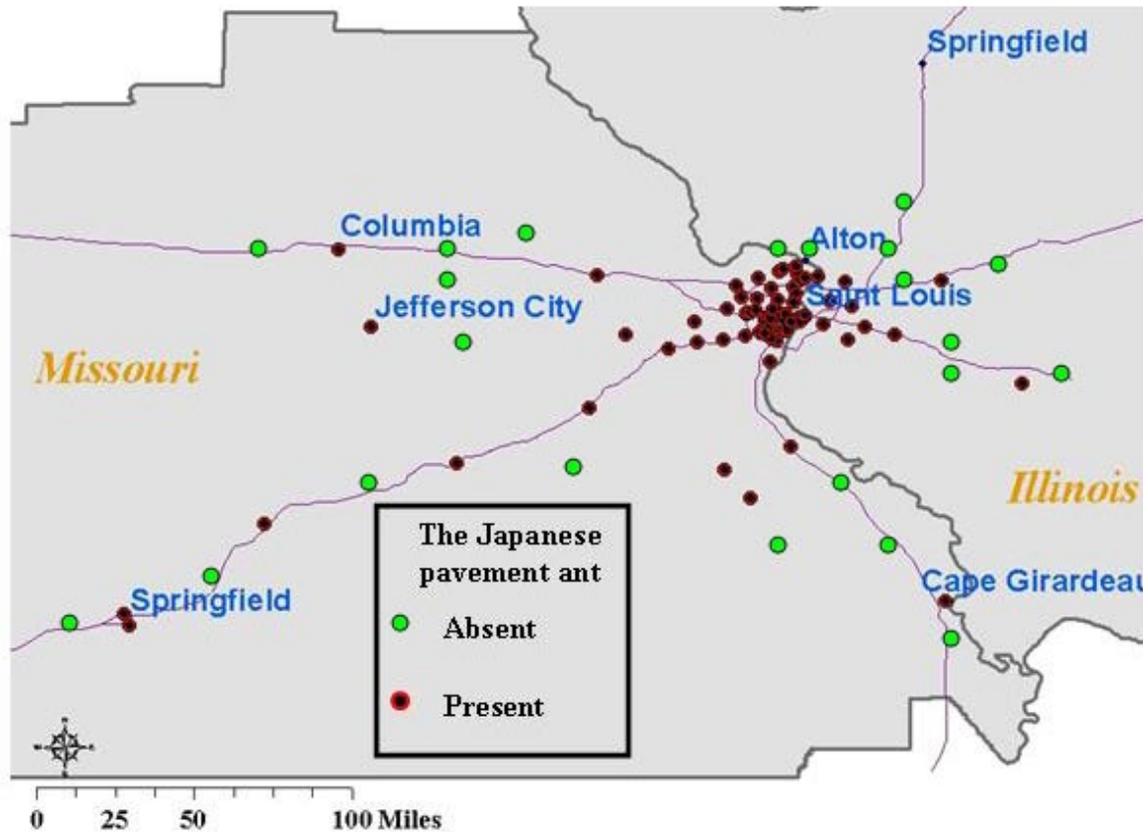


Figure 1. Distribution of the Japanese pavement ant, *Tetramorium tsushimae*, in 2006 as determined by presence/absence (N = 92; N = 22, respectively). Absence was determined by searching for 30 minutes in open canopy, disturbed, and well drained soils, the preferred nesting habitat (J. Trager pers. comm).

GenElute Mammalian DNA Prep Kit (Sigma-Aldrich) and eluted in 50 μ L of elution solution. Extracted DNA was stored at -20°C. Polymerase chain reactions (PCR) were conducted using a Qiagen HotStarTaq Plus Master Mix Kit in 20 μ L volumes. Forward primers were labeled with WellRED phosphodiesterase dyes D2, D3, D4 (Proligo). Each reaction contained 10 μ L HotStarTaq Plus Master Mix, 1.6 μ L of eluted sample DNA, 0.25 μ L-1 μ L of each primer, and ddH₂O. Due to differences in signal strength of the three different WellRED primer dyes, we used a 4:2.5:1 volume ratio of the D2, D3, and D4 labeled primers, respectively. I multiplexed 2 microsatellite loci per PCR using a

Eppendorf Mastercycler Gradient. After an initial 5 minute denaturation step at 95°C, we ran 32 cycles consisting of a 94°C denaturation step for 30 seconds, a 60°C annealing step for 1 minute, and a 72°C extension step for 45 seconds. This was followed by a final extension step at 68°C for 20 minutes. Fragment analysis was performed on a Beckman-Coulter CEQ8000. PCR and fragment analysis was repeated when sample analysis provided a poor or non-existent signal.

Genetic diversity and Hardy-Weinberg equilibrium

I used the programs Genepop 4.0 (Garnier-Gere and Dillmann 1992), FSTAT (Goudet 1995), and Arlequin 3.11 (Excoffier et al. 2005) to measure basic metrics of genetic diversity. Specifically, these analyses quantified for each of ten microsatellite loci: allele number (A), allelic richness (A_N), observed heterozygosity (H_O), expected heterozygosity (H_E), and the probability of deviation from Hardy-Weinberg equilibrium (P_{HWE}). Linkage disequilibrium was tested between all loci using the EM algorithm with 10,000 permutations. Each of these measures was calculated separately for the samples collected in North America and those originating from the native range in Japan.

Sub-population structure

Elucidating significant sub-population structure in geographic space not only provides insight into the dispersal and gene flow of a population, but also permits the use of higher order, hierarchical F-statistic analyses (e.g. AMOVA). In the case of the Japanese pavement ant in North America, the population is closely associated with anthropogenically disturbed habitats in Missouri and Illinois, so the potential for frequent

human-mediated jump dispersal is high. Therefore, *a priori* sub-population structure cannot be identified, based solely on geographic barriers. The programs Geneland and STRUCTURE 2.2 identify significant sub-population structure using two different algorithms. GPS coordinates and multilocus microsatellite genotypes were entered into the package Geneland (Guillot et al. 2005) in the R statistical environment (v.2.8.1; R Development Core Team 2008). By utilizing Markov Chain Monte Carlo-based inferences in a Bayesian model, genetic discontinuities are mapped in geographic space (Guillot et al. 2005; Guillot et al. 2005b). The program outputs a map containing putative subpopulation boundaries. Each nest location is assigned to a subpopulation at a specified probability.

STRUCTURE 2.2 utilizes a similar Markov Chain Monte Carlo model, but does not explicitly account for the spatial coordinates of samples (Pritchard et al. 2000). Also, unlike Geneland, STRUCTURE does not calculate the optimal number of subpopulations (K). I used the method of Evanno et al. (2005) to infer the optimal K value by calculating ΔK . This measure is the rate of change between successive K values of the log likelihood of the data [$\ln P(D)$]. I ran 10 iterations of the entire model for $1 \leq k \leq 15$. Each individual model was run for 100,000 MCMC iterations after an initial burn-in of 10,000 iterations. Final assignment of sub-population membership was based upon a consensus between multiple runs of both Geneland and STRUCTURE models.

Hierarchical F statistic analysis

To understand the structure of genetic variation at various biologically relevant scales, I performed a hierarchical AMOVA comparing genetic variation at four levels:

between sub-populations (as defined by Geneland and STRUCTURE), between nests, within nests, and within individuals. Using Arlequin 3.11, I performed a locus by locus AMOVA with 1000 permutations per locus (Excoffier et al. 2005). Confidence intervals of F-statistics were calculated by bootstrapping (Holzer et al. 2006). The levels were among sub-populations (F_{CT}), among nests (F_{ST}) within sub-populations (F_{SC}), within nests (F_{IS}), and within individuals (F_{IT}). I removed from the analyses nests that were identified by Geneland and STRUCTURE as belonging to different sub-populations.

Isolation by distance

Isolation by distance (IBD) is a positive correlation between geographic distance and genetic distance between nest pairs; IBD is indicative of short range natural dispersal (Suarez et al. 2001). Significant IBD can be measured by using a Mantel test to test for a significant correlation between pair-wise geographic and genetic distance matrices (Mantel 1967). I calculated a geographic distance matrix using data from all 520 individuals genotyped among 52 nests collected in the invaded range. Hawth's tools in ArcGIS 9.1 calculated the geographic distance matrix between collected nests (Beyer 2004). I calculated the pair-wise genetic distance between nests by constructing a matrix using Nei's D as a distance metric; this was performed using the adegenet package (Jombart 2008) in the R statistical environment (v.2.8.1; R Development Core Team 2008). Nei's D performs well when differentiating between groups at a fine geographic scale (Paetkau et al. 1997). I used the vegan package (Oksanen 2008) in the R statistical environment (v.2.8.1; R Development Core Team 2008) to calculate the two-tailed P value of a Mantel test run under 1000 randomizations.

Evidence of recent bottlenecks

Initial colonization of a species into a novel habitat often results in a genetic bottleneck, which is a sharp reduction in genetic variation resulting from the low effective population size of most invasive propagules (Lockwood et al. 2007). To determine whether a population has undergone a recent, significant genetic bottleneck without data from a reference source population, the program Bottleneck 1.2.02 finds heterozygote excess, a feature indicative of genetic bottlenecks (Cornuet and Luikart 1997). Heterozygote excess is when there is significantly more heterozygosity at a locus than is expected if the population is in Hardy-Weinberg equilibrium (Cornuet and Luikart 1997). Populations that have declined slowly or remained static over many generations reach an equilibrium state between allelic richness and heterozygosity. Populations that recently experienced a sharp decrease in size over few generations suffer from a reduction in allelic diversity, but levels of heterozygosity remain elevated, similar to those observed in the original source population (Cornuet and Luikart 1997). I used Bottleneck 1.2.02 under the two-phased model of mutation and the infinite allele model, as well as a Wilcoxon test to test for evidence of significant heterozygote excess within the whole North American population and within each nest (N=52). The Wilcoxon test is the most robust and powerful test associated with Bottleneck software and microsatellite data fits the two-phased model of mutation and the infinite allele model better than the step-wise mutation model (Di Rienzo et al. 1994; Luikart et al. 1997) The infinite allele model assumes that all possible alleles exists in a population between the smallest and largest microsatellite alleles (Luikart et al. 1997). The two-phased model allows for the

mutations greater in size than a single microsatellite repeat; therefore all alleles between the smallest and largest microsatellite alleles in a population do not necessarily exist (Di Rienzo et al. 1994).

Phylogenetic patterns

I created a neighbor-joining phylogram using Phylip 3.68 in order to test the monophyly of the North American invasion (Felsenstein 1989). The phylogram is built from a distance matrix based upon allele frequency data; I used the Cavalli-Sforza chord distance as a Euclidean measure of genetic distance, which has been shown to be accurate for microsatellites (Takezaki and Nei 1996).

Results

Genetic diversity, HWE, and Linkage Disequilibrium

I found all ten microsatellite loci to be polymorphic in both the invaded range and Japan. Within the U.S., allele number ranges from 8-31 with an average of 17.2 alleles among 520 individuals collected from 52 nests. While only 31 individuals were sampled from 5 nests in Japan, allele number ranges from 6-19 with a mean of 11.9 alleles per locus. There were more alleles present among the Japanese samples for loci Ttsu56d, Tspe52k, and Tspe53a than among the U.S. samples. Heterozygosity within the invaded range is 0.421 (range = 0.17-0.776) and 0.614 (range = 0.53-0.703) among the Japanese samples. Among all samples, expected heterozygosity is higher for all loci than observed heterozygosity (mean $H_E = 0.606$ [U.S.], 0.839 [Japan]). Among the sampled North American nests, nine of the ten loci are not in Hardy-Weinberg Equilibrium (Tspe51h,

$P=0.068$) because of significant heterozygote deficiency (Table 1). None of the ten loci are in linkage disequilibrium within the invaded range; thus, data for all loci were considered independent and were used for subsequent analyses.

Sub-population structure

The most effective software for measuring sub-population structure using genetic data rely on Bayesian Markov chain Monte Carlo (MCMC) methods (Chen et al. 2007). However, different programs are based upon distinct algorithms and assumptions; depending on the data, some models perform better than others (Chen et al. 2007; Coulon et al. 2006). For example, Geneland and Structure vary in their accuracy depending on the frequency of admixture, an often unknown quantity (Chen et al. 2007).

When optimizing K it is beneficial to utilize multiple methods and combine the results into a consensus model. Using STRUCTURE and the ΔK method proposed by Evanno et al. (2005) for determining the optimal value of K , I found that $K=2$ in the introduced Japanese pavement ant population. One sub-population was comprised of only two nests that were located in the far-eastern portion of the range (ID# 2237, Ashley, IL; ID# 2266, Troy, IL). The other sub-population included all other 500 individuals genotyped. Geneland indicated the presence of three subpopulations: ID# 2237, ID# 2266, and all other nests.

Genetic differentiation between the two far-eastern nests and the rest of the collected samples was significant for both models. Geneland and STRUCTURE are highly sensitive to the relative strength of genetic differentiation between sub-

Table 1: Genetic diversity of *Tetramorium tsushimae* in North America. We sampled 10 individuals from each of 52 nests located throughout the invaded north American range. The parameters calculated were: A, number of alleles; A_N , allelic diversity; H_E , expected heterozygosity; H_O , observed heterozygosity; P_{HWE} , P value testing against Hardy-Weinberg equilibrium.

Locus	U.S.A (N=520)				
	A	A_N	H_E	H_O	P_{HWE}
Tspe53a	10	9.863	0.403	0.205	0.003
Tspe51c	23	22.625	0.874	0.593	0.000
Tspe51a	10	9.766	0.312	0.17	0.000
Tspe51b	31	29.633	0.781	0.585	0.000
Ttsu54g	18	17.49	0.652	0.455	0.000
Tspe52b	26	25.162	0.768	0.514	0.000
Tspe51h	27	27	0.87	0.776	0.068
Tspe53b	8	8	0.478	0.313	0.005
Tspe52k	9	8.754	0.317	0.178	0.006
Ttsu56d	10	9.79	0.601	0.421	0.000
Mean	17.2	16.808	0.606	0.421	0.000

populations, so strong differentiation between the eastern and western portion of the range can mask significant genetic discontinuities elsewhere (i.e. F_{ST} ; Chen et al. 2007). Therefore, I tested for the existence of additional sub-population structure by running a STRUCTURE model with $K = 3$ and Geneland with the far-eastern nests excluded from the dataset. When $K = 3$, STRUCTURE indicates sub-population 1 (hereafter designated SP1) as including nests primarily collected within the suburbs of metropolitan St. Louis (Table 2; Figure 2). Sub-population 2 (SP2) included nests collected from downtown St. Louis and areas west of metropolitan St. Louis. Sub-population 3 (SP3) included nests ID# 2237 and ID# 2266.

When I removed nests ID# 2237 and ID# 2266 from the Geneland model, it determined that there are two significant sub-populations that roughly correspond to SP1 and SP2 of the previous STRUCTURE model (Table 2; Figure 3). I removed all nests

Table 2: Nests sampled for microsatellite analysis. Each nest has been assigned a geospatial location, nest ID# and sub-population membership based upon microsatellite analyses conducted with Geneland and Structure software. Sites names in bold face were designated different sub-population identities by Geneland and STRUCTURE.

Longitude	Latitude	Nest ID#	Location	State	Geneland			Structure											
					Sub-Population #	Sub-Pop. 1	Sub-Pop. 2	Sub-Pop. 1	Sub-Pop. 2	Sub-Pop. 3									
138.617	35.529	1201	Hirakawa, Japan																
140.883	38.267	1205	Sendai, Japan																
139.860	35.663	1207	Tokyo, Japan																
130.733	33.750	1243	Nogata, Japan																
136.487	34.711	1244	Tsu, Japan																
-90.366	38.622	2148	Tilles Park	MO		1			0.73		0.27								0.00
-90.266	38.565	2156	Carondolet park	MO		1			0.80		0.20								0.00
-90.344	38.623	2159	Strasser Rd. park	MO		1			0.76		0.24								0.00
-90.355	38.578	2162	Webster G. Mem. Park	MO		1			0.55		0.45								0.00
-90.369	38.556	2167	White Cliff Park	MO		1			0.86		0.14								0.00
-90.235	38.768	2169	Bellefontaine CA	MO		1			0.85		0.15								0.00
-90.497	38.775	2190	Boonelick Park	MO		1			0.43		0.57								0.00
-90.292	38.644	2191	Forest Park	MO		2			0.88		0.12								0.00
-90.278	38.642	2192	Forest Park	MO		2			0.59		0.41								0.00
-90.258	38.608	2193	Tower Grove Park	MO		2			0.28		0.72								0.00
-90.257	38.607	2194	Tower Grove Park	MO		2			0.60		0.26								0.14
-90.282	38.854	2199	Sioux Passage Cty Park	MO		2			0.82		0.17								0.00
-90.184	38.810	2202	Columbia Bottom	MO		2			0.91		0.08								0.01
-90.276	38.641	2205	Forest Park	MO		2			0.94		0.05								0.01
-90.807	38.480	2206	Shaw Nature Preserve	MO		2			0.09		0.90								0.00
-90.560	38.526	2207	Tyson Research Center	MO		2			0.93		0.07								0.00
-90.211	38.627	2209	Upper Limits	MO		1			0.83		0.17								0.00

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Table 2: (continued)

Longitude	Latitude	Nest ID#	Location	State	Geneland		Structure	
					Sub-Population #	Sub-Pop. 1	Sub-Pop. 2	Sub-Pop. 3
-89.967	38.677	2212	Glidden Park	IL	1	0.64	0.35	0.00
-89.907	38.586	2213	O'Fallon Com. Park	IL	1	0.58	0.42	0.00
-89.986	38.522	2214	Belleville Plst. Vly. Park	IL	1	0.89	0.10	0.01
-90.102	38.599	2215	Frank Holden SP	IL	1	0.96	0.03	0.01
-90.217	38.616	2216	Lafayette Park	MO	1	0.82	0.17	0.00
-90.273	38.641	2217	Park	MO	2	0.90	0.09	0.00
-90.273	38.642	2218	Park	MO	2	0.95	0.05	0.00
-90.542	38.665	2219	Faust Park	MO	1	0.89	0.11	0.00
-90.679	38.510	2225	Six Flags	MO	1	0.98	0.02	0.00
-90.182	38.631	2227	Laclede's Landing	MO	2	0.69	0.31	0.00
-90.183	38.631	2228	Laclede's Landing	MO	2	0.56	0.43	0.01
-90.183	38.632	2229	Laclede's Landing	MO	2	0.63	0.36	0.00
-90.190	38.626	2230	City hall Park	MO	2	0.86	0.14	0.00
-90.207	38.605	2233	Shenandoah @ 9th St.	MO	1	0.49	0.51	0.00
-90.251	38.606	2234	Tower Grove Park	MO	1	0.97	0.03	0.00
-90.250	38.606	2235	Tower Grove Park	MO	1	0.88	0.11	0.01
-89.773	38.548	2236	Rest Stop 64E	IL	1	0.90	0.10	0.00
-89.184	38.324	2237	Ashley City Park	IL	3	0.00	0.00	0.99
-91.006	38.548	2239	Washington HS	MO	2	0.21	0.79	0.00
-91.139	38.824	2243	Warrington	MO	2	0.28	0.72	0.00
-92.326	38.943	2246	Columbia	MO	2	0.23	0.77	0.00
-92.177	38.582	2247	Jefferson City	MO	2	0.31	0.67	0.01
-90.343	38.421	2257	Widmer Park	MO	2	0.08	0.91	0.00
-90.249	38.030	2258	Rest Stop I-55	MO	2	0.44	0.56	0.00

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Table 2: (continued)

Longitude	Latitude	Nest ID#	Location	State	Geneland			Structure		
					Sub-Population #	Sub-Pop. 1	Sub-Pop. 2	Sub-Pop. 1	Sub-Pop. 2	Sub-Pop. 3
-90.183	38.631	2264	Laclede's Landing	MO	2	0.22	0.78	0.00	0.00	0.00
-90.184	38.638	2265	Laclede's Landing	MO	2	0.22	0.77	0.01	0.01	0.01
-89.556	38.796	2266	Troy	IL	3	0.00	0.00	0.99	0.99	0.99
-90.223	38.861	2270	West Alton	MO	2	0.10	0.89	0.00	0.00	0.00
-92.664	37.670	2277	Lebanon	MO	2	0.62	0.38	0.00	0.00	0.00
-91.780	37.951	2280	Rolla	MO	2	0.01	0.99	0.00	0.00	0.00
-91.171	38.209	2281	Sullivan	MO	2	0.09	0.90	0.00	0.00	0.00
-90.244	38.637	2303	Laclede Ave. Post Office	MO	2	0.12	0.88	0.01	0.01	0.01
-90.257	38.643	2304	Maryland Ave. Dog Park	MO	2	0.07	0.92	0.00	0.00	0.00
-90.272	38.628	2305	Saint Louis Science Center	MO	2	0.31	0.69	0.00	0.00	0.00
-90.211	38.628	2307	Upper Limits	MO	2	0.47	0.53	0.00	0.00	0.00

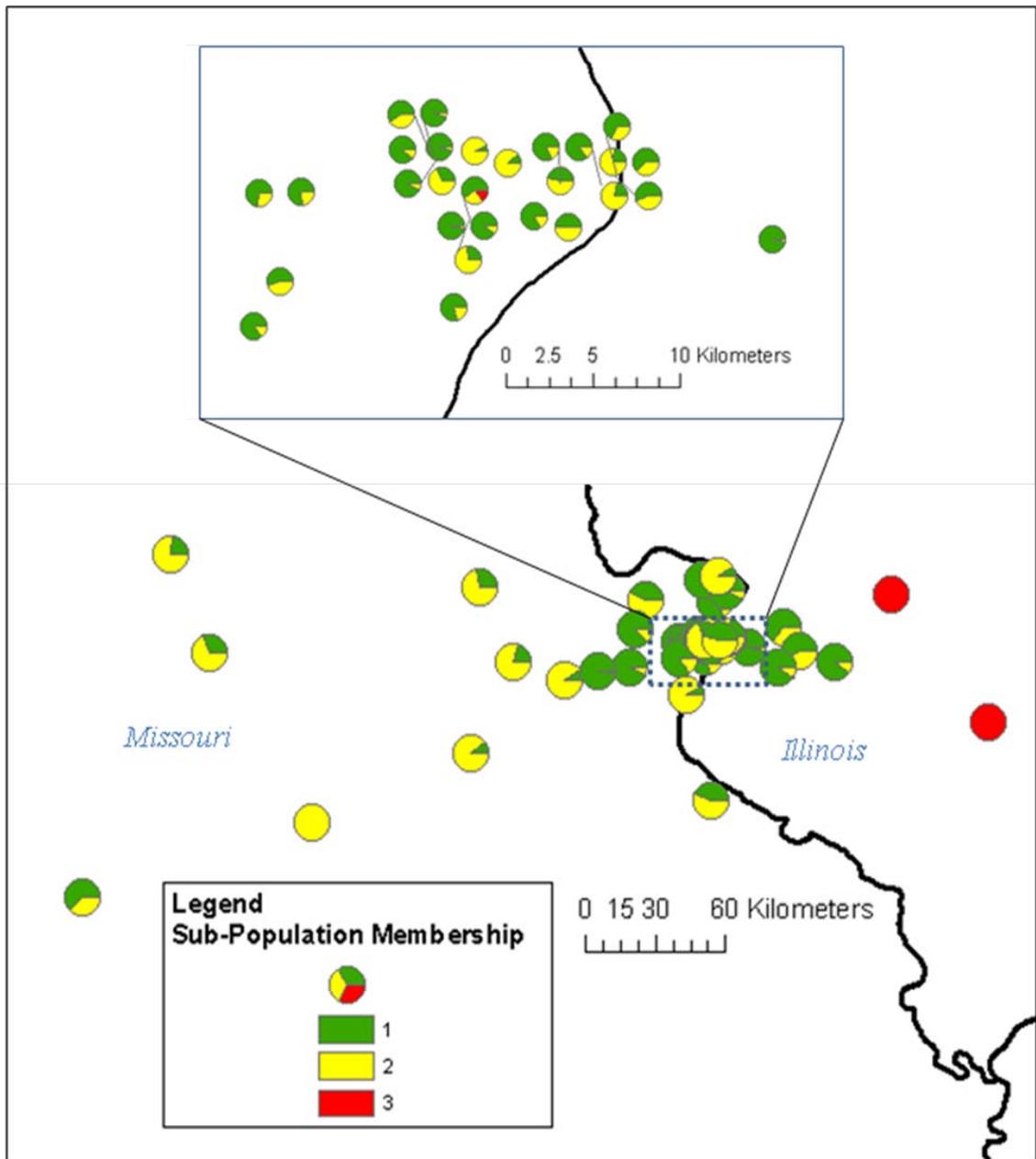


Figure 2. Map of nests collected for microsatellite analysis. The program STRUCTURE 2.2 identified three distinct sub-populations based upon the distribution of alleles at ten polymorphic microsatellite loci. Each pie chart represents a single nest of ten individual ants and displays the probability each nest is a member of each of the three sub-populations.

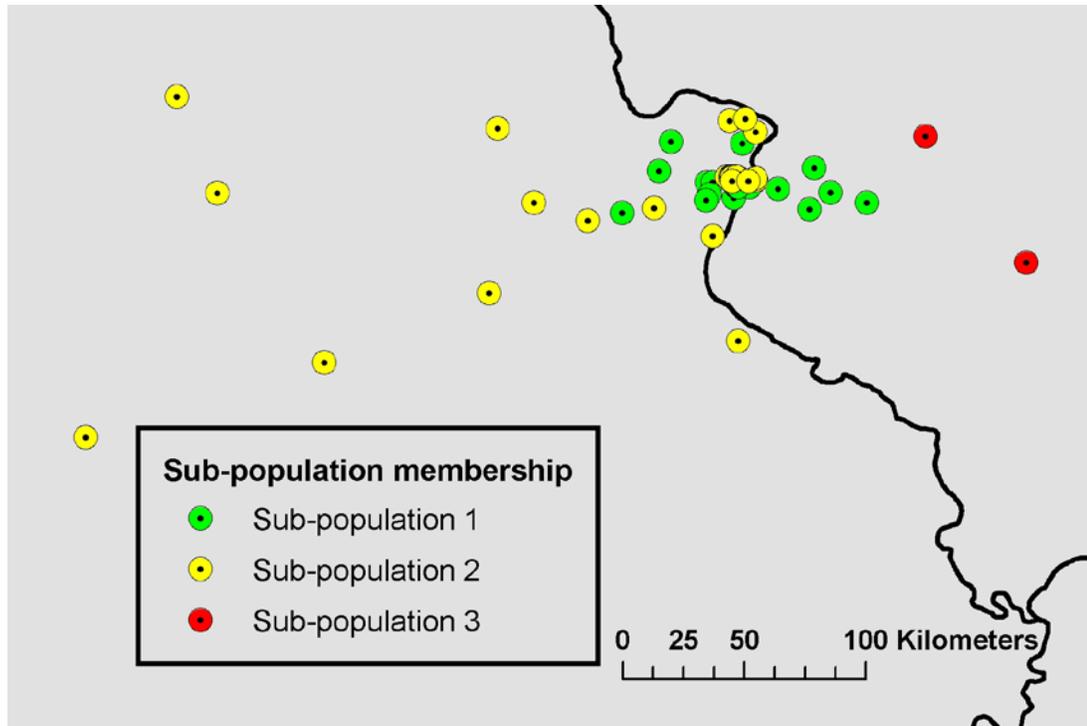


Figure 3. Map of nests collected for microsatellite analysis. The program Geneland identified significant genetic discontinuities in geographic space, thereby identifying three distinct sub-populations. Each individual was assigned sub-population membership with a probability of greater than 0.95.

from analyses that incorporate sub-population identity (e.g. hierarchical AMOVA) in which sub-population membership was incongruent between the Geneland and STRUCTURE models ($N = 16$ nests). The majority of these nests are located in the central and downtown portion of St. Louis, MO.

Table 3 provides information on the distribution of genetic diversity of SP1, SP2, SP3, the Japanese samples, and nests of which Geneland and STRUCTURE assigned to different sub-populations. All sub-populations in the introduced range have lower heterozygosity than expected at Hardy-Weinberg equilibrium. The per capita number of

Table 3: AMOVA table of hierarchical F-statistic analysis implemented in Arlequin 3.11. All F values have a $P < 0.0001$. Separate analyses were performed on various combinations of sub-populations as denoted table 2. When Geneland and STRUCTURE provided conflicting results regarding the sub-population identity of a nest it is designated as having an unknown sub-population identity (1 or 2). The designation "None" represents an AMOVA using all nests with no sub-population designation. When subpopulations were combined into a single group for AMOVA analysis, the '+' symbol is used.

Sub-population	Among Sub-populations		Among Nests		Within Nests		Within Individuals	
	Variation	F_{CT}	Variation	F_{SC}/F_{ST}	Variation	F_{IS}	Variation	F_{IT}
Sub-population	Among Sub-populations	FCT	Among Nests	FSC/FST	Within Nests	FIS	Within Individuals	FIT
None								
1, 2, 3	0.1136	0.1136	0.0942	0.1063	0.1878	0.2371	0.6043	0.3957
1			0.0901	0.0901	0.1707	0.1877	0.7391	0.2609
2			0.1103	0.1103	0.2268	0.2550	0.6629	0.3371
3			0.3204	0.3204	-0.0082	-0.0120	0.6878	0.3122
1 or 2			0.0919	0.0919	0.1608	0.1771	0.7473	0.2527
1, 2	0.0516	0.0516	0.0952	0.1003	0.2140	0.2508	0.6393	0.3607
(1+2), 3	0.3108	0.3108	0.0894	0.1297	0.1422	0.2371	0.4576	0.5424

unique alleles is higher in SP3 (0.7) than in both SP1 (0.050) and SP2 (0.056). The proportion of alleles shared between each sub-population and the Japanese nests ranges from (0.3896-0.4656). Despite having a greater proportion of unique alleles, SP3 does not share a greater proportion of its alleles with Japan than does SP2.

Hierarchical AMOVA and IBD

As predicted by Geneland and Structure, SP3 is highly distinct from SP2 and SP1 (Table 4). $F_{CT} = 0.3108$ between SP3 and all other samples combined. Within SP3, $F_{ST} = 0.3204$ between the two nest sites, indicating that gene flow is relatively low between ID# 2237 (Ashley, IL) and ID# 2266 (Troy, IL). Throughout the remainder of the range, gene flow between nests and SP1 and SP2 is relatively high; 63.9% of the genetic variation is partitioned at the individual level and not between nests or sub-populations (Table 5; $F_{IT} = 0.3270$). Despite high levels of gene flow and only 1.89% of the total genetic variation explained at the sub-population level between SP1 and SP2, there is significant isolation by distance throughout the entire introduced range (Mantel test, $r = 0.2779$, $P = 0.045$).

Tests for genetic bottlenecks

All ten loci were heterozygote deficient, showing no evidence of a range-wide genetic bottleneck ($P = > 0.999$). The only nest with significant heterozygote excess under the two-phased model of mutation was collected on the University of Missouri campus in Columbia, MO (ID# 2246, $P = 0.042$). *T. tsushimae* in this location is limited to disturbed soil around the Biology Department building and the nearest adjacent

Table 4: Measures of genetic variation among sub-populations of the Japanese pavement ant and Japanese samples. The sub-population designation "1 or 2" indicates nests that were assigned to different sub-populations by Geneland and STRUCTURE. The following data is listed in the table: number of genotyped individuals (N), mean allelic richness (A), the mean proportion of alleles in a sub-population that were found among the sampled Japanese nests (J.A.), the per capita number of unique alleles (U.A.), expected heterozygosity (H_E), observed heterozygosity (H_O), and the mean probability of being in Hardy-Weinberg equilibrium (P_{HWE}).

Sub-population	N	A	J.A.	U.A.	H_E	H_O	P_{HWE}
1	170	11.2	0.3896	0.0470	0.5100	0.4190	0.001
2	170	10.8	0.4656	0.0529	0.5160	0.4060	0.001
3	20	6.3	0.4204	0.7000	0.5873	0.5800	0.001
1 or 2	160	10	0.4231	0.0500	0.4482	0.4170	0.001
Japanese	31	11.9		1.8387	0.617	0.614	0.006

recorded presence locality is over 50km away in Jefferson City, MO. Under the infinite allele model, four other nests displayed significant heterozygote excess (ID# 2239, $P = 0.019$; ID# 2264, $P = 0.0005$; ID# 2281, $P = 0.012$; ID# 2258, $P = 0.014$). Three are nests located in the periphery of the known range and one (ID# 2264) is located near the Mississippi River in St. Louis, MO.

Phylogenetic analysis

A neighbor-joining tree rooted with nest ID# 1201 from Hirakawa, Japan groups all Japanese nests basal to the monophyletic North American population (Figure 4). Separate trees rooted with each of the other Japanese nests yield similar results. Within the invaded range, the three nests collected from the far eastern portion of the range are basal to the rest of the North American nests (ID#: 2266, 2237, and 2236). The rest of the samples separate into two general clades. One clade is comprised primarily of nests from SP2 which includes nests from downtown St. Louis and nests from the periphery of the range found along primary transportation corridors. The second clade is comprised of nests from both SP1, SP2, and those nests that harbor alleles from both SP1 and SP2.

Discussion

The aim of this study was to use methods in population genetic analysis to estimate characteristics of the invasion of the Japanese pavement ant, *T. tsushimae*, in North America. I analyzed 520 individuals from 52 nests located throughout the known introduced range, as well as 31 individuals from 5 colonies in native at ten polymorphic microsatellite loci. Results from this study provide evidence regarding the potential

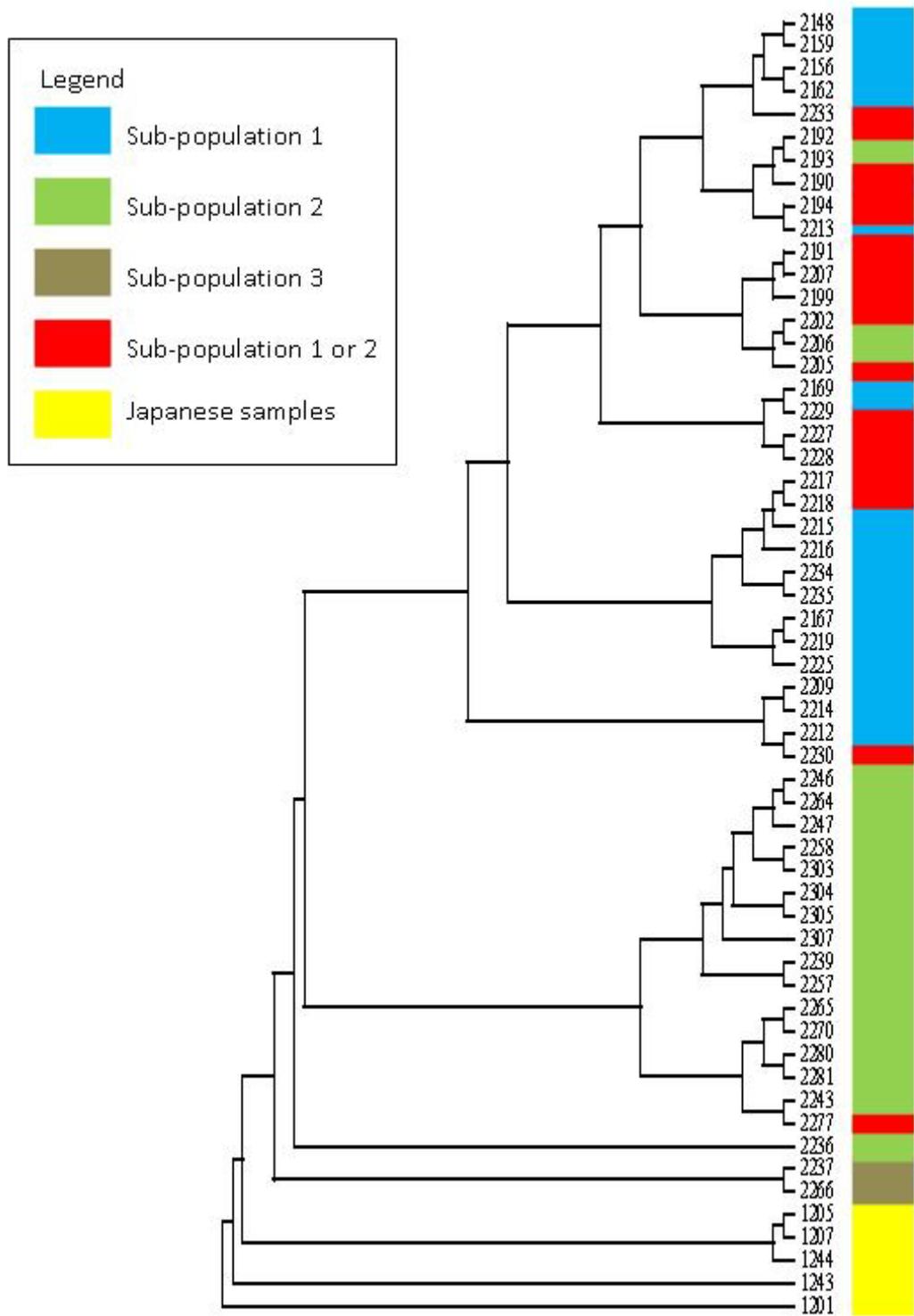


Figure 4. Neighbor-joining tree of North American and Japanese nests. The tree is based upon Cavalli-Sforza distance measures between the microsatellite genotypes of 5-10 individuals per nest. Sub-population assignments are based a consensus of Geneland and STRUCTURE models. Nests assigned different sub-population identities by both models are indicated in red.

number of historical introduction events, whether or not there was a significant lag period between colonization and range expansion, and the nature of dispersal patterns and gene flow.

Number of introductions

Knowledge of phylogenetic patterns, the scale of gene flow, and the geographic distribution of sub-populations can provide evidence for the number of introduction events that seeded an invasive population (Tsutsui et al .2001). A neighbor-joining tree based upon 10 microsatellite loci show that all North American samples formed a monophyletic group; all Japanese nests were basal to nests in the introduced range. A hierarchical AMOVA demonstrates that 15% of the total genetic variation is distributed between nests; the remaining 85% of microsatellite genetic variation is spread between individuals with no regard to nest of origin. The combined results of the neighbor-joining tree and AMOVA indicate significant gene flow throughout the introduced range (excluding SP3).

Despite high levels of gene flow between nests throughout most of the introduced population ($F_{IT} = 0.337$), Geneland and STRUCTURE models demonstrated the existence of three significant sub-populations. Sub-populations 1 and 2 (SP1, SP2) encompass most of the invaded range in Missouri and western Illinois. A hierarchical AMOVA shows low levels of genetic differentiation at the sub-population and nest levels between SP1 and SP2. 14.7% of the total genetic variation is explained by the distinction between SP1 and SP2. Conversely, this study demonstrates that a distinct genetic discontinuity separates

nests in the far eastern portion of the introduced range (SP3) from the remainder of the invasive population, as shown by analysis with Geneland. 31.1% of genetic variation in SP3 is distinct from the rest of the range ($F_{CT} = 0.311$). This is a greater proportion of genetic differentiation than between nests of in unicolonial populations of the Crazy ant, *Anoplolepis gracilipes* in Borneo ($F_{ST} = 0.230$) and the Argentine ant in the southeastern U.S. ($F_{ST} = 0.126 - 0.194$). Drescher et al. 2007; Buczkowski et al. 2004).

Despite the differentiation of SP3 from the rest of the introduced population, a few lines of evidence indicate that SP3 may not have resulted from a separate colonization event. SP3 does not share a greater proportion of alleles with Japanese colonies than do other sub-populations. The software STRUCTURE indicated that a single nest collected in St. Louis city at Tower Grove Park (ID# 2194) was admixed with SP3. While this could be the result of gene flow from Illinois to St. Louis, this sub-population could have also originated as a small propagule which was anthropogenically dispersed to Illinois. A genetic bottleneck and strong drift that resulted from isolation and small population size could have resulted in the genotypes of SP3 individuals being entirely composed of otherwise rare alleles (Ibrahim et al. 1996). The proportion of unique alleles in SP3 is 14 times larger than in any other sub-population.

Additional lines of evidence support the monophyly of the North American invasion. Steiner et al. (2006) used mitochondrial DNA to find that nests collected within the introduced range compose a monophyletic group with Japanese colonies in the Tokyo and Okayama, Japan area. A single introduction event is supported by the complete absence of *T. tsushimae* outside the native range of China, Japan, Korea, and Mongolia, except in Missouri and Illinois (Steiner et al. 2008).

Behavioral evidence supports a single introduction event. In chapter 3, I find that the North American population of the Japanese pavement ant may form a single unicolonial unit, with intraspecific aggression only present at low levels and in specific social contexts. This is in stark contrast to highly multicolonial Japanese colonies that defend territories that average only 30m² (Sanada-Morimura et al. 2006). Supercolonies that are derived from genetically distinct source populations are interact aggressively, despite a lack of aggression among individuals within a single supercolony (Giraud et al. 2002). Even if further invasion propagules arrived within the invaded range, strong negative selection would likely prevent establishment; aggression with a large, established unicolonial population would prevent incipient propagules from dispersing and growing (Giraud et al. 2002). However, it is unknown when unicolonialism evolved in this system. Separate propagules may have arrived between initial colonization and the evolution of a large, dense, unicolonial population. Future behavioral analysis of SP3 should ascertain whether or not the far-eastern portion of the introduced range is part of the main unicolonial population or if it is a distinct supercolony.

Age of the invasion

The North American population as a whole shows no evidence of heterozygote excess, with all ten genotyped microsatellite loci displaying a significant heterozygote deficit. The heterozygote excess method employed by Bottleneck software is the most accurate method for detecting the presence of recent bottlenecks in small populations (Balloux and Williams 2004). All ten microsatellite loci had significantly less heterozygosity than expected at Hardy-Weinberg equilibrium. Heterozygosity deficit can

be caused by inbreeding among small populations (Avisé 2004). This may indicate that the introduced population of the Japanese pavement ant existed for many generations in a restricted range at a relatively low population density. Future studies should make more detailed genetic comparisons between putative source populations in Japan and the North American population in order to determine if there was a significant genetic bottleneck subsequent to colonization.

Allelic diversity in the introduced range is greater than allelic diversity found in invasive Argentine ant and *Anoplolepis gracilipes* invasions that have lasted numerous decades (Buczkowski et al. 2004; Corin et al. 2007). There is an average of 17.2 alleles per locus among Japanese pavement ant nests. Microsatellite loci among populations of the Argentine ant in the Southeast U.S., California, and New Zealand have an allelic diversity of 3.3, 6.7, and 3.2, respectively (Buczkowski et al. 2004; Corin et al. 2007). A widespread population of *Anoplolepis gracilipes* in Sabah, Malaysia that was likely formed by multiple introductions has an average microsatellite allelic diversity of 7.3 (Drescher et al. 2007). The spread of these two invasive ant species has decreased allelic richness to levels lower than found within the Japanese pavement ant invasion. The greater numbers of alleles, particular those that may be unique to North America may be caused by mutation during a prolonged lag period. Further genetic testing of native Japanese colonies will provide a more accurate number of the proportion of uniquely North American alleles. However, a large propagule of numerous unrelated queens arriving in North America recently could account for high levels of allelic richness rather than mutations that evolved *in situ* over a long period of time.

Significant genetic differentiation between samples collected in downtown St. Louis and suburban St. Louis County are additional lines of evidence that the Japanese pavement ant may have existed in St. Louis over many generations. If the invasion was the result of a single introduction event, a rapidly expanding population does not commonly harbor significant genetic structure or IBD (Slatkin 1993). A Mantel test showed significant isolation by distance (IBD), a characteristic not common among recently established unicolonial ant invasions (Ingram and Gordon 2003). Many generations and potentially many decades pass before patterns of IBD form at the scale of 10-100km in a unicolonial population (Sundstrom et al. 2005). This effect may be increased by the absence of long-distance mating flights by both males and queens; long-distance dispersal is limited to anthropogenic vectors in the North American population (J. Trager pers. comm.). The general geographic separation of SP1 in suburban St. Louis County and SP2 in downtown St. Louis and Central Missouri as described by Geneland and STRUCTURE analyses also supports a historical origin of the Japanese pavement ant invasion.

Despite the possibility that the Japanese pavement ant may have existed in North America for over 100 years, the population has undergone rapid range expansion during the past two decades. In 1988, *T. tsushimae* was present in 600km² of urban habitat in St. Louis, MO. By 1990, it had advanced through the suburbs of Ballwin and Glencoe, Missouri; by the year 2004 it inhabited an area of 4000km² (Steiner et al. 2006). In 2005, satellite populations were found at the known extent of the current range, in Springfield and Columbia, MO, 348km and 203km from the invasion's epicenter, respectively. In this study, I found significant heterozygote excess in 3 nests found in the periphery of the

range, which is an indicator of recent genetic bottlenecks (Luikart et al 1997). Genetic bottlenecks can result from long distance jump dispersal (Suarez et al. 2001). Since winged dispersal of the Japanese pavement ant has never been observed in the introduced range, it is likely that dispersal vectors were anthropogenic (J. Trager pers. comm.). The source of these peripheral populations may be downtown St. Louis. SP2 includes nests located at the periphery and the urban center of the current range and are separated by nests in SP1 that primarily inhabit suburban St. Louis. Recent anthropogenic dispersal along major transportation corridors would explain geographically separated nests having relatively similar microsatellite genotypes (Suarez et al. 2001).

The combination of recent range expansion and the overall age of the invasion suggest a considerable lag period during which the Japanese pavement ant inhabited St. Louis, MO without undergoing rapid population growth and long-distance dispersal. A lag period is a common stage among biological invasions (Sakai et al. 2001; Crooks 2005; Catford et al. 2009). There are three primary hypotheses that explain extended lag phases during invasions. First, a small population undergoing exponential population growth inherently takes many generations for the rate of population growth to accelerate (Hengeveld 1989). The invasion of the European collared dove, *Streptopelia decaocto*, throughout the Bahamas and Florida is a well documented example of how exponential population growth of a small, incipient invasion can have a significant lag between colonization and range expansion (Hengeveld 1993). While this mechanism is inherent to any lag period, it does not fully explain lag in a system that is unicolonial and dominant in invaded ant communities (Chapter 3 and 4). After initially colonizing Japan in 1993, the unicolonial Argentine ant rapidly spread across coastal Western Japan in only a

decade (Sunamura et al. 2009). The red fire ant rapidly invaded the Southeastern U.S. over 30 years without a significant lag period even though the invasion was likely initiated with only 9-20 unrelated queens (Ross and Shoemaker 2008).

Changes in the biotic or abiotic environment that promote the spread of an invader can drive an invasion out of a static lag period (Crooks 2005). Climate change, local extinction of predators, or an increase in anthropogenic dispersal can modify an environment to a state that promotes the growth and dispersal of an invasive population (Cohen and Carlton 1998; Mooney and Hobbs 2000; Shea and Chesson 2002). An increase in urban sprawl and movement of landscaping and nursery materials that could harbor ant propagules may have had a significant effect on recent range expansion of the Japanese pavement ant. Missouri is composed of at least 135,000 acres of private residential yards, many of these acres have been recently developed (McKinney 2002).

Lastly, evolutionary changes may be necessary for an introduced species to become highly invasive. This could be in the form of selection, drift, or increased genetic diversity due to subsequent introduction events after initial colonization (Crooks 2005). Among invasive ants, unicoloniality is the primary factor driving ecological success and population growth (Holway et al. 2002). Most often, unicoloniality arises from genetic bottlenecks that homogenize chemical cues used for recognition (Suarez et al. 2008). Since native populations of *T. tsushimae* are multicolonial, unicolonialism in the North American population evolved *in situ* (Sanada-Morimura et al. 2006; Chapter 3). Nests that inhabit St. Louis city – the potential site of initial colonization – have intermediate levels of intraspecific aggression between other introduced nests and multicolonial native Japanese colonies (Sanda-Morimura et al. 2006; Chapter 3). Intraspecific aggression is

reduced among nests further from the St. Louis, MO city center (Chapter 3). This indicates that serial genetic bottlenecks caused by jump dispersal may have led to the evolution of unicolonialism over time, a pattern found among North American Argentine ant populations in the Southeast and California (Buczowski et al. 2004). Therefore, a combination of exponential population growth, increased anthropogenic dispersal, and the evolution of unicoloniality are all factors that may have contributed to elevate this invasion from a static lag phase to rapid range expansion.

Potential invasion scenario

Analysis of microsatellite variation of *T. tsushimae* in both Japan and North America has provided evidence to support the following hypotheses: 1) the invasion is the result of a single introduction event; 2) initial colonization did not occur recently; 3) and after a significant lag period, rapid range expansion extended the invasion outside the St. Louis metropolitan area.

A probable scenario that would explain the geographic structure of genetic variation begins with an initial colonization in downtown St. Louis, MO on the shores of the Mississippi River or in Forest Park where large volumes of unregulated plant material were imported from Japan for the 1904 World's Fair (Steiner et al. 2006). Plant material is the primary transport vector of ants worldwide and multiple species, including the tree *Pseudolarix amabilis*, were imported and planted at the Japanese Pavilion in St. Louis around the turn of the century (Steiner et al. 2006; Ward et al. 2006; Suarez et al. 2005). St. Louis is the major trade hub of the American Midwest. St. Louis has the second largest inland port and is the third largest rail center in the U.S. (STLRCGA 2009). This

makes downtown St. Louis a likely location for the frequent arrival of exotic species. Future studies should search for associations between imported Japanese vegetation and Japanese pavement ant nests.

After colonization of St. Louis, *T. tsushimae* likely spread slowly through budding and/or anthropogenic dispersal within the urban limits of St. Louis. Significant differentiation between SP1 and SP2 and IBD provide evidence for long-term reduced gene flow between urban and suburban St. Louis. It was potentially at this time that a small propagule was transported from Tower Grove Park in St. Louis city to an isolated location in Illinois where SP3 was established. Subsequent to a significant lag period, rapid range expansion spread the invasion to its current extent throughout Eastern Missouri. Shared SP2 membership between peripheral nests and urban downtown St. Louis nests suggests recent, long-distance jump dispersal westward from sources in urban St. Louis.

The invasion of the Japanese pavement ant, *T. tsushimae*, in North America represents one of the few known temperate ant invasions and suitable habitat is found throughout the Midwestern states (Steiner et al. 2008). Understanding the dynamics of this invasion not only provides valuable information that might help slow its spread, but also gives insight into the evolution of invasions entering the ultimate stage of widespread dispersal and impact. Future studies should monitor the mechanisms of dispersal in this system; even rare anthropogenic jump dispersal can be the primary driver of range expansion and unicolonialism (Suarez et al. 2001; Shigesada et al. 1995). Identifying transportation vectors within the invaded range will provide an efficient means to slow range expansion. Also, future studies of the population genetic structure

and ecology studies of native populations of *T. tsushimae* will provide a valuable baseline for gauging the changes that occur in the early stages of an invasion (Suarez et al. 2008; Holway et al. 2002).

CHAPTER 3 - UNICOLONIALITY IN THE NORTH AMERICAN INVASIVE
POPULATION OF THE JAPANESE PAVEMENT ANT, *TETRAMORIUM*
TSUSHIMAE

Chapter Abstract

Unicoloniality is characterized by a lack of intraspecific aggression throughout an entire population. This otherwise rare trait is a common characteristic among the world's most invasive ant species and is correlated with competitive success in invaded habitats. This study examines whether the recently discovered invasion of the Japanese pavement ant, *Tetramorium tsushimae*, in St. Louis, Missouri is unicolonial. Discovered in 1988, the Japanese pavement ant has rapidly spread throughout Eastern Missouri and Western Illinois. I conducted laboratory aggression assays between nests collected throughout the invaded range to determine the degree of unicolonialism present in the invaded range. I also measured microsatellite distance, differences in cuticular hydrocarbon profile, and geographic distance to understand how these factors correlate with levels of intraspecific aggression. I find that the North American population of the Japanese pavement ant is unicolonial, exhibiting intraspecific aggression at levels significantly lower than found among multicolonial conspecifics in the native Japanese range. There was an increase in aggression when at least one nest was collected from urban, downtown St. Louis, MO. Generalized estimating equations (GEE) determined that genetic distance best explained variation in aggression, followed by differences in cuticular hydrocarbon profile and geographic distance. I conclude that unicoloniality likely evolved *in situ* and may have been the result of changes in patterns of genetic diversity as the incipient invasion expanded.

Introduction

Invasive ant species have been directly linked to dramatic decreases in biodiversity, loss of ecosystem function, and severe economic costs (Pimentel et al. 2000; McGlynn 1999; Holway et al. 2002; Gurevitch and Padilla 2004). Reduction of native ant biodiversity resulting from ant invasions can have far reaching effects. Ants often comprise a large proportion of animal biomass within a community (~15% of animal biomass worldwide) and are important predators, prey, pollinators, mutualists, and detritivores that significantly alter habitat structure and composition (e.g. nutrient cycling and soil turnover; Hölldobler and Wilson 1990; Underwood and Fisher 2006).

A suite of natural history characteristics ubiquitous among invading ant populations have been shown to be significant drivers of population growth, ecological impact, and geographic spread (Holway et al. 2002; Tsutsui and Suarez 2003). Invasive ant colonies usually consist of a large network of interconnected nests (i.e. polydomy), many containing multiple reproductive queens (i.e. polygyny; Hölldobler and Wilson 1990; Tsutsui and Suarez 2003). Polygyny and polydomy both result in part from an overall decrease in intraspecific aggression. Intraspecific aggression varies across a continuum; while most ant colonies have single nests, a single queen, and are highly territorial, some have no territorial boundaries and entire populations can freely exchange individuals and share nest space (Suarez et al. 2008). This extreme level of cooperation, known as unicoloniality, is common among the planet's most notorious ant invasions (Tsutsui et al. 2003; Holway et al. 2002).

Unicoloniality may be the primary factor driving the expansion and ecological success of invasive ants in native communities (Holway et al. 2002). Nest density increases sharply in unicolonial populations, since intraspecific competition and territorial behavior is a primary determinant of colony density (Holldobler and Wilson 1990). Density of polygynous *Solenopsis geminata* and *S. invicta* nests is twice as large as that found among monogyne nests (MacKay et al. 1991; Macom and Porter 1996). Nest density and biomass of a unicolonial ant population that has monopolized a habitat can even be greater than the combined sum of all native ant individuals in an uninvaded habitat (Porter and Savignano 1990; Hoffmann et al. 1999; Human and Gordon 1997). Numerical superiority and the ability to redirect time and energy normally dedicated to intraspecific aggression combine to drastically alter invaded landscapes (Suarez et al. 2008).

Due to the significant impact of unicolonial ant invasions, it is imperative to understand the characteristics underlying this trait and the forces that drive its evolution. Mechanistically, unicoloniality results from an inability to accurately distinguish nestmates from non-nestmates. Individuals have a chemical odor derived both genetically and environmentally (Liang and Silverman 2000; Suarez et al. 2008). This odor is expressed by a suite of hydrocarbons embedded in the cuticle of the ant (Torres et al. 2007). The sum of these odors within a nest combine to form a template by which all strangers can be compared (Suarez et al. 2008; Torres et al. 2007). In the case of a territorial, multicolonial population, two interacting foragers compare the foreign scent to their own nest odor. If the difference is greater than a specific threshold, aggression is

initiated. In unicolonial societies, that recognition threshold is greater than the range of odors found within the population (Suarez et al. 2008).

The Japanese pavement ant, *Tetramorium tsushimae*, was discovered in the Saint Louis, Missouri metropolitan area in 1988 (Steiner et al. 2006). Since its discovery, the invasion has spread throughout Eastern Missouri and Western Illinois. Where the Japanese pavement ant is abundant, common ant genera such as *Crematogaster*, *Formica*, *Camponotus*, *Myrmica*, *Lasius*, *Tapinoma*, *Prenolepis*, and *Paratrechina* are rare or absent (Steiner et al. 2006; Chapter 4). Native to Eastern Asia, an analysis of mitochondrial and microsatellite DNA supports a single invasion event originating from Central Japan (Steiner et al. 2006; Chapter 2). In its native range, *T. tsushimae* is highly aggressive and nest territories have an average area of 32m² (Sanada-Morimura et al. 2006). While no studies have quantified aggression in the introduced range, intraspecific aggression has rarely been witnessed and this invasion may be unicolonial (Trager per. comm.).

The aim of this study is to use the Japanese pavement ant in North America as a model system to understand the characteristics of unicoloniality in a system where it may have recently evolved. I will address the following questions: i) how does intraspecific aggression vary across the invaded range? ii) How does social context affect aggression? iii) Does aggression correlate with differences in geographic distance, genetic diversity, and/or cuticular hydrocarbon profile? Studying a system where unicoloniality may be actively evolving for the first time will provide the best evidence for the circumstances that are associated with proliferation of this important and successful trait.

Methods

Collection and Laboratory Maintenance of Nests

I collected twenty-two nests of *Tetramorium tsushimae* between June and September of 2006 and 2007 to use as subjects in laboratory aggression assays. I collected large nests comprised of multiple queens and thousands of larvae and workers in order to match laboratory social conditions with those found in a natural setting as close as possible. Individuals were shoveled and aspirated into plastic containers for transport to the laboratory. I sampled nests from throughout the interior of the introduced range, concentrating on portions of the range where the Japanese pavement ant is most prolific (Figure 5). I attempted to sample and pair nests so that different spatial scales were equally represented in the data (e.g. 1m-60km; Figure 6). All nests were collected from moderately disturbed turf grass habitat. Latitude and longitude of each nest was recorded with a Garmin Geko 201 GPS device (Garmin, USA). Nests were collected and assayed in five blocks or sets of four nests each and a single block of two nests to prevent nests from remaining in the laboratory more than 3 weeks. Extended captivity tends to homogenize scent profiles and artificially decrease intraspecific aggression; because nest odor is partially a product of the environment (Liang and Silverman 2000; Chen and Nonacs 2000).

All laboratory nests contained at least one reproductive gyne, 100 larvae, and 500 workers. Individuals were transported to the laboratory and placed in nest boxes filled with moist Hydrostone. All nests contained glass tubes partially filled with water and plugged with cotton, as well as cardboard to provide shelter for larvae. Each nest box was

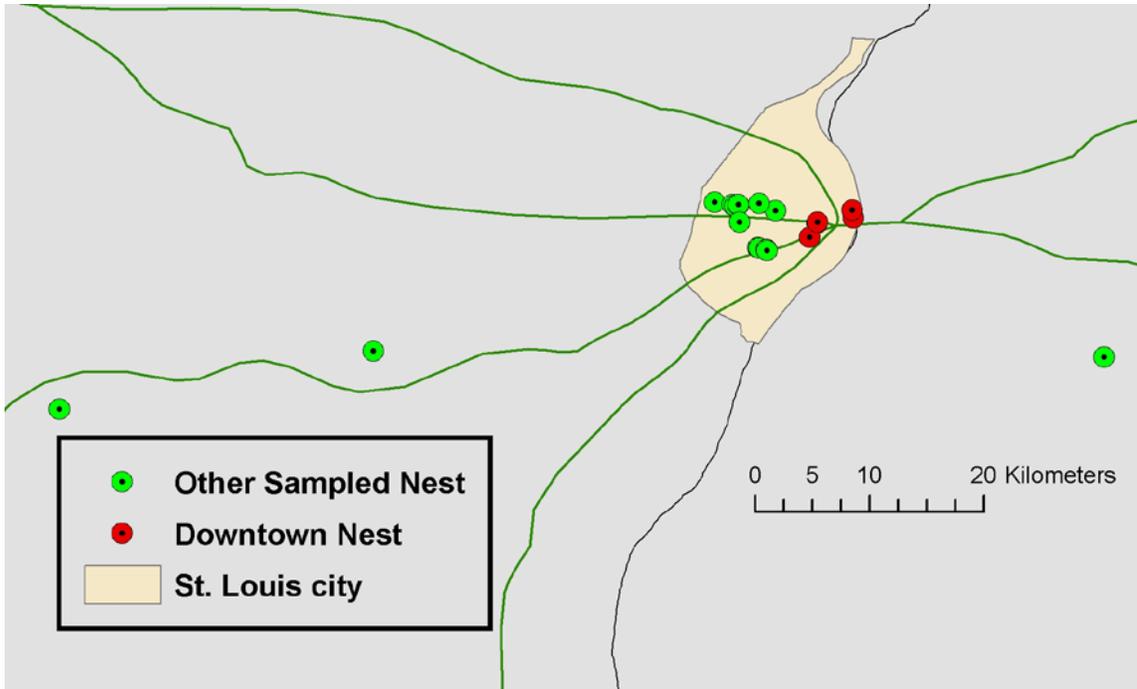


Figure 5. Map of 22 collected nests. Individuals from each nest were used in three aggression assays of varying social context. Cuticular hydrocarbon profile and genotypes at ten microsatellite loci were measured for each nest.

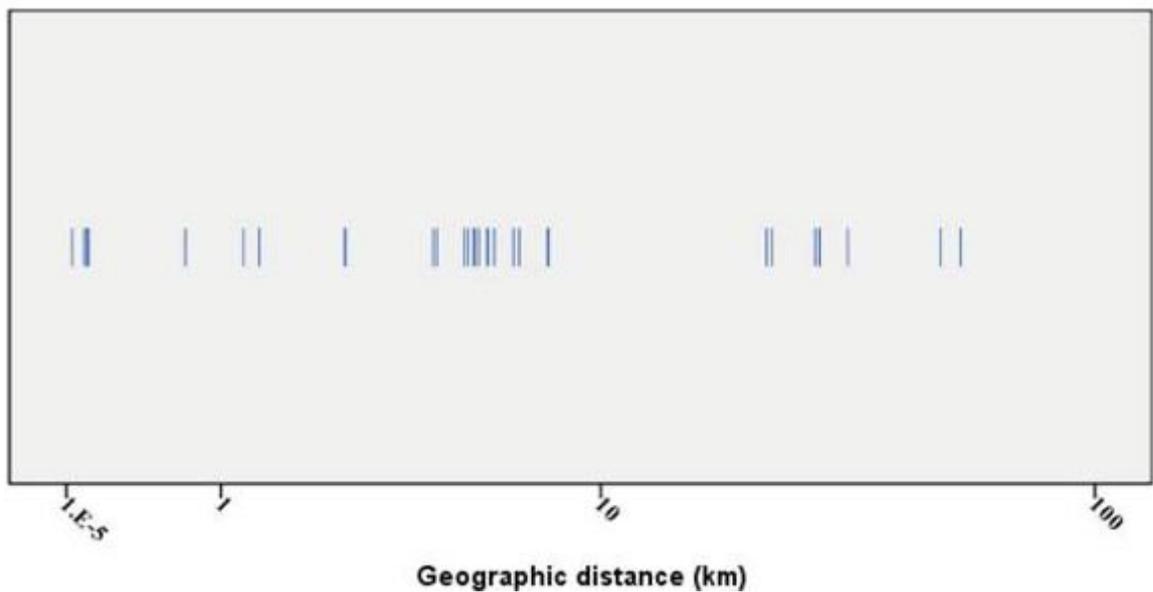


Figure 6. Distribution of geographic distance between sampled nest pairs. Each line represents the distance between two nests that were paired in aggression assays (N=31 nest pairs).

placed inside a foraging arena lined with Fluon to prevent escape. Colonies were maintained at a 12h:12h light cycle at a temperature of $23 \pm 2^\circ\text{C}$ and a relative humidity of $60 \pm 10\%$. Nests were provided with sugar water every 2 days and food was provided *ad libitum*. Diet consisted of a mixture of a prepared Bhatkar ant diet, crickets, peanut butter, diluted honey, and pecan sandie cookies (Bhatkar and Whitcomb 1970).

Aggression Assays

For each block of nests, all possible nest combinations were matched in aggression assays under three social contexts. Because social context is known to affect aggression levels, multiple aggression assays were performed to minimize the bias of any single experimental protocol (Buczowski and Silverman 2005). Thus, I varied the number and location of subjects in three different aggression assays. Isolated individuals experience increased risk in an aggressive encounter and are often farther from defensible resources, such as the nest; therefore, isolated individuals are less likely to engage in aggressive behavior (Reeve 1989; Buczowski and Silverman 2005). The likelihood of aggression increases with the number of allies and the quality and proximity of defensible resources. This is due to changes in the risk and benefit of behaving aggressively in different contexts (Brown 1964). Performing multiple assays in different social contexts increases the chance of detecting natural patterns in aggression within the laboratory. I generally followed the methods of Buczowski and Silverman (2005). Aggression assays were either a) dyadic interactions, b) group interactions between 10 ants from each nest, or c) nest interactions during which a single intruder is placed inside a foreign nest. Individual ants were tested only once and then removed from the study. Nests habituated

for at least 24 hours prior to experimentation. Controls for all assays consisted of pairing individuals randomly taken from the same nest. All pairings, including controls, were replicated five times. I used individuals from 18 nests for the dyadic and group interaction assays. I tested 22 nests for the nest intruder assay.

Assay 1: Dyadic Interaction

Single workers were taken from nests with flexible forceps at random and placed at opposite sides of a circular arena (diameter = 1cm) lined with Fluon, a slippery polymer that prevents escape. An interaction consisted of the two subjects coming to within one body length of each other. Interactions were quantified based upon a standard 0-4 scale commonly used in ant aggression experiments (Suarez et al. 1999). A score of “0” represents no change in direction or speed of movement and no initiation of new behavior. “1” represents a non-aggressive touch, usually with the antennae. “2” represents an avoidance behavior, such as a change in walking direction or an increase in speed after contact. “3” represents a temporary aggressive behavior, such as biting, pulling, mounting, or rearing. “4” represents prolonged fighting. The first interaction was recorded. This assay was conducted in 4 blocks of 4 nests each and 1 block of 2 nests. Subjects from each nest were paired with subjects from every other nest within each block. Each block of four nests consisted of six experimental pairings and four control pairings. The block of two nests consisted of one experimental pairing and two control pairs. The assay was replicated for each nest pairing five times (43 total nest pairings including controls; 215 trials).

Assay 2: Group Interaction

In order to quantify aggression in the context of two interacting groups, ten randomly chosen workers were aspirated into a vial from each of the two subject nests. Both vials were inverted and placed inside a circular arena (diameter = 10cm) lined with Fluon. I left each vial untouched at opposite ends of the arena for 5 minutes, in order to acclimatize the ants after aspiration. Vials were then lifted, releasing all the ants simultaneously into the arena. After 1 minute, I counted the number of individuals (0-20) involved in aggressive interactions using the aggression assay rating scale previously described. Preliminary testing demonstrated that waiting one minute permitted enough time to elapse for all individuals to discover the presence of the other test group. Roulston et al. (2003) found that continuously scanning and recording behavior over time does not provide more accurate results than recording aggression at a single time. Aggression ratings of either “3” or “4” were considered aggressive behavior while scores of “0”, “1”, and “2” were considered non-aggressive behavior. I used the standardized 0-4 aggression scale to differentiate between aggressive and non-aggressive behavior. This assay was also conducted in 4 blocks of 4 nests each and 1 block of 2 nests. Subjects from each nest were paired with subjects from every other nest within each block; control replicates including pairing groups of individuals from the same nest. The assay was replicated for each nest pairing five times (43 total nest pairings including controls; 215 trials).

Assay 3: Nest Intruder

To test aggression levels in the presence of defensible resources, single individuals were chosen randomly and moved with flexible forceps to open space inside

the nesting box of another nest. The single ant was observed for 30 seconds after its first interaction. Preliminary trials indicated that individuals that were not aggressively attacked within 30 seconds were never attacked. All aggressive interactions were recorded using the 0-4 aggression scale and the ant was subsequently removed from the nest box. Nests were untouched for at least 10 minutes between trials. After completing five trials between two nests, the roles of intruder and host nest were reversed and another 5 trials were completed for a total of 10 trials per nest pair. Controls were conducted for each nest and consisted of removing an individual with flexible forceps and replacing it in its own nest; controls were replicated 5 times. This assay was conducted in 5 blocks of 4 nests each and 1 block of 2 nests (31 experimental nest pairings of 10 trials each; 115 control trials; 425 total trials).

Cuticular Hydrocarbon Analysis

In order to analyze the nest's cuticular hydrocarbon profile, I collected 15-40 workers from 17 of the assayed nests. Individuals were collected in either the field or immediately after arrival in the laboratory. No cuticular hydrocarbon data was collected from nests 2206 (Shaw Nature Reserve), 2209 (Downtown St. Louis), 2214 (Belleville, IL), 2228 (Downtown St. Louis), and 2229 (Downtown St. Louis). Ants were stored dry at -20°C until analysis. Each sample for cuticular hydrocarbon analysis constituted of ten individuals placed in .5mL of hexane for 24 hours; each nest produced 1-4 samples of 10 individuals each (55 replicates; 550 individuals). Variation in sample size was due to the need to aggregate ten individuals in a single sample to achieve a strong enough GC-MS signal. I evaporated the hexane using nitrogen gas and hydrocarbons were reconstituted

in 200 μ L of hexane. I quantified cuticular hydrocarbon profiles using a 12m by 0.2mm by 0.33 μ m DB-1 capillary column in a Hewlett Packard 6890 Series Gas Chromatography/Mass Spectrometry System. After splitless injection of 1 μ L of sample, the temperature was held at 60°C for 4 minutes, ramped up to 250°C at 20°C per minute, then to 300°C at 2.5°C per minute, and finally held at 300°C for 2 minutes. I used helium as a carrier gas. Cuticular hydrocarbons were identified using standard GS/MS chemical databases.

Microsatellite Analysis

I placed 10 individuals from each of the 22 nests tested in the aggression assays in 95% ethanol and stored them at -20°C for later genetic analyses. Adult worker ants were genotyped at 10 microsatellite loci: Tspe51a, Tspe51b, Tspe51c, Tspe51h, Tspe52b, Tspe52k, Tspe53a, Tspe53b, Ttsu56d, and Ttsu54g (Steiner et al. 2008b). Genomic DNA was extracted using a Sigma GenElute Mammalian DNA Prep Kit (Sigma-Aldrich) and eluted in 50 μ L of elution solution. Extracted DNA was stored at -20°C. Polymerase chain reactions (PCR) were conducted using a Qiagen HotStarTaq Plus Master Mix Kit in 20 μ L volumes. Forward primers were labeled with WellRED phosphodiesterase dyes D2, D3, D4 (Proligo). Each reaction contained 10 μ L HotStarTaq Plus Master Mix, 1.6 μ L of eluted sample DNA, 0.25 μ L-1 μ L of each primer, and ddH₂O. Due to differences in signal strength of the three different WellRED primer dyes, I used a 4:2.5:1 volume ratio of the D2, D3, and D4 labeled primers, respectively. I multiplexed 2 microsatellite loci per PCR reaction using a Eppendorf Mastercycler Gradient. After an initial 5 minute denaturation step at 95°C, I ran 32 cycles consisting of a 94°C denaturation step for 30

seconds, a 60°C annealing step for 1 minute, and a 72°C extension step for 45 seconds. This was followed by a final extension step at 68°C for 20 minutes. Fragment analysis was performed on a Beckman-Coulter CEQ8000. PCR and fragment analysis was repeated when sample analysis provided a poor or non-existent signal.

Statistical Analysis

To test for geographic patterns in aggressive behavior, I measured whether there was a significant correlation between geographic distance and aggression between nest pairs for all three assays. Preliminary results indicated regional differences in aggression, so I decided to test for the significance of these emergent patterns. I used a Mann-Whitney U test to measure differences in aggression scores between nest pairings that did and did not include at least one nest collected from urban downtown St. Louis, MO (Figure 5). The metric of aggression in the group assay was the proportion of individuals engaged in aggressive behavior after 1 minute. The metric of aggression in the colony intruder assay was the proportion of replicates where aggression occurred within 30 seconds of the intruder's first interaction. These tests as well as descriptive statistics were performed using SPSS 16.1 (SPSS Inc. 2007).

I measured the cuticular hydrocarbon profile for each nest by recording the relative area under the peak of the twenty most common cuticular hydrocarbon compounds (Appendix A). To reduce the number of variables and create an orthogonal dataset, a principle component analysis (PCA) was performed on the hydrocarbon profile of all replicates using SPSS 16.0.1 (SPSS Inc., 2007). The first four principle components explain 92% of the total hydrocarbon variation. A discriminant analysis was performed

on all PCA factor scores grouping together samples from the same nest as in Ugelvig et al. (2008). Use of a grouping variable in discriminant analysis creates four orthogonal discriminant functions around a centroid for each nest, thus accounting for the differences in replicate number (1-4 replicates per nest). I calculated pair-wise differences in cuticular hydrocarbon profiles between nests using the Mahalanobis distances between nest centroids. Mahalanobis distances were calculated with the “stats” package in the R statistical computing environment (R Development Core Team 2008).

I examined the correlation between genetic distance, cuticular hydrocarbon profiles, and geographic distance by conducting a series of mantel and partial mantel tests. I calculated the pair-wise genetic distance between nests by constructing a matrix using Nei’s D as a distance measure. Nei’s D performs well when differentiating between groups at a fine geographic scale, as is the case in this study (Paetkau et al. 1997). I used the “adegenet” package in the R statistical computing environment to calculate the pair-wise genetic distance between nests using Nei’s D as a metric (Jombart 2008; R Development Core Team 2008). I used Hawth’s tools in ArcGIS 9.1 to calculate the geographic distance matrix between collected nests (Beyer 2004). Mantel tests were performed for all pair-wise combinations of genetic, geographic, and cuticular hydrocarbon distance using 1000 randomizations – two-tailed *P*-values are reported. I used a partial Mantel test to test for a significant correlation between microsatellite and cuticular hydrocarbon distance while controlling for geographic distance. All mantel and partial mantel tests were performed using the “vegan” package in the R statistical computing environment (Oksanen 2008; R Development Core Team 2008).

I measured the independent effects of geographic location, genetic variation, and cuticular hydrocarbon variation on aggression using a specific type of generalized linear model (GLM) called generalized estimating equations (GEE). A GLM is a multivariate test that measures the individual effects of multiple independent variables (i.e. genetic, geographic, and cuticular hydrocarbon distances) on a single dependent variable (i.e. aggression) with few of the assumptions inherent to multiple regression (Liang and Zeger 1986). GEE is a type of GLM that accounts for potential correlations in data that may have resulted from the blocked sampling design of the aggression assays (Liang and Zeger 1986). The dependent variables were the mean aggression measures per nest pairing in the group and nest intruder assays. In both tests, the independent variables were geographic, genetic, and cuticular hydrocarbon distances; sampling block ID number was used as the confounding, correlated variable. Due to limits in sample size, the robust Z score was reported and used to calculate a two-tailed *P*-value (Rochon 1998). GEE analyses were performed using the “gee” package in the R statistical computing environment (Carey 2002; R Development Core Team 2008).

Results

Aggression Assays

Mean aggression score for the dyadic assay was 0.98 ± 0.27 on the the 0-4 aggression scale (mean \pm 1 S.D). Of all assayed nest pairs, the maximum mean score over 5 trials was 1.6 and there was not a single incidence of aggressive behavior – biting, rearing, or gaster flagging (i.e. aggression score \geq 3). Individuals moved freely throughout the arena and rarely avoided each other (“2” score). Generally, when

individuals moved within one body length of each other, subjects either did not alter course (“0” score) or they approached each other and antennated briefly (“1” score). No control replicates exhibited any aggressive behavior.

In the group assay, where 10 individuals faced 10 individuals in a neutral arena, $21\% \pm 17.6\%$ (mean ± 1 S.D) of individuals engaged in aggressive behavior 1 minute after being released (i.e. aggression score ≥ 3). This did not include control replicates, during which no aggressive behavior was observed. Individuals moved freely around the arena and first contact between groups usually occurred within 30 seconds. Once aggressively engaged individuals continued fighting until removal at the conclusion of the trial. Fighting mostly involved small groups of 2-4 individuals as contact occurred. In no cases did more than four individuals total congregate and fight in a cohesive melee. Non-aggressive individuals moved freely throughout the arena.

Similarly, $22.3\% \pm 22.3\%$ (mean ± 1 S.D) of experimental replicates in the nest intruder assay resulted in aggression directed toward the foreign intruder. This does not include control replicates, during which no aggressive behavior was observed. Generally, the intruder moved immediately once placed in the foreign nest, but not significantly faster than surrounding individuals. Contact between the intruder and nest workers occurred every few seconds. Some intruders were not attacked until they had passively interacted with up to 15 different individuals; this is potentially due to variation in acceptance thresholds between individual workers within a single nest (Errard et al. 2006). Once aggression occurred, it would continue until the individual was removed at the end of each 30 second trial before which up to 5 additional workers would simultaneously attack the intruder.

In all three assays, there was no correlation between aggression and geographic distance between nest pairs (Figure 7; dyadic assay, $r = -0.26$, $P > 0.99$; group assay, $r = 0.042$, $P > 0.99$; nest intruder assay, $r = 0.064$, $P > 0.99$). Further analysis indicated that the replicates that did experience elevated aggression in the group and nest intruder assays were primarily comprised of nest pairings that included at least one nest collected from downtown St. Louis, MO. In both the group and nest intruder assays, there was a significant increase in aggression when pairings involved at least one nest collected from downtown St. Louis, MO (Figure 8; group assay: Mann-Whitney $U = 26.5$, $P = 0.004$; nest intruder assay: Mann-Whitney $U = 59$, $P = 0.015$).

Effect of geographic, microsatellite, and cuticular hydrocarbon distances between nests on aggression

Although there were limited levels of aggression between the sampled nests, GEE analysis demonstrates that differences in geographic location, genetic distance, and cuticular hydrocarbon profile between paired nests all had a significant independent effect ($P \leq 0.05$) on aggression in at least one of the two social aggression assays (Table 6). I did not include the results from the dyadic assay in this analysis due to the absence of aggression between all nests pairs. Specifically, as genetic distance between nests increased, there was a subsequent increase in aggression for both the group and nest intruder assay ($P < 0.0001$). GEE analysis estimated the relative effect of genetic distance to be more than 100 times greater than other metrics (Table 6). Geographic distance was

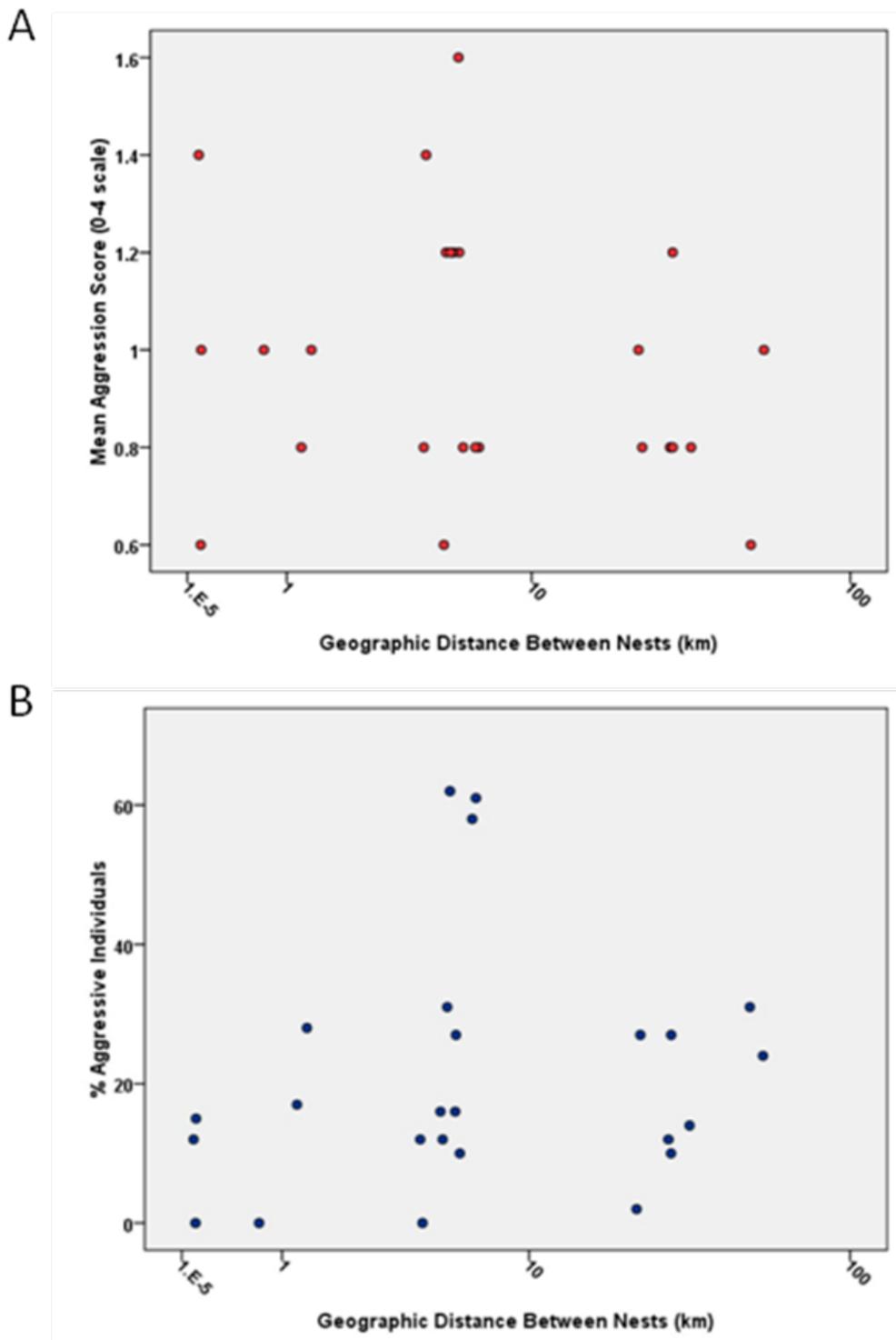


Figure 7. Scatterplots for each of the three aggression assays showing the relationship between aggression and the geographic distance between nest pairs. The three assays varied in social context: (A) dyadic interaction, (B) group interaction, (C) nest intruder interaction.

C

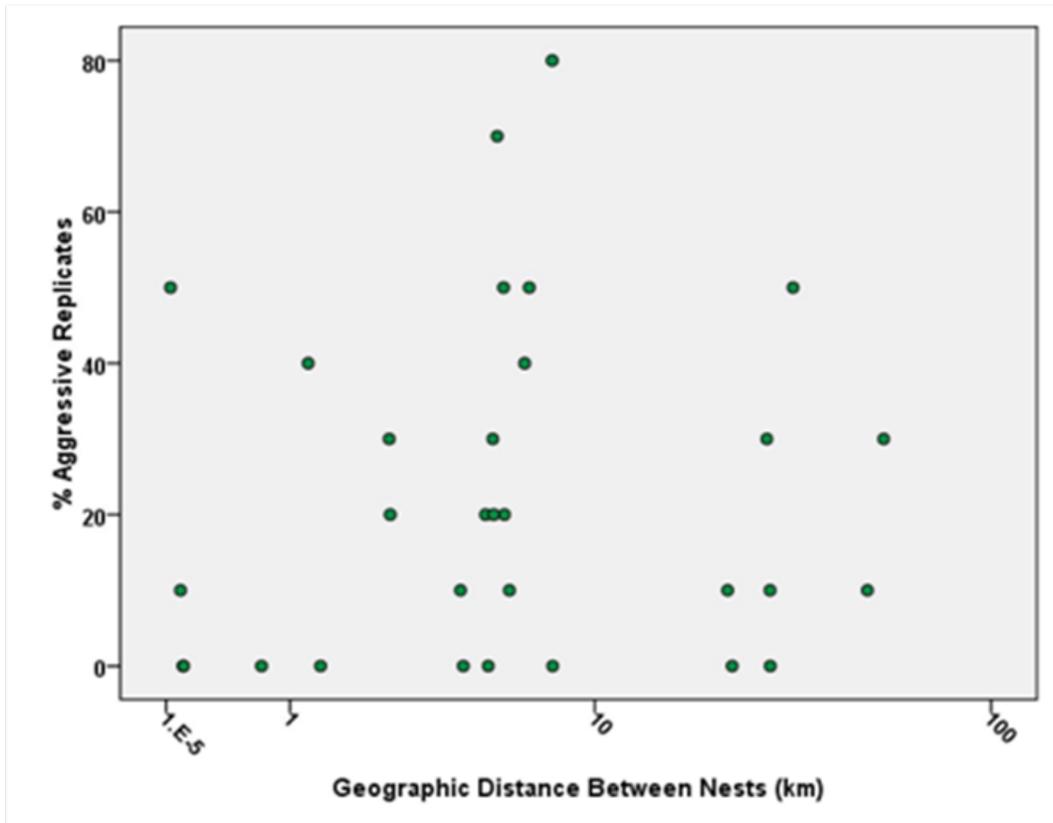


Figure 7 cont.

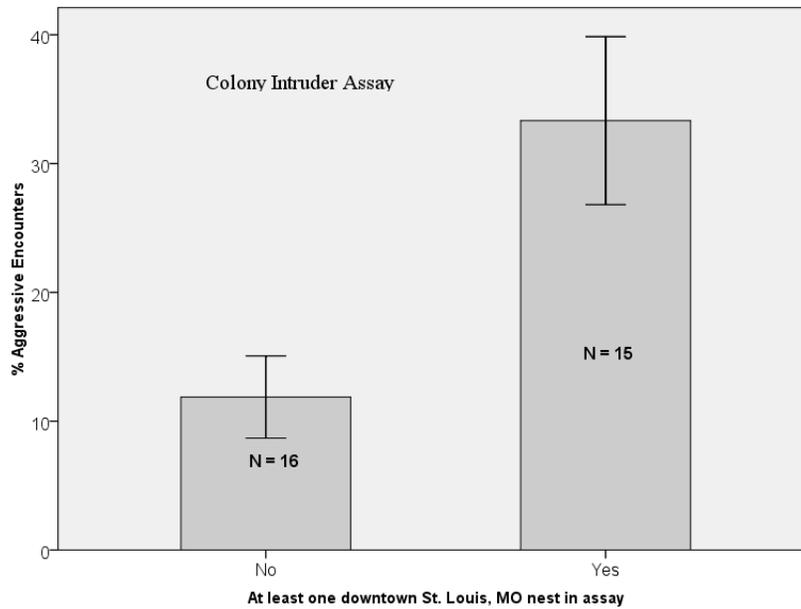
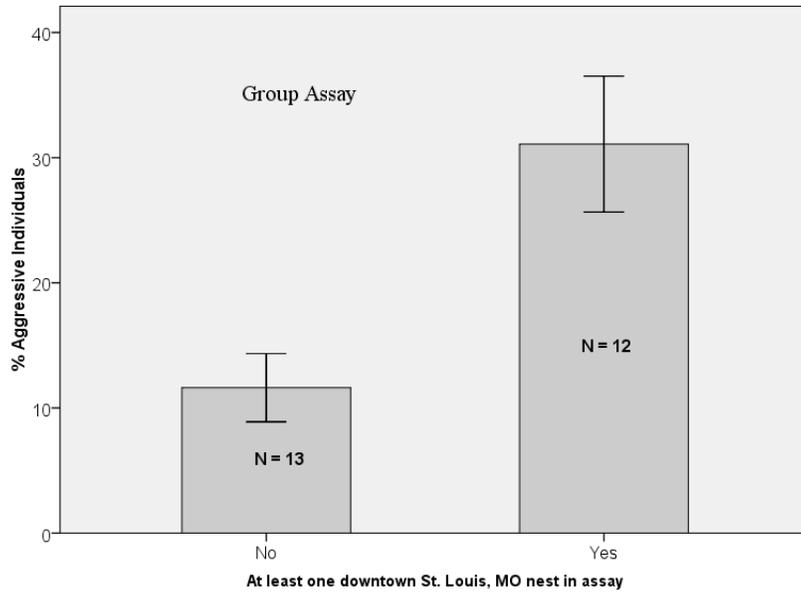


Figure 8. Bar plots displaying differences in aggression between nest pairs that did and did not include at least on nest collected from downtown St. Louis, MO. Error bars signify ± 1 standard error.

only a significant factor in the group assay and was negatively associated with aggression ($P = 0.011$) and cuticular hydrocarbon profile distance was only a significant factor in the nest intruder assay ($P = 0.009$). However, while the effect of geographic distance significantly affects group aggression, the magnitude of this effect is much smaller than the estimated effect of genetic distance (Table 5). When a separate GEE analysis was performed without genetic distance as an explanatory variable, the effect estimate of cuticular hydrocarbons increased for both assays (group assay: estimate = 1.59; $P = 0.41$; nest intruder assay: estimate = 2.61, $P < 0.01$). This suggests that a portion of hydrocarbon variation as it pertains to aggression is explained by microsatellite variation. While each distance variable had a significant effect on aggression, partial Mantel and Mantel test results show that none of the three distance measures were significantly correlated with each other, although microsatellite and geographic distances were nearly significantly correlated (Table 6; $P = 0.056$). I conducted more detailed population genetic analyses on a greater sample of nests in Chapter 2.

Discussion

Unicoloniality in the introduced Japanese pavement ant

I found that when single individuals from different nests collected from the introduced range of the Japanese pavement ant were placed together, aggression did not occur, regardless of their point of origin within the invaded range. When I altered the social context to make aggression a more profitable choice, aggression increased, but only to moderate levels; 20.96% of individuals were aggressive when 2 sets of 10

Table 5: Results of Generalized Estimating Equation (GEE) analysis. The GEE analysis quantified the separate effects of geographic distance (km), genetic distance (Nei's D), and cuticular hydrocarbon profile distance (Mahalanobis distance) on inter-nest aggression. Separate analyses were performed for the group aggression assay and the nest intruder assay.

Nest Intruder Aggression Assay				
Effect	Estimate	Robust S.E.	Robust z	Sig.
(Intercept)	-22.818	6.485271	-3.51843	<0.0001
geographic distance	-0.31106	0.259255	-1.19981	0.2302
genetic distance	329.472	46.5342	7.080213	<0.0001
hydrocarbon distance	1.44686	0.553631	2.613399	0.009
Group Aggression Assay				
Effect	Estimate	Robust S.E.	Robust z	Sig.
(Intercept)	-17.6497	4.424852	-3.98877	<0.0001
geographic distance	-1.3735	0.53998	-2.54361	0.011
genetic distance	389.2082	78.09139	4.984008	<0.0001
hydrocarbon distance	-2.99844	1.652447	-1.81454	0.0702

Table 6: Results of pair-wise Mantel and partial Mantel tests between microsatellite genetic distance (G), cuticular hydrocarbon profile distance (H), and geographic distance (S). Distance matrices were calculated by Nei's D, Mahalanobis distance, and kilometers, respectively. A partial Mantel measures the correlation between the X and Y matrix while controlling for the effect of the Z matrix.

X matrix	Y matrix	Z matrix	Mantel r	Sig.
G	H		-0.0091	0.385
G	S		0.3083	0.056
H	S		-0.106	0.816
G	H	S	-0.0188	0.395

nestmates were paired, while only 22.26% of intruders were attacked when introduced into a foreign nest.

The intensity of intraspecific aggression is similar to levels found in the world's most destructive unicolonial invasive species, such as the red fire ant, Argentine ant, and little fire ant (Holway et al. 2002; Hölldobler and Wilson 1977; Morel et al. 1990).

Aggression levels for all three aggression assays used in this study are similar or lower than those found among unicolonial populations of the Argentine ant, *Linepithema humile* under similar conditions (Suarez et al. 2008; Buczkowski and Silverman 2005; Roulston et al. 2003). Among Argentine ant nests collected from the Southeastern U.S., the mean aggression score in a dyadic context was 1.1 ± 0.1 , but increased significantly in group ($76.2\% \pm 3.6\%$ individuals fighting) and nest intruder interactions (3.2 ± 0.1 mean aggression score; Buczkowski and Silverman 2005). This complete absence of intraspecific aggression in dyadic encounters is present in unicolonial populations of *Wasmannia auropunctata*, *L. humile*, and *Lasius neglectus* (Errard et al. 2005; Holway et al. 1998; Buczkowski and Silverman 2005; Ugelvig et al. 2008).

Intraspecific aggression between individuals from separate colonies of multicolonial ants is ubiquitous and elevated regardless of the social context (Hölldobler and Wilson 1990). During territorial encounters between non-nestmate workers of the green tree ant, *Oecophylla smaragdina*, individuals chemically recruit soldiers and other nestmates to hold and kill foreign conspecifics (Hölldobler 1983). Elevated levels of aggression among multicolonial workers are maintained in dyadic encounters within laboratory settings (Mercier et al. 1997). Native populations of *T. tsushimae* are highly territorial and polygynous, polydomous colonies occupy a mean territory size of 32m^2 (Sanada-Morimura et al. 2006). Pavement ants are well known for battles between thousands of individuals and have relatively high levels of intraspecific aggression (Hölldobler and Wilson 1990; Morisita 1939; Sanada-Morimura et al. 2006).

The invasive population is unicolonial, due to its overall low levels of intraspecific aggression. However, aggression was present in two of the three aggression

assays: group interactions and nest intruder interactions. I found that altering social context had a direct effect on the frequency of aggressive interactions; intraspecific aggression increased significantly with group size and nest proximity. In the group aggression assay, individuals placed with 9 nestmates and 10 non-nestmates were more likely to act aggressively when individuals were placed with only a single non-nestmate. These results mirror those found by Buczkowski and Silverman (2005) in a unicolonial population of the Argentine ant under similar social circumstances.

I found a similar pattern in the nest intruder assay, where intruders were more likely to be attacked than individual subjects in the dyadic assay. In this case, defensible resources (i.e. the nest, stored food, queens, and larvae) are present, thus increasing the benefit of attacking intruders. Simultaneously, risk is significantly decreased due to overwhelmingly disparity in the number of intruders (1) and nestmates (1000's). In the Argentine ant, intraspecific aggression between non-nestmates is significantly higher when the interaction occurs within a nest (Roulston et al. 2003; Buczkowski and Silverman 2005).

The costs and benefits of aggression depend on the number of nestmates (i.e. allies) and non-nestmates (i.e. opponents). Individuals are more likely to engage in aggressive behavior when the risk can be shared among nestmates (Buczkowski and Silverman 2005). In addition, the presence of multiple nestmates can be perceived as a proxy for nest proximity; aggression increases as the risk of losing resources increases (Starks et al. 1998; Venkataraman and Gadagkar 1992). When comparing multiple social contexts among unicolonial Argentine ants, aggression was highest in assays that involved the greatest number of ants (Roulston et al. 2003). Proximally, individuals

stimulate aggression among nestmates through transfer of alarm pheromones and cuticular hydrocarbons (Wilson 1976; Tanner 2008).

Mechanisms of aggression

This study demonstrates that genetic distance, cuticular hydrocarbon profile, and geographic distance between nest pairs affect levels of intraspecific aggression. GEE analyses show that genetic distance based upon microsatellite genotype is the most significant predictor of intraspecific aggression, explaining variation in aggression for both the group and nest intruder assays. Differences in cuticular hydrocarbon profiles did explain a significant proportion of aggressive variation in the nest intruder assay, while geographic distance explained a significant proportion of aggressive variation in the group assay. However, the strength of these effects was relatively minor compared to the effect of genetic distance.

Similar to invasive populations of Argentine ant and *Lasius neglectus*, I found the proximal mechanism influencing aggression to be differences in genetic and cuticular hydrocarbon profiles (Suarez et al. 2008; Ugelvig et al. 2008). Studies have compared aggression with genetic and cuticular hydrocarbon variation in the ants *L. neglectus* and *Plagiolepis pygmae* (Ugelvig et al. 2008; Thurin and Aron 2008). In both studies genetic differences explained more variation in aggression than differences in cuticular hydrocarbon profile. Ugelvig et al. (2008) found that genetic distance was more highly correlated to aggression than either cuticular hydrocarbon profile or geographic distance ($r = 0.375$, Mantel test: $P = 0.001$). Suarez et al. (2002) found that after Argentine ant nest fragments had been reared in identical laboratory environments for one year,

intraspecific aggression remained relatively unchanged. Chemical odors involved in recognition are derived both genetically and environmentally, but these studies suggest that genetic differences may be the primary driver of intraspecific aggression, even though environmental differences can modulate the intensity of such aggression (Holway et al. 2002; Suarez et al. 2008; Thurin and Aron 2008; Hölldobler and Wilson 1990; Sorvari et al. 2008; Liang and Silverman 2001).

I found no significant correlation between genetic diversity, cuticular hydrocarbons, and geographic distance. This pattern is similar to that found among invasive *Lasius neglectus* populations (Ugelvig et al. 2008). This pattern may seem counterintuitive, because cuticular hydrocarbons are at least partially derived from an individual's genotype (Brandt et al. 2009; Dronnet et al. 2006). Two factors may explain the lack of correlation between genetic distance and cuticular hydrocarbon distance. First, environmental factors may have such a large overall effect on cuticular hydrocarbon composition that a larger dataset is needed to show a significant correlation. Second, the metric used in this study used to quantify cuticular hydrocarbon distance accounts for every single common hydrocarbon peak – not all compounds are genetically derived. Genetic variation is primarily expressed by a few compounds, but these may be key determinants of aggressive behavior (Martin et al. 2008; Dronnet et al. 2006).

Regional variation in aggression

I found a significant increase in aggression when assays involved at least one nest collected from downtown St. Louis, MO. When considered simultaneously with the significant positive relationship between genetic distance and aggression found in this

study, nests sampled downtown may have greater genetic variation than other nests in the invaded range. The relative absence of aggression among nests collected outside of downtown St. Louis – even when separated by over 100km – may signify greater genetic diversity among nests in downtown St. Louis. High levels of gene flow among nests in central St. Louis city found in chapter 2 support this supposition.

A potential scenario that explains these reductions in intraspecific aggression involves a step-wise reduction in genetic variation as a propagule successfully colonized downtown St. Louis and eventually dispersed throughout the present range.

Mitochondrial and microsatellite analyses of nests collected from the native and invaded ranges indicate that *T. tsushimae* was transported from central Japan to St. Louis, MO in a single introduction event (Steiner et al. 2006, Chapter 2). This model of unicolonial evolution is supported by behavioral and genetic differences among North American populations of the Argentine ant (Buczkowski et al. 2004). Populations of the Argentine ant in the SE United States have intermediate genetic variability and intraspecific aggression compared to populations collected in the native range and in California. The fact that alleles found among California supercolonies are a subset of those found in the SE U.S. suggests that the California population is derived from a secondary introduction originating from the east coast.

Further behavioral and population genetic research is warranted to fully understand the evolution of decreased intraspecific aggression at the local scale. In chapter 2, I identify a distinct sub-population located in central Illinois, the far eastern portion of the invaded range. It would be informative to test aggression levels between those nests and the rest of the range. Additionally, future studies should note aggressive

asymmetry. Nests with less genetic variability are more likely to initiate aggression when matched against more diverse nests (Tsutsui et al. 2003). Since less diverse nests have a more homogenous nest odor, individuals can be more sensitive to odor differences in strangers. I hypothesize that *T. tsushimae* individuals collected outside downtown St. Louis would initiate aggression when paired with individuals collected from downtown.

Unicolonial ants account for 5 of the 100 worst invasive species worldwide, directly lead to the extirpation of native populations, and incur significant economic costs (Lowe et al. 2001; Holway et al. 2002; Lard et al. 2002). Understanding the novel evolution of unicolonialism and identifying potential avenues of control are necessary to attenuate the dominance of invasive ants. The absence of multiple introductions and recent geographic spread make the Japanese pavement ant in North America a unique model system for understanding the evolution of unicoloniality.

CHAPTER 4: THE EFFECT OF THE JAPANESE PAVEMENT ANT ON NATIVE ANT COMMUNITY STRUCTURE IN PRAIRIE AND TURF GRASS ECOSYSTEMS

Chapter Abstract

The Japanese pavement ant, *Tetramorium tsushimae*, is one of the most recently documented exotic ant species in the world. Native to East Asia, it was discovered in the St. Louis, MO metropolitan area in 1988. As of 2009, the population has expanded its range throughout Eastern Missouri and Western Illinois, often reaching high densities and exhibiting natural history characteristics indicative of a successful invasive ant species. The goal of this study is to quantify the effect of the Japanese pavement ant on native ant communities and test whether competitive superiority plays a significant role in shaping these patterns. I used an artificial introduction competition experiment to test whether the Japanese pavement ant can usurp food resources that are actively attended by native species through interference competition. I measured recruitment speed and volume of the Japanese pavement ant to food baits in invaded habitats. While unable to displace *Monomorium minimum* from food baits, the Japanese pavement ant did displace and monopolize resources from *Tapinoma sessile* and *Lasius neoniger*. Japanese pavement ants recruited to 70-100% of bait stations at invaded sites. In order to quantify the effect of Japanese pavement ant abundance on ant community structure, I compared ant diversity in environmentally matched invaded and uninvaded prairie and turf grass habitats. Canonical correspondence analyses (CCA) indicate that abundance of the Japanese pavement explains more variation in ant diversity between invaded and uninvaded sites than climatic, soil, and microhabitat variables. Widespread native genera

such as *Formica*, *Camponotus*, *Crematogaster*, *Monomorium*, *Tapinoma*, *Myrmica*, and *Lasius* are rare or absent from invaded habitats. Subterranean cryptic species are least affected by the invasion, while opportunistic ground-foraging ant abundance is significantly reduced in invaded habitats. This study is the first to quantify the effect of the Japanese pavement ant on native ant communities and demonstrates the severity of the most widespread ant invasion in the U.S. Midwest.

Introduction

Highly invasive species not only undergo rapid population growth, but also have significant adverse effects on biodiversity and community structure (Huber 2002; Sanders et al. 2003; Gurevitch and Padilla 2004). Invasive ant species in particular have a significant negative impact on entire ecosystems by homogenizing native ant communities (Holway et al. 2002). Ant invasions can result in a nearly 90% reduction in native ant biodiversity, and generally the species that remain are often cryptic, rare species, or do not forage on the soil surface (i.e. hypogeic and arboreal species; Porter and Savignano 1990; Human and Gordon 1997; Ross 1994; Sanders et al. 2003). Ants frequently comprise a large proportion of animal biomass - ~15% worldwide - as well as being important predators, prey, detritivores, granivores, pollinators, and mutualists, changes in ant communities can have drastic effects on entire ecosystems (Hölldobler and Wilson 1990; Holway et al. 2002; Diamond and Case 1986; Parker 1999). Five of the 100 world's most destructive invasive species are ants (Lowe et al. 2001). 32% of scientific journal articles documenting the ecological effects of invasive insects focus on only two

ant species: the Argentine ant, *Linepithema humile*, and the red fire ant, *Solenopsis invicta* (Kenis et al. 2009).

The superior competitive ability of invasive ants is attributed to a distinct lack of intraspecific aggression and territoriality (Suarez et al. 2008; Holway et al. 2002; Tsutsui and Suarez 2003). All five of the most pervasive invasive ants worldwide can exist in supercolonies, which are dense populations without any distinct colony boundaries (Lowe et al. 2001; Suarez et al. 2008). Cooperation among conspecifics has two primary consequences. First, aggressive behavior toward other species is often significantly elevated in the absence of intraspecific aggression (Hölldobler and Wilson 1990; Passera 1994). Second, the numerical advantage in such densely populated invasions allows invaders to excel at both resource discovery and resource dominance (Feener 2000). Most native ant species are limited in colony size and must choose a strategy that either widely distributes foragers for quick discovery of resources or concentrates individuals in space to elevate recruitment and resource defense (Feener 2000). Since the biomass of invasive ant populations can be greater than the combined biomass of all native ant species in an uninvaded habitat, invasive ants can dominate through both interference and exploitative competition (Porter and Savignano 1990; Holway 1999; Human and Gordon 1996; Rowles and O'Dowd 2007).

The negative effect of invasive ants on native ant diversity tends to decrease over time; recent invasions tend to have the greatest impact on native ant communities (Morrison 2002; King and Tschinkel 2006). For example, after the initial invasion of the Brakenridge Field Laboratory in Central Texas in the 1980s, the red fire ant, *S. invicta*, caused a sharp reduction in arthropod diversity (Porter and Savignano 1990). However,

when the area was resampled a decade later, ant diversity had increased to levels present prior to the invasion (Morrison 2002). While the red fire ant is still the most common ant at Brackenridge Field Laboratory, most native ant species reestablished viable populations (Morrison 2002). In other locations where invasions were established decades earlier, red fire ant colonies did not competitively exclude native ant species (King and Tschinkel 2006; Morrison and Porter 2003). Analysis of stable nitrogen isotope data shows that Argentine ant nests along active invasion fronts consume more arthropods than nests that occupy the interior of the invaded range (Tillberg et al. 2007).

Evidence indicates that recent, incipient invasions have the greatest effect on native ant communities, yet recent ant invasions are poorly studied and the effects and mechanisms driving success are relatively unknown. Since a primary focus in invasion biology is to study the characteristics of early invasions – older, established invasions are nearly impossible to eradicate – it is important to examine the relationship between early stage ant invasions and native ant communities.

The Japanese pavement ant, *Tetramorium tsushimae*, is one of the most recently documented ant invasions worldwide (Steiner et al. 2006; Holway et al. 2002). Its native range encompasses Japan, eastern China, Korea and southeastern Russia (Steiner et al. 2008). First discovered in Missouri in 1988, its range has rapidly expanded throughout eastern Missouri and western Illinois (Steiner et al. 2006; Chapter 2). In both the native and invaded range, Japanese pavement ant colonies inhabit primarily open canopy habitats, such as grassland and disturbed urban areas, where they nest near the surface (Imai et al. 2003). An omnivorous species, the Japanese pavement ant has been observed foraging on arthropods, seeds, and honeydew derived from both plants and insects (J.

Trager pers. comm.). In Japan, it actively defends extrafloral nectaries and excludes potential herbivores (Katayama and Suzuki 2004).

In Chapter 3 I showed that in North America, most nests of the Japanese pavement ant lack intraspecific aggression, existing as one large unicolonial supercolony. Unicoloniality is the primary characteristic of the most widespread invasive ant species (Holway et al. 2002). The combination of unicoloniality, omnivory, generalist nesting requirements, and an affinity for disturbance make the Japanese pavement ant a candidate for being a successful and damaging invader (Sakai et al. 2001). Thus, it has the potential to spread throughout the Midwest and have a significantly negative impact on native ant communities (Steiner et al. 2008).

The aim of this study is to quantify the effect of the Japanese pavement ant, an early-stage invasive species, on native ant communities. Previous studies of other ant invasions have measured impact through historical accounts, longitudinal studies, mapping of mutually exclusive distributions, and experimentation (Holway et al. 2002). However, most early invasions lack historical records. Quantifying differences between invaded and uninvaded communities is a short-term method for measuring landscape scale effects of this invasion on ant diversity. However, correlation between diversity and invader abundance does not imply causation (King and Tschinkel 2006; Thomson 2006). Therefore, experimental studies should be paired with such distributional studies to show that the invader actively affects the foraging ability of native ants (King and Tschinkel 2006; Thomson 2006). I accomplish this by pairing competition experiments with an examination of ant community structure in invaded and uninvaded habitats.

Methods

Artificial introduction experiment

I collected 10 nests of the Japanese pavement ant from Forest Park and Tower Grove Park in Saint Louis, MO, as well as Tyson Research Center in Eureka, MO. Each nest contained 1000-1500 workers, brood, and at least one reproductive queen and originated from a nest network that covered at least one acre. Nests were contained in 64-ounce plastic boxes filled with soil from the source nest. All nests were kept in the laboratory for 1 month before experimentation. In the laboratory, nest boxes were kept in a plastic foraging arena lined with Fluon to prevent escape (Bioquip). A single tube in the nest box allowed foragers to exit the nest. Nests were fed every other day with a sugar water solution and *ad libitum* with pecan sandie cookies, tuna, and live mealworms. No food was provided for five days prior to experimentation.

Laboratory nests of the Japanese pavement ant were taken into the field where a competition experiment was staged using standard methods (Schaffer et al. 1983, Human and Gordon 1996, Holway 1999, Rowles and O'Dowd 2007). All experiments were conducted at sites at least 100m from the nearest Japanese pavement ant nest; colony size of native ants may be reduced at invasion boundaries (Sanders et al. 2003; Holway 1999). A sealed Japanese pavement ant nest box was placed 40cm from a native ant species nest entrance. I then placed 1cm² of crushed pecan sandie cookie on bare soil equidistant between both nest entrances. The native species was allowed to forage at the bait continuously for 30 minutes. Recruits were defined as all workers within 5cm of the bait and those walking between the bait and the nest entrance (Human and Gordon 1996; Holway 1999). Once recruitment reached equilibrium, the number of foragers was

recorded. Immediately afterward, *T. tsushimae* was released and allowed to forage at the bait. I then recorded the number of recruits for both species every 10 minutes for 30 minutes after *T. tsushimae* discovered the bait. Afterward, all Japanese pavement ant workers were aspirated and placed back in the nest box. 30 minutes after the removal of Japanese pavement ants, I recorded the number of native individuals to return to the bait.

The Japanese pavement ant was matched with three native species: *Monomorium minimum* (Myrmicinae), *Lasius neoniger* (Formicinae), and *Tapinoma sessile* (Dolichoderinae). These species represent each of the three most prevalent ant sub-families found in the invaded range. Each is a common, omnivorous ant with large colonies that forage on the soil surface under little canopy cover, similar to the Japanese pavement ant (Trager pers. comm.). While locally common outside of the invaded range, each of these species is rarely found in the presence of the Japanese pavement ant (Steiner et al. 2006). I replicated each species pairing 10-12 times, using each Japanese pavement ant nest only once per native ant species per experiment. This experiment tested the competitive ability of the Japanese pavement ant to supplant food resources already discovered by another species. I carried out experiments between May and July of 2008 when all tested species actively foraged.

I used a repeated-measure multivariate analysis of variance (MANOVA) with linear and quadratic contrasts to test for significant changes in the number of recruited foragers (Holway 1999; Gurevitch and Chester 1986). This analysis was performed using SYSTAT 12 (SYSTAT 2007). Independent analyses were conducted for each native ant species. I tested two null hypotheses. First, that there is no difference between native ant recruitment before the introduction and after the removal of the Japanese pavement ant. I

utilized a linear contrast MANOVA to test this hypothesis. Second, there is no difference between native ant recruitment when Japanese pavement ants are present and absent (measured as mean recruitment before introduction and after removal). In this case, a quadratic contrast MANOVA was used.

Native vs. Invaded Ant Communities

I compared ant species richness and abundance between sites where the Japanese pavement ant was either present or absent. Since the Japanese pavement ant prefers inhabiting open spaces with relatively high soil temperatures, I used sites that comprised of either turf grass or prairie (Sanada-Morimura et al. 2006). The study included ten turf grass sites and ten prairie sites, with the Japanese pavement ant present at half of the sites (Figure 9). The prairie sites where the Japanese pavement ant was present included Sioux Passage County Park, Forest Park (Kennedy Woods and Deer Lake), Bellefontaine Conservation Area, and Route 66 State Park. The prairie sites where the Japanese pavement ant was absent included St. Stanislaus Conservation Area, Shaw Nature Preserve, Litzsinger Road Ecology Center, Bellefontaine Conservation Area, and Southern Illinois University at Edwardsville. The turf grass sites where the Japanese pavement ant was present included Carondelet Park, Forest Park, Lafayette Park, Tower Grove Park, and Sioux Passage County Park. The turf grass sites where the Japanese pavement ant was absent included West Tyson County Park, Litzsinger Road Ecology Center, Rockwoods Conservation Area, and Southern Illinois University at Edwardsville.

Data was collected at each site annually between May and August in 2007 and 2008. Each site comprised four 25m² square plots, each separated from the nearest plot

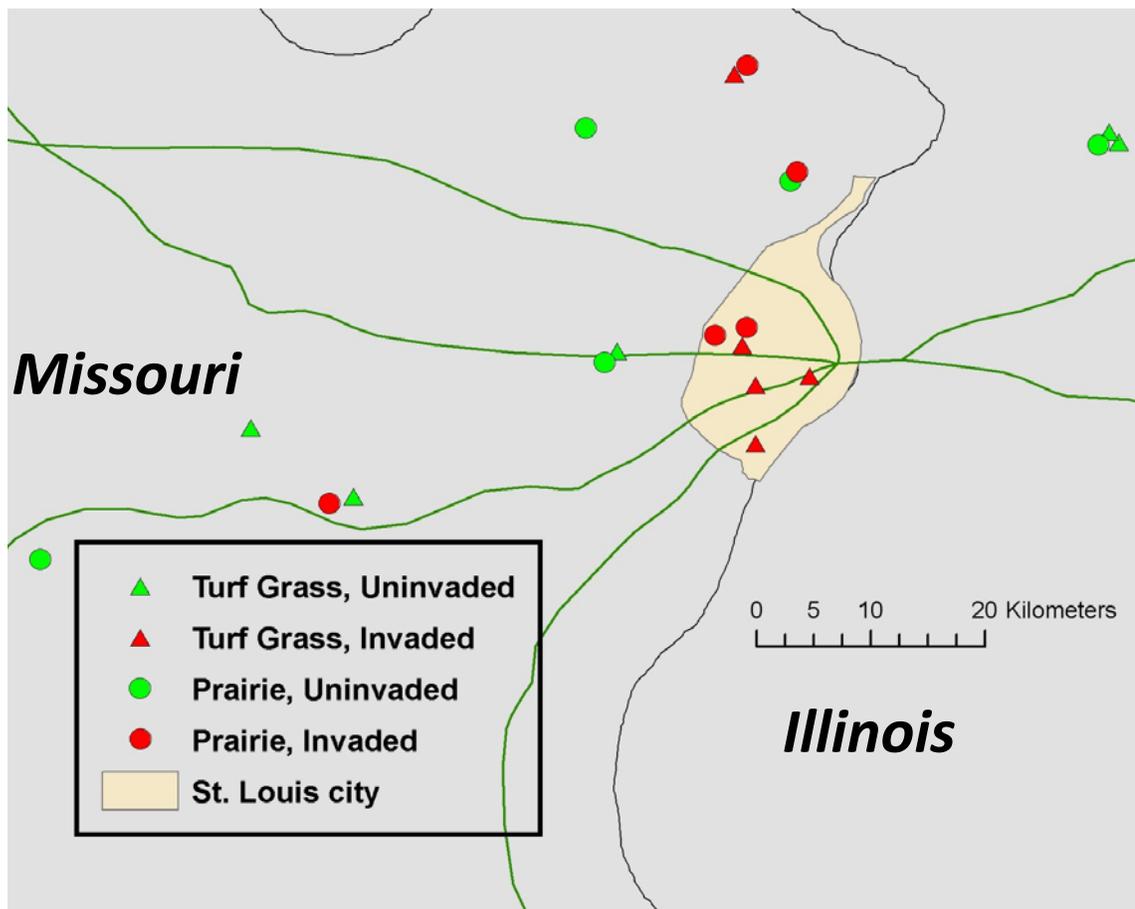


Figure 9. Map of turf grass and prairie sites where ant community structure was analyzed in 2007 and 2008 using pitfall trap and cookie bait sampling. One site, St. Stanislaus Conservation Area was invaded by *T. tsushimae* in 2008.

by 15m. I positioned the plots on an unfragmented site with relatively uniform vegetation in order to minimize microhabitat variation. At each site, I used pitfall traps and food baits to quantify local ant diversity. Pitfall traps and baits provide a comprehensive representation of surface-dwelling ant species, especially in locations with little leaf litter (e.g. turf grass and prairie; Agosti et al. 2000). I placed 1cm³ of bait at the corners of each of the four 25m² square plots and recorded the genus and abundance of foragers observed within 10cm of the bait after 20 minutes, 1 hour, and 2 hours. Ant abundance was

measured on an ordinal scale (1 ant, 2-9ants, 10-25 ants, >25 ants). Pitfall traps consisted of 50mL tubes placed in a hole with its edge flush with the soil surface. Pitfall traps were collected after 72 hours (Bestelmeyer et al. 2000). The combination of pitfall and baiting data was used to measure species diversity. Bait data was also used to calculate the foraging ability of *T. tsushimae* (i.e. time to bait discovery, total foraging time, and maximum number of foragers recruited to bait).

Individuals were identified to species and assigned to a functional group based upon the classification of Andersen (1997) and Stephens and Wagner (2006). The four groups include generalist Myrmicinae, opportunist, cryptic, and cold climate specialist. Cryptic species, such as *Solenopsis molesta*, forage primarily under the soil near their nest and are of small body size. Opportunists, such as *Lasius neoniger*, forage over large distances and specialize in resource discovery rather than resource defense. Generalist Myrmicinae, such as *Monomorium minimum*, mass recruit and aggressively defend nearby resources. Cold climate specialists, such as *Prenolepis imparis*, forage in cooler temperature and thereby avoid competition from species that require warmer temperature for activity (Hölldobler and Wilson 1990; Andersen 1997; Stephens and Wagner 2006).

Since I compared ant communities at separate locations, it was necessary to determine whether there were significant differences in environmental parameters between invaded and uninvaded sites. Climatic data at the landscape scale was collected from the Worldclim database and included the following variables: altitude, annual mean temperature, annual temperature range, minimum temperature of the coldest month, maximum temperature of the warmest month, temperature seasonality, isothermality, mean diurnal temperature range, annual precipitation, precipitation seasonality,

precipitation of the wettest month, and precipitation of the driest month (Hijmans et al. 2005). At the local scale, I measured microhabitat parameters known to affect ant diversity. These included: % vegetative cover, canopy cover, number of plant morphotypes present, and maximum vegetation height (Wang et al. 2001; Pfeiffer et al. 2003; Armbrrecht et al. 2004; Lubertazzi and Tschinkel 2003; Perfecto and Vandermeer 1996). I accessed the Missouri Cooperative Soil Survey database for soil characteristics at each site, which included measures of clay, silt, sand, organic matter, moisture penetration (Ksat), cation exchange capacity (CEC), and pH (<http://soils.missouri.edu/index.asp>). Environmental data for all sites are tabulated in appendix B.

Statistical analysis of ant communities

I used principle components analysis (PCA) to determine whether invaded and uninvaded sites were environmentally similar (Figure 10). The first two principle components account for 43% and 56% of the total variation for prairie and turf grass sites, respectively. Since there is no apparent clustering of sites by invasion status, I considered invaded sites environmentally similar to uninvaded sites. I estimated ant species richness (S) and species diversity (H' , Shannon Diversity Index, Weaver and Shannon 1949). Both, diversity and richness were calculated with the package *vegan* (v. 1.12; Oksanen 2008) in the R statistical environment (v.2.8.1; R Development Core Team 2008). I used a Mann-Whitney U test to examine differences in ant richness and diversity between invaded and uninvaded sites and Kendall's Tau to measure the correlation between individual species and Japanese pavement ant abundance. MANOVA analysis

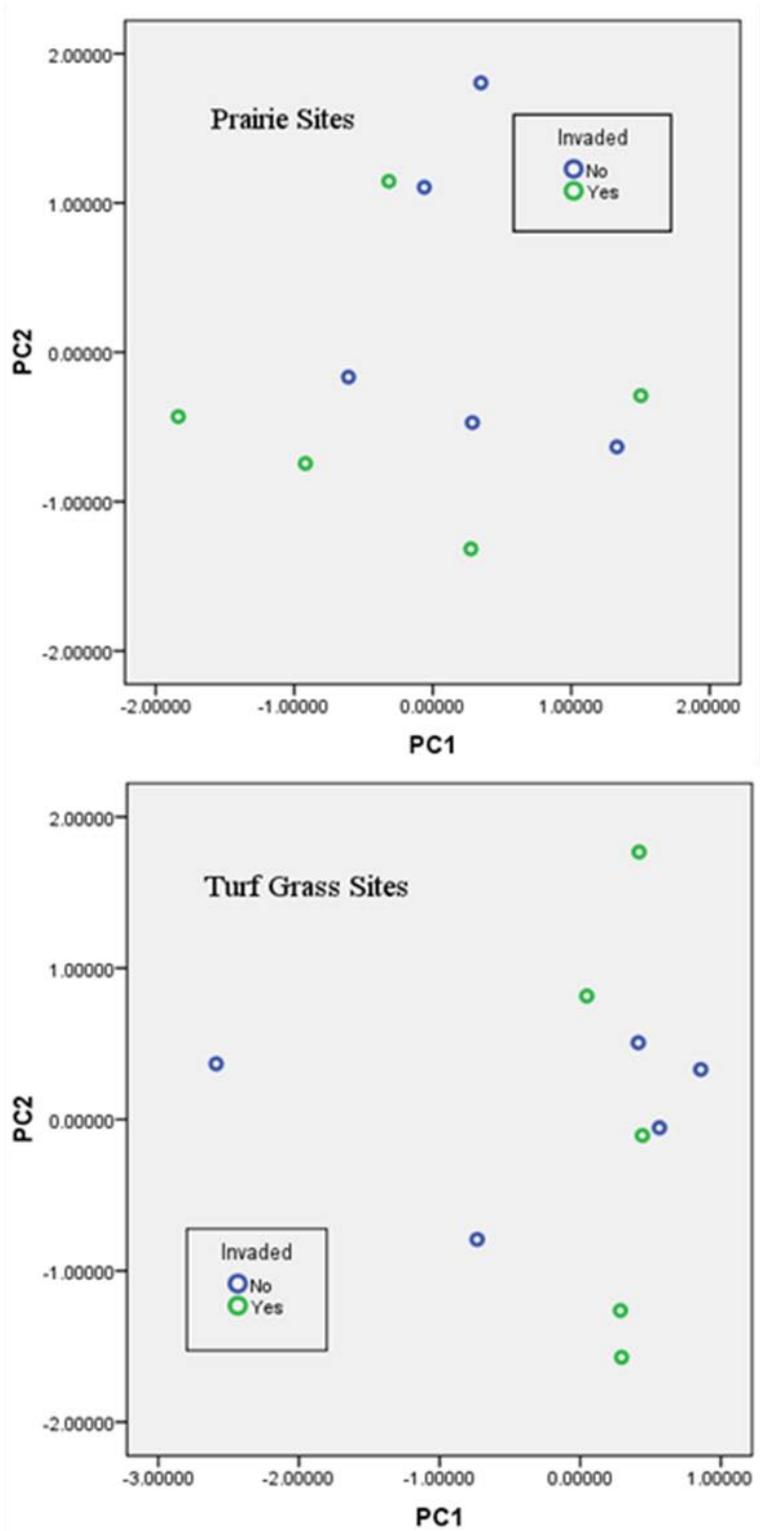


Figure 10. Scatterplot of site scores along the first and second principle components. Sites are separated by ecotype and *T. tsushima* invasion status.

was used to test for significant differences in the abundance of each ant functional group between invaded and uninvaded sites. Separate F and P values were calculated for each ecotype (i.e. prairie and turf grass).

I used a canonical correspondence analysis (CCA; ter Braak 1986) to quantify the effect of Japanese pavement ant abundance on ant community structure. CCA determines the relationship between species abundance and environmental gradients by creating ordination axes for both sets of variables and measuring correlations between them under the assumption that species abundance reacts to environmental gradients in a unimodal fashion (ter Braak 1986). These analyses were performed with the package *vegan* (v. 1.12; Oksanen 2008) in the R statistical environment (v.2.8.1; R Development Core Team 2008). For the CCA analysis, I considered Japanese pavement ant abundance as an environmental variable. The CCA was repeated on data collected from all sites in both 2007 and 2008.

To prevent overfitting of the CCA, the most orthogonal environmental factors with the greatest eigenvalues from a PCA were chosen as representative variables (Eklöv et al. 1999). In the CCA with individual species as dependent variables, the environmental variables were: % clay, % silt, K_{sat} (cm/hr), minimum annual temperature (C), annual precipitation (cm), % canopy cover, plant species richness, vegetation height (cm), and Japanese pavement ant abundance. In the CCA using ant functional groups as dependent variables, the environmental variables used in the 2007 analysis were: annual precipitation, vegetation height, and Japanese pavement ant abundance. The environmental variables used in the corresponding 2008 analysis were: % silt, vegetation height, and Japanese pavement ant abundance.

The resulting models from the CCA were contrasted with an information-theoretical approach (Burnham and Anderson 2004). In the information-theoretical paradigm, there is no hypothesis testing; instead, multiple potential models are compared to see which one most closely describes real patterns/conditions (Burnham and Anderson 2004). Kullback-Leibler (K-L) information is a measure of how closely a model approximates reality and maximized log-likelihood functions provide a statistical method of estimating K-L information (Kullback and Leibler 1951; Akaike 1974). Akaike's Information Criterion (AIC) is a metric that compares the relative information contained within competing models attempting to describe the identical real conditions. The model with the lowest AIC value contains the most K-L information using the smallest number of parameters (Akaike 1974; Burnham and Anderson 2004). I used AIC to quantify the ability of each environmental variable (including Japanese pavement ant abundance) to explain variation in native ant community structure by comparing the AIC of models with different combinations of explanatory parameters (Richards 2005). The difference between the AIC of a complete model and the AIC of the same model minus one parameter provides a metric of the independent effect of the removed parameter. Multimodel inference analysis was performed with the function "step" in the R statistical environment (v.2.8.1), which measures changes in AIC after every parameter had been removed from the model.

Results

Artificial introduction experiment

The number of recruited *L. neoniger* and *T. sessile* foragers decreased in the presence of the Japanese pavement ant. Both species experienced a significant reduction in recruitment 30 minutes after the introduction of the Japanese pavement ant (ANOVA, $P < 0.001$). I did not observe overt aggressive behavior between Japanese pavement ant foragers and either *L. neoniger* and *T. sessile*. Prior to the introduction of the Japanese pavement ant, *L. neoniger* recruited 29 ± 14.30 individuals to cookie baits (Figure 11; mean ± 1 S.D.). Ten minutes after the release of the Japanese pavement ant, forager abundance dropped to 14 ± 26.12 individuals. After 30 minutes, one of ten replicates still had active foragers at the bait. Foraging levels remained high at this one replicate (80 individuals). Ten minutes after being released, 59.5 ± 39.5 Japanese pavement ant foragers attended the bait. This level increased to 96 ± 42.2 individuals after 30 minutes. After the removal of the Japanese pavement ant, *L. neoniger* forager abundance increased to 28 ± 22.8 individuals. *L. neoniger* foraging abundance was not significantly different before the introduction and after the removal of the Japanese pavement ant ($P = 0.770$).

T. sessile recruited 45.4 ± 14.30 individuals to bait before the release of the Japanese pavement ant (mean ± 1 S.D.). Ten minutes after the release of the Japanese pavement ant, forager abundance decreased to 33.8 ± 30.8 individuals. In 3 of 12 replicates, *T. sessile* abundance increased in the same period of time. After 30 minutes, two of twelve replicates still had five active *T. sessile* foragers at the bait; no native foragers remained for the other ten replicates. Forager recruitment of *T. sessile* 30 minutes after removal of the Japanese pavement ant was significantly lower than prior to

the release of the Japanese pavement ant ($P < 0.001$). Ten minutes after being released, 57.5 ± 36.4 Japanese pavement ant foragers attended the bait. This level increased to 112.1 ± 32 individuals after 30 minutes. After the removal of the Japanese pavement ant, *T. sessile* forager abundance increased to 30.4 ± 11.8 individuals.

The number of *M. minimum* foragers present prior to the release of the Japanese pavement ant was 78.5 ± 24.6 (mean \pm S.D.) and remained high 30 minutes after the release of the Japanese pavement ant (77 ± 63.1 individuals). The presence of the Japanese pavement ant did not significantly alter *M. minimum* forager numbers (ANOVA, $P > 0.999$). Direct fighting and physical contact was rarely observed; *M. minimum* repelled Japanese pavement ant foragers primarily through gaster flagging. While the abundance of the Japanese pavement ant averaged 77 ± 55.3 individuals, individuals were located at the periphery of the foraging area and only a few workers were able to carry away cookie. The abundance of *M. minimum* before and after the introduction of the Japanese pavement ant did not significantly change (ANOVA, $P = 0.381$); however, there was a nearly significant increase in *M. minimum* foragers 30 minutes after the removal of the Japanese pavement ant (89.5 ± 16.8 individuals; ANOVA, $P = 0.072$).

Ant community impact

Mann-Whitney U tests demonstrate that invaded and uninvaded turf grass sites differed significantly in both ant species richness (2007: $U < 0.001$, $P = 0.007$; 2008: $U < 0.001$, $P = 0.007$) and ant diversity (2007: $U < 0.001$; $P = 0.008$; 2008: $U < 0.001$, $P = 0.008$; Figure 12). Among uninvaded sites, ant species richness was 10.2 ± 2.7 (Mean \pm

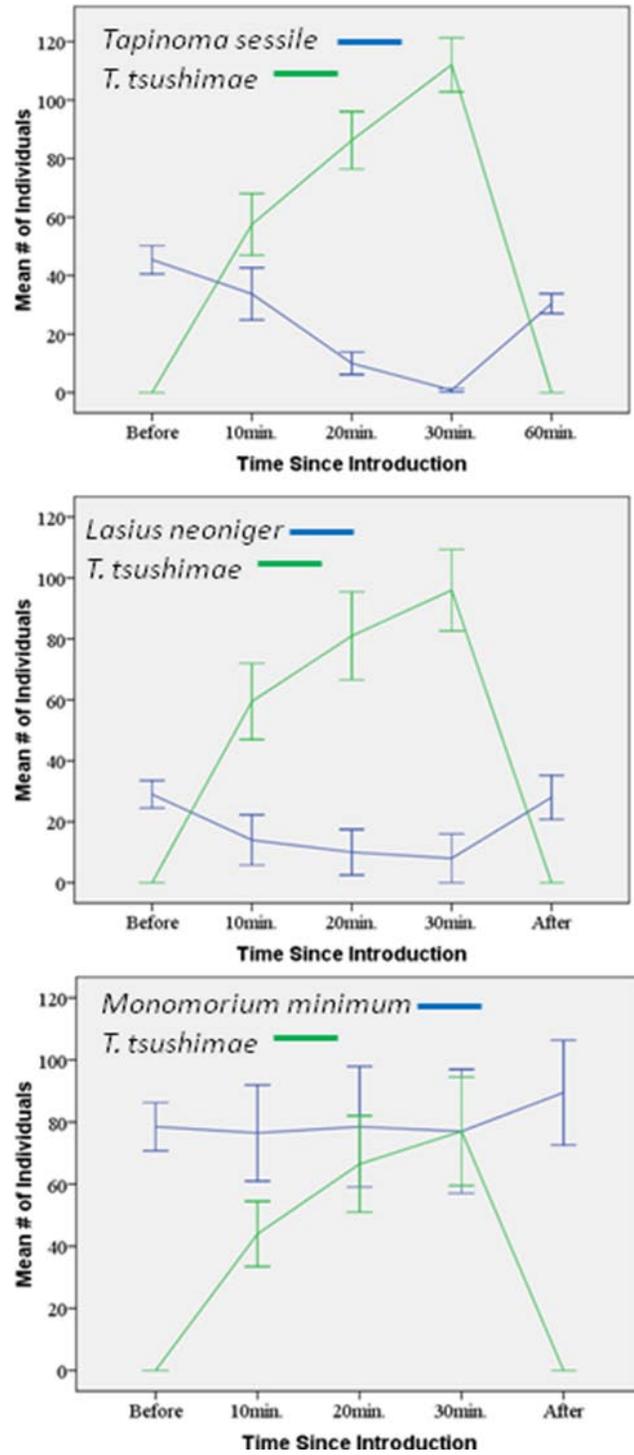


Figure 11. Forager recruitment over time in an artificial introduction experiment. The abundance of each focal species was measured 30 minutes after bait discovery and then every 10 minutes after *T. tsushimae* was allowed to forage. Focal species abundance was again measured 30 minutes after the removal of *T. tsushimae*.

S.D.) in 2007 and 8.4 ± 1.1 in 2008. In the presence of the Japanese pavement ant, ant richness decreased to 2.8 ± 0.8 in 2007 and 2.2 ± 1.1 in 2008. Ant diversity at uninvaded turf grass sites was 1.59 ± 0.22 in 2007 and 1.65 ± 0.2 in 2008. These values decreased among invaded sites in 2007 (0.08 ± 0.17) and 2008 (0.18 ± 0.4).

In 2007 and 2008, there was a significant difference between invaded and uninvaded prairie sites ant species richness (2007: Mann-Whitney $U = < 0.001$, $P = 0.008$; 2008: $U = 2.000$, $P = 0.031$; Figure 12). In 2007 ant diversity significantly differed between sites ($U = 1.000$, $P = 0.015$), but not in 2008 ($U = 3.000$, $P = 0.052$). Among uninvaded sites, ant species richness averaged 9 ± 2.5 (Mean \pm S.D.) in 2007 and 7.3 ± 2.6 in 2008. In the presence of the Japanese pavement ant, ant richness decreased to 3 ± 2.3 in 2007 and 3 ± 2.1 in 2008. Mean ant diversity at uninvaded prairie sites was 1.6 ± 0.5 in 2007 and 1.47 ± 0.55 in 2008. As in turf grass habitats, these values decreased among invaded prairie sites in 2007 (0.53 ± 0.6) and 2008 (0.55 ± 0.65).

Table 7 outlines the individual ant species I collected in this study and the proportion of invaded and uninvaded prairie and turf grass sites they were present. Among turf grass habitats, 25 species were only collected in uninvaded habitat. Three species were found in both invaded and uninvaded turf grass sites; all are subterranean cryptic species (*Solenopsis molesta*, *Hypoponera opacior*, and *Brachymyrmex depilis*).

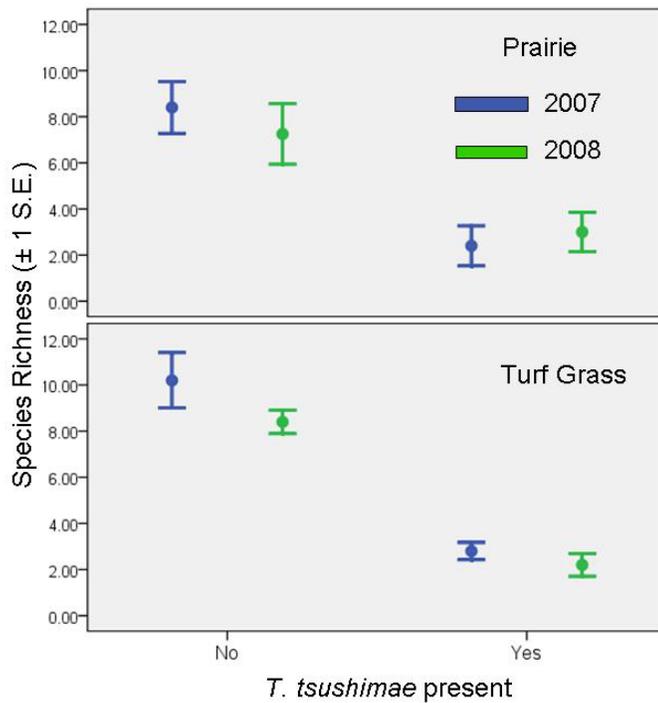
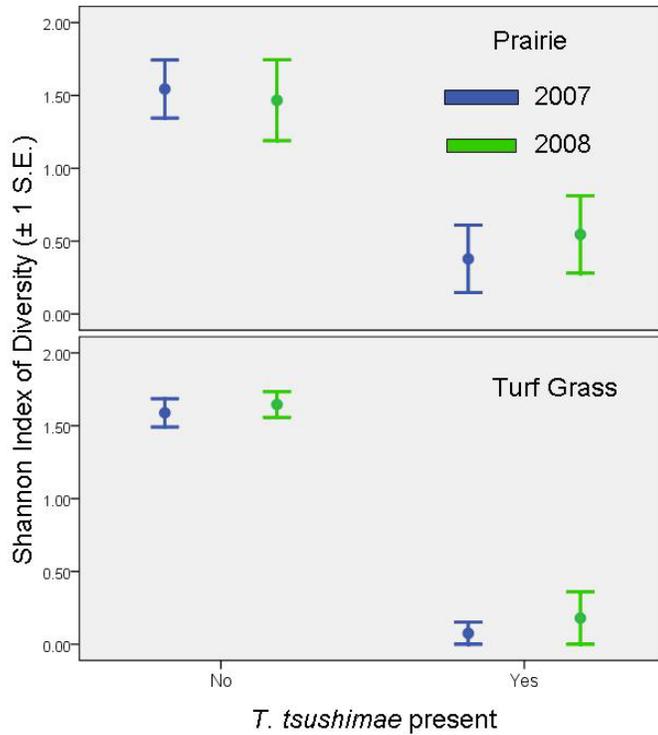


Figure 12. Error plot of ant species richness and ant diversity (Shannon's Index of Diversity) in 2007 and 2008 in prairie and turf grass habitats where the Japanese pavement ant was either present or absent.

Table 7: List of species collected by pitfall traps and cookie baits. Each species is assigned to an ecological functional group based upon Andersen (1997), Stephens and Wagner (2006), and Trager (unpublished data). The number of sites where each species was collected is listed and separated by *Tetramorium tsushimae* presence (P), absence (A) and habitat type.

Species	Functional Group	Prairie		Turf Grass	
		P	A	P	A
<i>Prenolepis imparis</i>	Cold Climate Specialist				4
<i>Stenamamma impar</i>	Cold Climate Specialist	1			
<i>Brachymyrmex depilis</i>	Cryptic			3	1
<i>Hypoponera opacior</i>	Cryptic			2	2
<i>Ponera pennsylvanica</i>	Cryptic	2	2		
<i>Solenopsis molesta</i>	Cryptic	3	2	5	5
<i>Solenopsis texana</i>	Cryptic		2	1	
<i>Crematogaster ashmeadi</i>	Generalist Myrmicine	1			
<i>Crematogaster cerasi</i>	Generalist Myrmicine		1		
<i>Crematogaster lineolata</i>	Generalist Myrmicine		1		1
<i>Crematogaster missouriensis</i>	Generalist Myrmicine				1
<i>Monomorium minimum</i>	Generalist Myrmicine	1	3		5
<i>Pheidole pilifera</i>	Generalist Myrmicine				1
<i>Pheidole tysoni</i>	Generalist Myrmicine		2		4
<i>Aphaenogaster N16</i>	Opportunist	1			
<i>Aphaenogaster N17</i>	Opportunist				1
<i>Aphaenogaster N19</i>	Opportunist	1	3		
<i>Camponotus americanus</i>	Opportunist				1
<i>Camponotus castaneus</i>	Opportunist		1		1
<i>Camponotus chromaiodes</i>	Opportunist				2
<i>Camponotus nearcticus</i>	Opportunist		1		4
<i>Camponotus pennsylvanicus</i>	Opportunist				1
<i>Formica biophilica</i>	Opportunist		2		3
<i>Formica incerta</i>	Opportunist	1	3		2
<i>Formica pallidefulva</i>	Opportunist		1		1
<i>Formica subsericea</i>	Opportunist		2		4
<i>Formica vinculans</i>	Opportunist				1
<i>Lasius alienus</i>	Opportunist		4		1
<i>Lasius claviger</i>	Opportunist		1		
<i>Lasius neoniger</i>	Opportunist		1		3
<i>Myrmica evanida</i>	Opportunist		5		3
<i>Paratrechina faisonensis</i>	Opportunist	1	3		4
<i>Paratrechina parvula</i>	Opportunist				1
<i>Tapinoma sessile</i>	Opportunist	2	4		2
<i>Temnothorax curvispinosus</i>	Opportunist		1	1	
<i>Temnothorax longispinosus</i>	Opportunist	1	1		
<i>Temnothorax pergandei</i>	Opportunist				1
<i>Temnothorax schaumii</i>	Opportunist				1
<i>Tetramorium tsushimae</i>	Opportunist	6		5	

Brachymyrmex and *Solenopsis* were the only genera to inhabit more than one of the ten invaded sites in both 2007 and 2008. The cryptic *Solenopsis molesta* was collected at all ten turf grass sites. *Temnothorax curvispinosus* was the only opportunistic ant species present at an invaded turf grass site.

Among prairie habitats, 15 species were only present at uninvaded prairie sites. The genera *Lasius*, *Monomorium*, *Myrmica*, *Camponotus* and *Pheidole* were absent from all invaded sites. *Tapinoma sessile* only existed in the presence of the Japanese pavement ant at 2 prairie sites within St. Louis city limits (Kennedy Woods and Deer Lake). The only *Formica* individuals collected from invaded sites were three *Formica incerta* individuals found at the Deer Lake invaded prairie in 2008. Three species were collected only from a single invaded prairie site (*Aphaenogaster* N16, *Crematogaster ashmeadi*, and *Stenamma impar*). Seven species were present at both invaded and uninvaded prairie sites and one, *S. molesta*, was located at three invaded sites and two uninvaded sites.

Among all species collected in this study, the Myrmicines *Monomorium minimum* and *Myrmica evanida* have the lowest significant coefficient of correlation with Japanese pavement ant abundance (Table 8). In addition to *M. minimum* and *M. evanida*, *Pheidole tysoni* abundance negatively correlates with Japanese pavement ant abundance in both 2007 and 2008. All three species are in the subfamily Myrmicinae.

At both turf grass and prairie sites in 2007 and 2008, the average abundance of individuals within each ant functional species group was lower when the Japanese pavement ant was present (Figure 13). Among ant functional groups, only cryptic species were sympatric with the Japanese pavement ant at turf grass sites. In 2007, an average of 16.2 ± 20.1 (mean \pm S.D.) cryptic individuals were collected per site compared to $575.8 \pm$

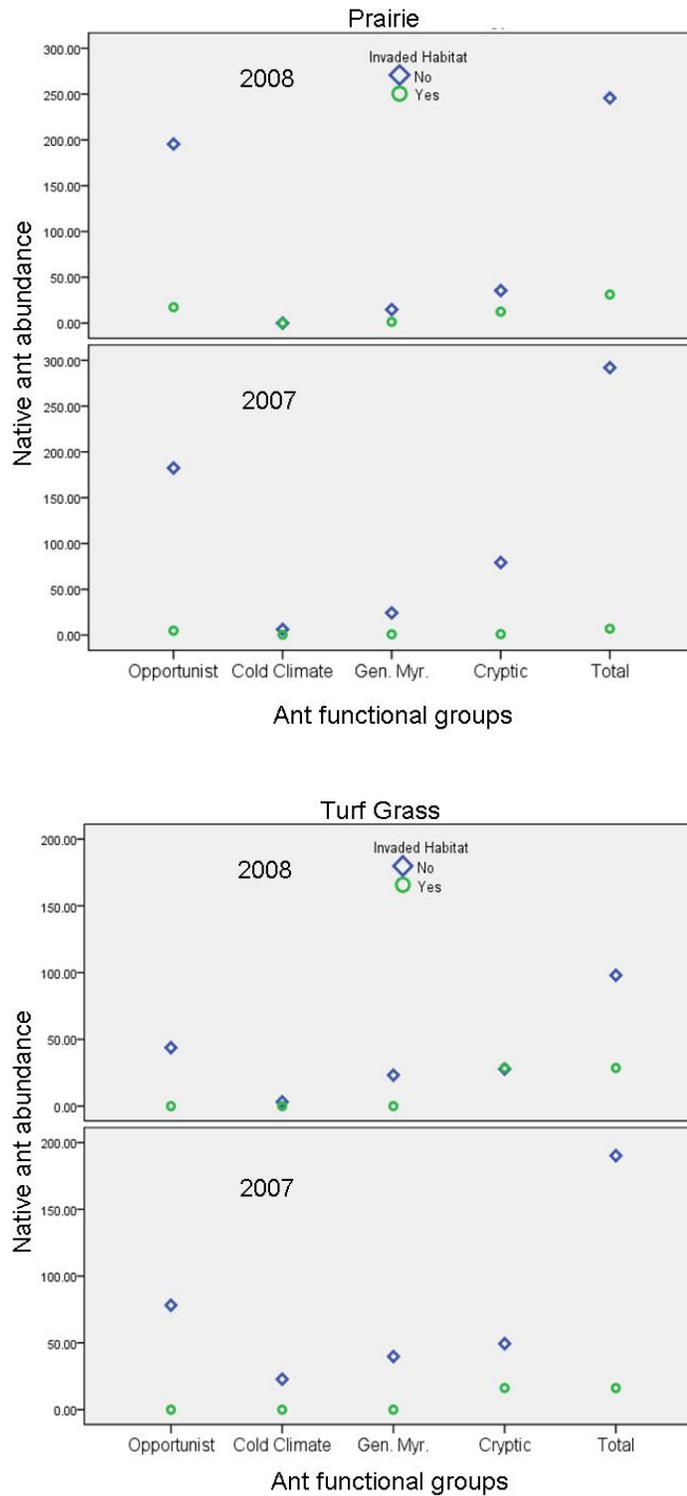


Figure 13. Plot of mean abundance of different ant functional species groups in 2007 and 2008. Mean abundance is isolated habitat type (prairie and turf grass) and the presence or absence of the Japanese pavement ant.

Table 8: List of species correlated with Japanese pavement ant abundance. Coefficients of correlation were calculated using Kendall's Tau.

Species	Coefficient of Correlation (r)	
	2007	2008
<i>Monomorium minimum</i>	-0.597	-0.541
<i>Myrmica evanida</i>	-0.549	-0.535
<i>Tapinoma sessile</i>	-0.447	ns
<i>Paratrechina faisonensis</i>	-0.411	ns
<i>Formica biophilica</i>	-0.397	ns
<i>Lasius alienus</i>	-0.397	ns
<i>Pheidole tysoni</i>	-0.397	-0.537
<i>Formica subsericea</i>	ns	-0.427

419.4 Japanese pavement ant individuals. The pattern was similar in 2008 where invaded turf grass site pitfall traps yielded an average 28.6 ± 34.7 cryptic individuals and 549.4 ± 178.2 Japanese pavement ant individuals. MANOVA analyses demonstrate that the reduction in mean abundance of all native ants is significant at both prairie and turf grass sites in 2007 ($P = 0.008$; $P = 0.016$, respectively) and 2008 ($P < 0.001$; $P = 0.047$, respectively; Table 9). In 2007, the abundance of the following functional groups was not significantly different between invaded and uninvaded sites: opportunists in turf grass and generalist Myrmicines and cold climate specialists in prairie sites. In 2008, ant abundance was not significantly different in both prairie and turf grass communities for any functional species group. Cryptic ant abundance was significantly lower at both ecotypes in 2007 (prairie: $P = 0.05$; turf grass: $P = 0.038$) and neither in 2008 (prairie: $P = 0.211$; turf grass: $P = 0.964$).

The mean number of individuals collected from pitfall traps located at invaded sites was greater than at uninvaded sites. An average of 606 ± 139 Japanese pavement ant

Table 9: ANOVA table testing for significant differences in ant abundance between environmentally similar sites where *Tetramorium tsushimae* is either present or absent. Ant species were divided into ecological functional groups based upon Anderson (1997), Stephens and Wagner (2006), and Trager (unpublished data).

Functional Group	Ecotype	2007		2008	
		F	Sig.	F	Sig.
Opportunists	Prairie	10.979	.011	8.105	.022
	Grass	4.885	.058	2.450	.156
	All Sites	13.422	.002	6.461	.020
Cold Climate Specialists	Prairie	1.026	.341		
	Grass	5.911	.041	1.882	.207
	All Sites	5.929	.026	2.141	.161
Generalist Myrmicines	Prairie	4.556	.065	4.571	.065
	Grass	8.784	.018	251.514	.000
	All Sites	13.575	.002	32.434	.000
Cryptic	Prairie	5.345	.050	1.851	.211
	Grass	6.192	.038	.002	.964
	All Sites	9.549	.006	.947	.343
Total	Prairie	12.226	.008	9.324	.016
	Grass	32.059	.000	5.476	.047
	All Sites	26.650	.000	9.892	.006

individuals were trapped at invaded sites, while only collecting an average of 204 ± 32 native ants from uninvaded sites. However, these differences were not significant in 2007 (t test: $P = 0.068$) or 2008 (Mann-Whitney: $P = 0.175$). At invaded sites, 70% of bait stations were attended by >25 individuals and less than 16% of bait stations failed to recruit any foragers (Figure 14). At Bellefontaine Conservation Area (prairie) and Sioux Passage County Park (turf grass) every bait station recruited over 25 individuals during 2007 and 2008.

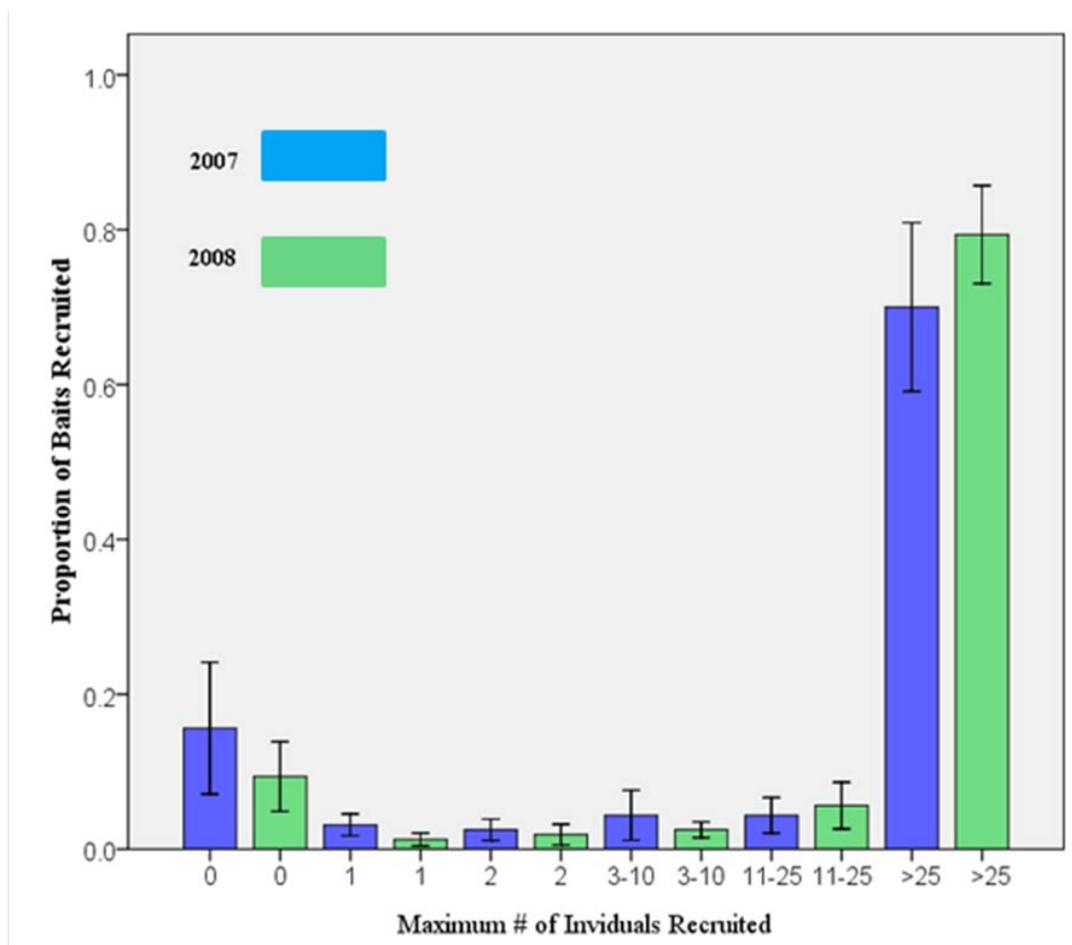


Figure 14. Barplot of *T. tsushimae* foraging response to baits placed at 20 prairie and turf grass sites. Each bar represents the average proportion of baits per site that *T. tsushimae* recruited to within 2 hours per abundance class. Error bars represent ± 1 standard error.

In the four CCA models (i.e. individual species response to environmental variation in 2007 and 2008, as well as ant functional species group response to environmental variation in 2007 and 2008), three accounted for 62.1% - 66.4% of the total variation in native ant community structure. The CCA comparing ant functional groups to environmental variation in 2007 accounted for 38% of the total variation. In both 2007 and 2008, all invaded sites cluster together and are best explained by the eigenvector describing variation in Japanese pavement ant abundance (Figure 15). Of the

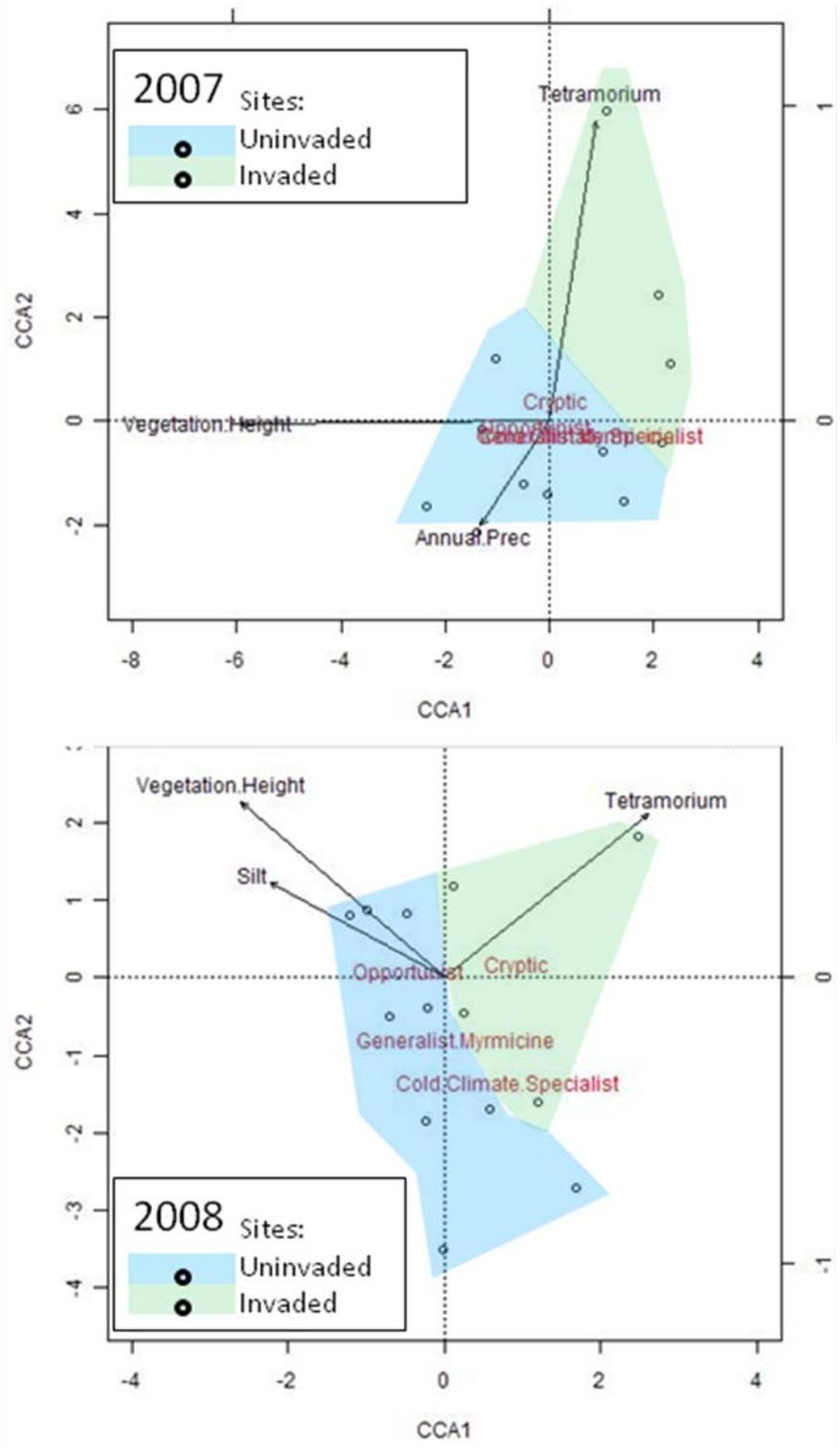


Figure 15. Plot of the first and second eigenvectors of a canonical correspondence analysis comparing the effect of *T. tsushimae* and the environment on ant community structure. Sites that received the same score appear as a single circle. In both 2007 and 2008, five sites received identical scores, each with the highest CCA1 and CCA2 scores.

Table 10: The effect of *Tetramorium tsushimae* on ant community structure based upon Canonical Correspondence Analysis (CCA). Akaike's Information Criterion (AIC), Δ AIC, and weight (ω) are given, representing the goodness of fit of each CCA model with and without *Tetramorium tsushimae* as an explanatory environmental variable. Lower AIC values indicate more accurate models. Weight represents the proportion of replicates where the specified model is the best fit after bootstrapping. Individual CCA was performed for individual ant species (SP) and functional species groups (FG). Values in bold indicate a model that is more accurate without *Tetramorium tsushimae* abundance as an explanatory variable.

		AIC		Δ AIC		ω	
Year		2007	2008	2007	2008	2007	2008
SP	Total Model	111.19	101	0.45	0	0.132736	0.143909
	<i>T. tsushimae</i> removed	110.74	102	0	1	0.166228	0.087285
FG	Total Model	72.577	62.86	1.403	0	0.261471	0.702982
	<i>T. tsushimae</i> removed	73.507	70.796	2.333	7.936	0.164239	0.013294

four functional species groups in the CCA biplot, cryptic species are most closely associated with increased Japanese pavement ant abundance and invaded ant communities. No native species were present at three invaded sites in both 2007 and 2008; therefore, these sites could not be incorporated in CCA models. Of the four CCA models, three performed better (i.e. AIC values were greater) when Japanese pavement ant abundance was included among the explanatory variables (Table 10). Only the model that included individual native species in 2007 had a lower AIC value when Japanese pavement ant abundance was removed as a predictor variable.

Discussion

Competitive ability

In this study, the presence of Japanese pavement ants reduced the number of *Lasius neoniger* and *Tapinoma sessile* foragers that recruited to a cookie bait. After native species discovered and recruited foragers to a bait located 20cm from a nest

entrance, introduction of the Japanese pavement ant significantly reduced the number of native foragers within thirty minutes. Native species recruitment remained low until at least 30 minutes after the removal of Japanese pavement ant foragers.

No significant interspecific aggression was observed during these interference competition trials. *Tetramorium* fights are conspicuous and well-known; large-scale intraspecific battles are commonplace among *T. sp. E* and native populations of the Japanese pavement ant (Hölldobler and Wilson 1990; Morisita 1939). Instead of noxious chemical sprays, *Tetramorium* species generally rely on conspicuous biting and stinging in agonistic interactions, behavior unlikely to be missed by an observer (von Sicard et al. 1989). The lack of aggression is likely due to the propensity of *T. sessile* and *L. neoniger* to abdicate food resources when in contact with competitors. Both species are classified as “opportunists” and “submissives,” relying on resource discovery rather than defense (Savolainen and Vepsäläinen 1988; Andersen 1997).

Similar to *T. sessile* and *L. neoniger*, the Japanese pavement ant is classified as an opportunistic species that has generalist nesting and food requirements (Passera 1994; Hölldobler and Wilson 1990). It is possible that niche overlap, as well as competitive dominance of the Japanese pavement ant over *T. sessile* and *L. neoniger* may explain the apparent absence of these species from invaded habitats. This is supported by evidence that cryptic species are least affected by the invasion. Species such as *Solenopsis molesta* are subterranean, can nest and forage in moist soil not preferred by the Japanese pavement ant, and require limited foraging territories (Hölldobler and Wilson 1990). Cryptic species coexist with the Argentine ant and make up a significantly larger portion

of ant diversity in invaded habitats than those that remain uninvaded (Human and Gordon 1997).

Despite the success of the Japanese pavement ant in usurping food resources from *L. neoniger* and *T. sessile*, artificial introductions of the Japanese pavement ant failed to significantly affect the foraging ability of *M. minimum*. *M. minimum* workers aggressively defended cookie baits and frequently repelled Japanese pavement ants by gaster flagging, which releases a noxious substance from the sting (Adams and Traniello 1981). The genus *Monomorium* is classified as a generalist Myrmicine, which specializes in mass recruitment and interference competition at food resources located near nest entrances (Anderson 1997). Interspecific aggression occurs nearly 100% of the time when other species attempt to remove larger food items from foraging workers (Adams and Traniello 1981). Despite small body size, *M. minimum* can aggressively defend and acquire resources from the two most common and invasive ant species in North America, the Argentine ant, *Linepithema humile*, and the red imported fire ant, *Solenopsis invicta* (Alder and Silverman 2005; Keck et al. 2005; Holway et al. 2002). During this study, Japanese pavement ant workers attempted to approach baits, yet were unable to approach baits due to the aggressive behavior of *M. minimum*.

The failure of the Japanese pavement ant to change foraging patterns in *M. minimum* in an artificial introduction experiment fails to explain why these two species did not coexist at any of the invaded sites measured in this study. The apparent lack of *M. minimum* nests in invaded sites may be partially explained by high population densities of the opportunistic and unicolonial Japanese pavement ant. The ability of the Japanese

pavement ant to excel at resource discovery and mass recruitment in a natural setting may restrict the foraging territory and food resources available to *M. minimum* colonies.

Aside from competition for food resources, competition for nesting space may limit the dispersal and ultimately the persistence of *M. minimum* nests. In the Argentine ant, competition for nest sites is not thought to be a factor limiting the dispersal of native ant species, because nest raids are rarely observed and the patchy distribution of Argentine ant nests at small scales leaves significant amounts of available nest space (Holway 1999; Heller et al. 2008; Menke and Holway 2006). Nest raiding is not uncommon in the Japanese pavement ant and occurs underground as expanding nests open into native ant colony galleries (J. Trager pers. comm.). Also, this study showed that Japanese pavement ant in some locations recruited to 100% of bait stations, suggesting high population density in a species that constructs networks of interconnected shallow nests in open, warm soil (Sanada-Morimura et al. 2006). Both *M. minimum* and the Japanese pavement ant prefer high soil temperatures for nesting (J. Trager pers. comm.). *M. minimum* may be unable to successfully disperse if all nearby nesting sites are occupied by highly dense groups of the Japanese pavement ant. *M. minimum* is highly territorial and not known to reproduce by budding; instead, individual queens undergo mating flights and must establish colonies alone. Mortality is high during this reproductive phase and may be nearly 100% in a landscape dominated by the Japanese pavement ant (Hölldobler and Wilson 1990). In this fashion, highly competitive native ants such as *M. minimum* may be unable to maintain population growth and eventually disappear. Testing this hypothesis would require long-term monitoring of newly invaded

habitat, mapping the distribution of nests over time and further knowledge of nesting preferences.

Effect on ant community structure

In this study, I found that the presence of Japanese pavement ants in prairie and turf grass habitats was associated with a significant reduction in ant species richness and diversity. I did not find the widespread genera *Lasius*, *Monomorium*, *Myrmica*, *Camponotus* and *Pheidole* at any invaded study sites, either prairie or turf grass. In addition to a reduction in the number of species, all species functional groups (i.e. opportunist, cold climate specialist, generalist Myrmicine, and cryptic) were less abundant at invaded sites for at least one year of the study. Canonical correspondence analyses (CCA) demonstrated that Japanese pavement ant abundance explained more variation in ant community composition than climatic, soil, and microhabitat variables.

By choosing environmentally similar sites and explicitly incorporating existing environmental variation into analyses, I isolated the Japanese pavement ant as a potential causal agent leading to changes in ant communities within the invaded range. Without accounting for the effect of environmental differences between sites, species associations can be merely an artifact of environmental gradients (Ter Braak 1987). For example, anthropogenic disturbance can alter landscapes to fit the niche of exotic species rather than native biota (Didham et al. 2007).

The Japanese pavement ant is a common ant that can inhabit most dry, open canopy environments in both its native and invaded range (Steiner et al. 2006). Ecological niche modeling has shown it has the potential to occupy these habitats east to

Ohio and west to the Rockies (Steiner et al. 2008). The narrow geographic distribution of study sites and relatively similar microhabitat characteristics between study sites make environmental variation an unlikely candidate driving variation in ant community structure.

Despite the significant differences in species composition between invaded and uninvaded habitats, these differences were not uniform between collection years. Cryptic species, predominantly *Solenopsis molesta* and *S. texana*, and cold climate specialists were significantly rarer in invaded turf grass and prairie sites during 2007, but the effect disappeared in 2008. The increase in abundance among native species and concurrent decrease in Japanese pavement ant abundance may be due to climatic changes. The 2007 summer months in St. Louis, MO were warmer than in 2008 (NOAA 2009). The optimal soil temperature to rear reproductive brood of the Japanese pavement ant is 30°C (Sanada-Morimura et al. 2006; Cerdá et al. 1998). Conversely, cryptic species tend to nest in moist leaf litter and soil and are not as heat tolerant (Holldobler and Wilson 1990). Therefore, the warmer 2007 summer potentially resulted in an increase in Japanese pavement ant population growth and cryptic ant mortality. To the best of my knowledge, no site underwent significant microhabitat changes or disturbance between 2007 and 2008.

Aside from temporal differences in native ant diversity, ant communities within prairies were generally less affected than turf grass sites, particularly prairies with elevated canopy cover and vegetation height. Cryptic species were the only native ants I collected in invaded turf grass sites. This difference may be the result of a combination of factors acting in concert. First, the Japanese pavement ant prefers high levels of

disturbance and is ubiquitous in urban St. Louis, MO; since turf grass is mowed and otherwise disturbed frequently, turf grass in developed areas are preferred habitat. Prairie in Missouri and Illinois is less disturbed and more isolated from urban areas. Second, the invaded prairie sites with the highest concentration of native species possessed the greatest canopy cover and vegetation height. Increased shade at the soil level decreases soil temperature and increases moisture retention. Japanese pavement ants in the native range prefer open canopy, warm, dry soils (Sanada-Morimura et al. 2006). Third, an increase in vertical complexity and plant diversity may provide more available nest space and food resources, which can increase the amount of resources available to native ant species (Hölldobler and Wilson 1990; Trager 1998).

The Japanese pavement ant significantly impacts native ant communities found in prairie and turf grass ecosystems. This unicolonial species outcompetes the opportunistic, ground-dwelling native species *T. sessile* and *L. neoniger* for food resources. In some locations, the Japanese pavement ant is widespread and coexists with no native species. A comparison of invaded and uninvaded habitats show that Japanese pavement ant abundance is linked to a decrease in ant species richness and diversity, despite an overall increase in the number of total individuals collected. The combination of competition experimentation and a comparison of invaded and uninvaded communities indicate that the Japanese pavement ant may be driving the decline of native ant populations in Missouri and Illinois. Further study is necessary to understand the exact mechanisms eliminating native ant populations and help attenuate one of the only temperate and early-stage ant invasions worldwide.

Appendix A. Table of cuticular hydrocarbon concentration for individual compounds found in 17 collected nests tested in intraspecific aggression assays. Values are the mean relative area under the curve for the compound of each peak as measured by GC-MS.

Nest ID	Cuticular Hydrocarbon Compounds									
	1	2	3	4	5	6	7	8	9	10
2191	0.0221	0.1168	0.0372	0.1310	0.0836	0.0747	0.0067	0.0105	0.0673	0.0012
2192	0.0127	0.0700	0.0206	0.0822	0.0925	0.0331	0.0026	0.0121	0.0664	0.0030
2193	0.0051	0.0651	0.0247	0.0939	0.0943	0.0252	0.0030	0.0109	0.0776	0.0028
2194	0.0044	0.0651	0.0151	0.0816	0.1291	0.0119	0.0025	0.0063	0.0664	0.0037
2205	0.0311	0.1242	0.0314	0.1005	0.0987	0.0394	0.0034	0.0064	0.0691	0.0022
2207	0.0046	0.0664	0.0249	0.0941	0.0656	0.0331	0.0036	0.0139	0.0531	0.0017
2216	0.0046	0.0731	0.0178	0.0831	0.1503	0.0200	0.0030	0.0090	0.0796	0.0041
2217	0.0170	0.1065	0.0331	0.1380	0.0825	0.0616	0.0036	0.0088	0.0625	0.0018
2218	0.0340	0.0932	0.0340	0.1087	0.0691	0.0413	0.0041	0.0149	0.0647	0.0017
2234	0.0124	0.0708	0.0203	0.0976	0.0714	0.0233	0.0021	0.0087	0.0635	0.0019
2235	0.0043	0.0647	0.0154	0.1077	0.1023	0.0149	0.0011	0.0058	0.0542	0.0020
2264	0.0021	0.0532	0.0126	0.0852	0.1272	0.0160	0.0023	0.0058	0.0714	0.0036
2265	0.0034	0.0777	0.0154	0.0612	0.1632	0.0147	0.0022	0.0056	0.0707	0.0046
2303	0.0098	0.1359	0.0173	0.0763	0.1639	0.0051	0.0050	0.0130	0.0372	0.0032
2304	0.0109	0.0776	0.0334	0.0866	0.0550	0.0438	0.0090	0.0161	0.0613	0.0028
2305	0.0019	0.0565	0.0111	0.0895	0.0591	0.0166	0.0026	0.0109	0.0189	0.0015
2307	0.0105	0.0685	0.0210	0.1052	0.0769	0.0153	0.0114	0.0149	0.0319	0.0023

Appendix A. cont.

Nest ID	Cuticular Hydrocarbon Compounds									
	11	12	13	14	15	16	17	18	19	20
2191	0.0032	0.0097	0.2815	0.0045	0.0251	0.0246	0.0021	0.0124	0.0694	0.0162
2192	0.0086	0.0151	0.3236	0.0100	0.0169	0.0310	0.0052	0.0326	0.1226	0.0394
2193	0.0133	0.0181	0.2957	0.0146	0.0128	0.0247	0.0058	0.0396	0.1303	0.0425
2194	0.0086	0.0127	0.3259	0.0182	0.0092	0.0206	0.0065	0.0359	0.1284	0.0479
2205	0.0038	0.0096	0.2927	0.0101	0.0205	0.0148	0.0038	0.0223	0.0880	0.0278
2207	0.0203	0.0261	0.2514	0.0070	0.0157	0.0333	0.0043	0.0621	0.1837	0.0351
2216	0.0173	0.0136	0.2595	0.0207	0.0145	0.0196	0.0085	0.0441	0.1199	0.0378
2217	0.0042	0.0134	0.2751	0.0081	0.0265	0.0223	0.0022	0.0193	0.0925	0.0210
2218	0.0053	0.0159	0.2938	0.0067	0.0187	0.0314	0.0032	0.0276	0.1052	0.0266
2234	0.0077	0.0143	0.3404	0.0121	0.0182	0.0309	0.0055	0.0341	0.1306	0.0341
2235	0.0119	0.0156	0.3306	0.0123	0.0075	0.0161	0.0053	0.0422	0.1444	0.0417
2264	0.0157	0.0153	0.2581	0.0219	0.0076	0.0189	0.0110	0.0473	0.1661	0.0595
2265	0.0121	0.0134	0.2296	0.0210	0.0076	0.0170	0.0128	0.0439	0.1492	0.0748
2303	0.0108	0.0280	0.2048	0.0190	0.0095	0.0103	0.0309	0.0272	0.0654	0.1273
2304	0.0242	0.0317	0.2149	0.0149	0.0227	0.0387	0.0196	0.0532	0.1526	0.0310
2305	0.0279	0.0527	0.2222	0.0084	0.0132	0.0387	0.0037	0.0880	0.2131	0.0635
2307	0.0257	0.0373	0.2426	0.0098	0.0106	0.0393	0.0032	0.0709	0.1614	0.0412

Appendix B. Table of mean estimates of environmental variables measured at ten prairie and ten turf grass sites that differ by invasion status. Variables included are as follows: *T. fusiformis* presence in 2007 (Inv. 2007) and 2008 (Inv. 2008); soil composition as measured by proportion of clay, silt, and sand; moisture penetration in soil (Ksat), soil organic matter (Org.), pH; cation exchange capacity (CEC); mean diurnal temperature range (MDR), annual precipitation (AP), altitude (Alt); canopy cover (Can. C); vegetation richness (Veg. Rich.); maximum vegetation height (Veg. H.); vegetation cover (Veg. C); *T. fusiformis* abundance in 2007 (T.t. 2007) and 2008 (T.t. 2008); maximum annual temperature (Max. T.); minimum annual temperature (Min. T.); precipitation of the driest month (PDM).

Site name	Habitat	Inv. 2007	Inv. 2008	Clay (%)	Silt (%)	Sand (%)	Ksat(in/h r)	Org. (%)	pH	CEC (meq/100g)	MDR (C)
Bellefontaine CA	prairie	Yes	Yes	21.8	72.6	5.6	1.28	2.6	6.6	16	128
Bellefontaine CA2	prairie	No	No	23	73	4	1.28	0.5	5.1	24	114
Carondelet	turfgrass	Yes	Yes	21.8	73	5.2	1.28	2	7.2	22	114
Deer Lake	prairie	Yes	Yes	23	73	4	1.28	0.5	5.1	24	128
Forest Park	turfgrass	Yes	Yes	21.8	72.6	5.6	1.28	2.6	6.6	16	120
Kennedy Woods	prairie	Yes	Yes	21.8	72.6	5.6	1.28	2.6	6.6	16	138
Lafayette	turfgrass	Yes	Yes	23	73	4	1.28	0.5	5.1	24	120
LREC 1	turfgrass	No	No	21.8	73	5.2	1.28	2	7.2	22	138
LREC 2	prairie	No	No	21.8	73	5.2	1.28	2	7.2	22	138
Rockwoods CA	turfgrass	No	No	15	58.5	26.5	1.28	1.5	6.5	15	115
Route 66 SP	prairie	Yes	Yes	22.5	52.5	25	0.43	1.25	6.5	15	114
Shaw NP	prairie	No	No	15.2	78.9	5.9	1.28	2	5.3	17	133
Sinix Passage 1	turfgrass	Yes	Yes	12	79	9	1.28	2.5	7.2	10.1	114
Sinix Passage 2	prairie	Yes	Yes	25.3	69.1	5.6	1.28	3.5	5.5	20.5	138
SIUE Gardens	turfgrass	No	No	24	72	4	1.3	2	6.5	12.5	120
SIUE 2	prairie	No	No	24	72	4	1.3	2	6.5	12.5	114
SIUE UP	turfgrass	No	No	22	74	4	1.3	2	6.2	13	138
St. Stanislaus CA	prairie	No	Yes	30.7	68.4	0.9	0.38	3.4	7.6	22.5	131
Tower Grove Park	turfgrass	Yes	Yes	26	64.9	9.1	1.28	3.5	6.5	33	138
West Tys on Cty Park	turfgrass	No	No	49	47	4	0.03	7	7	34	115

Appendix B cont.

Site name	Habitat	AP (cm)	Alt. (ft)	Can. C. (%)	Veg. Rich.	Veg. H. (cm)	Veg. C. (%)	T.t. 2007	T.t. 2008	Max. T. (C)	Min. T. (C)	PDM (cm)
Bellefontaine CA	prairie	985	198	0	7	80	83.3	946	755	317	-79	50
Bellefontaine CA2	prairie	988	172	0	3	130	97.9	0	0	314	-68	50
Carondelet	turfgrass	965	160	15.9	7	10	93.8	1244	837	315	-68	49
Deer Lake	prairie	994	192	17.9	9	130	100	120	15	308	-77	51
Forest Park	turfgrass	973	161	17.9	4	10	93.8	150	573	319	-68	49
Kennedy Woods	prairie	960	138	16.4	13	170	100	142	210	322	-62	50
Lafayette	turfgrass	965	135	70.2	5	10	72.9	304	292	321	-65	50
LREC1	turfgrass	964	151	25.9	5	10	91.7	0	0	322	-64	50
LREC2	prairie	964	154	0	7	180	100	0	0	321	-64	50
Rockwoods CA	turfgrass	962	149	65.3	5	10	95.8	0	0	316	-72	47
Route 66 SP	prairie	966	164	0	8	80	100	338	63	315	-68	50
Shaw NP	prairie	991	185	0	14	120	100	0	0	319	-79	51
Sioux Passage 1	turfgrass	962	169	9.81	4	10	100	619	639	317	-67	48
Sioux Passage 2	prairie	955	132	0	12	100	95.8	2370	2132	319	-72	47
SIUE Gardens	turfgrass	973	161	0	5	10	97.9	0	0	319	-68	49
SIUE2	prairie	962	169	0	7	110	100	0	0	317	-67	48
SIUEUP	turfgrass	969	170	0	5	20	83.3	0	0	319	-65	50
St. Stanislaus CA	prairie	987	135	0	5	170	100	0	18	321	-78	51
Tower Grove Park	turfgrass	964	148	55.6	6	10	85.4	562	406	321	-64	50
West Tyson Cty Park	turfgrass	962	149	46	2	10	81.3	0	0	316	-72	47

CHAPTER 5: SYNTHESIS AND CONCLUSIONS

Discovered in 1988, the Japanese pavement ant, *Tetramorium tsushimae*, has rapidly spread from St. Louis, Missouri and become a ubiquitous pest capable of altering native ant communities in the U.S. Midwest (Steiner et al. 2006). In its native Japan, populations are members of diverse and stable ant communities (Sanada-Morimura et al. 2006; Onoyama 1980; Suzuki et al. 2004). The characteristics, evolution, and effects of this invasion after colonization of North America are poorly studied. The purpose of my research is to better understand the causes, consequences, and evolution of this system and how the dynamics of this invasion relate to other ant invasions.

In chapter one, I review the literature on the early stages of successful ant invasions. After transportation to novel habitats, exotic species must survive an initial colonization stage. Few invasions ever become invasive; different traits and conditions are necessary to pass through each invasion stage. Knowledge of these traits and the role human society plays in promoting the spread of species is essential to preventing further loss of biodiversity. A greater understanding of the initial stages of invasion is vital, because conservation efforts are most effective when invasions are incipient (Holway et al. 2002). Unfortunately, few successful ant invasions in the early stages of spread are known. Small, potentially innocuous exotic species often escape notice. Studying the dynamics of the Japanese pavement ant invasion in North America is a rare opportunity to study a successful invasion that has only recently begun rapid geographic spread.

In chapter 2, I conducted a population genetic analysis of the Japanese pavement ant invasion by genotyping 52 North American nests and 5 Japanese nests at ten microsatellite loci. The goal of this study was to use geographic patterns in genetic

diversity to deconstruct the history of this invasion. The invasion likely is the result of only a single introduction event. A neighbor-joining tree supports the monophyly of the introduced population, while a hierarchical AMOVA indicates low levels of genetic differentiation at the level of the sub-population and nest. This single introduction event probably did not occur recently even though rapid range expansion has only occurred in the past 20 years. There is no evidence of a recent genetic bottleneck; there is significantly less heterozygosity than would be expected under Hardy-Weinberg equilibrium. The presence of significant sub-population structure indicates that local evolutionary forces and restricted gene flow acting over many generations have created distinct genetic boundaries between downtown St. Louis, St. Louis County, and the rest of the introduced range, particularly the far eastern portion of the range.

A potential invasion scenario that would explain patterns of genetic diversity in the introduced range is as follows: A propagule may have arrived in St. Louis, MO well over 20 years ago, when rapid range expansion was first documented. Colonization could have occurred as early as during the 1904 World's Fair when a large amount of plant material was imported from Japan. During a considerable lag period, individuals dispersed throughout the metropolitan area and east into Illinois. Limited gene flow combined with genetic drift over many generations established distinct sub-populations, particularly in the eastern portion of the range.

During the late 1980s, rapid range expansion began, at least partially the result of anthropogenic jump dispersal from downtown St. Louis westward along major transportation corridors. Evidence indicates that both recent and historical range expansion originated from source populations in the industrial center of St. Louis, MO.

This highlights the influence of anthropogenic dispersal in the expansion of species invasions and that identification of source nests and associated transportation vectors might be an efficient and effective means of slowing invasive spread.

In chapter three, I examine intraspecific aggression in the introduced Japanese pavement ant, a key characteristic absent in the world's most cosmopolitan invasive ants (Holway et al. 2002). Generally, ant colonies are highly territorial, which constrains population density (Hölldobler and Wilson 1990). However, the most destructive and widespread ant invasions are unicolonial – they lack significant territorial behavior between nests at the population level. In the absence of territorial boundaries, population density can increase orders of magnitude (MacKay et al. 1991). Numerical superiority over native ant species provides invaders, such as the Argentine ant, an advantage in both interference and exploitative competition, thus potentially leading to the local extinction of native ant populations (Human and Gordon 1996; Ward 1987).

I tested Japanese pavement ant individuals in three aggression assays of varying social context to determine whether the introduced population is unicolonial. In all assays, intraspecific aggression was rare, indicative of a unicolonial population. This is in stark contrast to native Japanese populations of *T. tsushimae*, which are highly territorial and fights between colonies are commonplace (Sanada-Morimura et al. 2006). Dyadic interactions never resulted in overt aggression, while the limited aggression I observed between groups of individuals did not correspond with the geographic distance between nests. Nests collected from locations separated by over 100km were often passive when placed together in laboratory, regardless of social context.

The only nest combinations that elicited elevated levels of aggressive behavior included at least one nest collected from urban downtown St. Louis, MO. Since aggression is based upon differences in genetically based chemical recognition cues, specifically cuticular hydrocarbons, I hypothesized that the increased aggression associated with downtown nests is the result of a correlated increase in microsatellite and cuticular hydrocarbon profile differences. Restated, an increase in aggression would be positively correlated with nest differences in microsatellite genotype and chemical recognition cue profile. I measured the genotype of each sampled nest at ten microsatellite loci as well as used gas chromatography to identify the composition of each nest's cuticular hydrocarbon profile. A generalized estimating equation (GEE) analysis showed that microsatellite and cuticular hydrocarbon distance both explained a significant portion of variation in aggression. However, microsatellite variation explained 100 times more variation in aggression than either geographic or cuticular hydrocarbon differences. This supports the hypothesis that the decision to be aggressive is based upon genetic differences.

If downtown St. Louis nests are more genetically distinct and diverse than nests throughout the rest of the invaded range then the invasion likely first colonized St. Louis and a series of genetic bottlenecks occurred as the Japanese pavement ant dispersed throughout the current range. The debate over whether genetic bottlenecks, release from natural enemies, or negative selection on rare recognition alleles are responsible for the evolution of unicolonialism is strong and unresolved (Giraud et al. 2002; Suarez et al. 2008). While this study supports the relationship between genomic diversity and

intraspecific aggression, finer scale analysis of each potential hypothesis in both the native and introduced range is warranted.

In chapter 4, I quantify the ecological effect of the Japanese pavement ant on native ants and test whether competitive superiority is a potential mechanism leading to the homogenization of invaded ant communities. I used a combination of pitfall traps and cookie baits to measure ant diversity in invaded and uninvaded prairie and turf grass sites. Invaded sites of both ecotypes exhibit significantly reduced native ant diversity and species richness. This decline is best explained by increased abundance of the Japanese pavement ant rather than environmental differences between sites. Cryptic ant genera such as *Solenopsis* were least affected by the invasion, while opportunist ant genera that had the greatest niche overlap with the Japanese pavement ant were generally absent from invaded sites. These genera include *Formica*, *Lasius*, *Tapinoma*, *Myrmica*, *Crematogaster*, and *Camponotus* – some of the most common genera in the U.S. Midwest.

I used an artificial introduction experiment to test the ability of the Japanese pavement ant to usurp resources from common native species. While the Japanese pavement ant significantly reduced the foraging ability of the opportunistic *Tapinoma sessile* and *Lasius neoniger*, it had no effect on *Monomorium minimum*, a highly competitive ant species with strong chemical defenses. These results demonstrate that while competitive superiority is a primary mechanism driving the local extinction of native ant populations, it is not the sole force. The Japanese pavement ant can blanket suitable habitats, occupying most available nest space and exploiting food resources. Territorial species such as *M. minimum* disperse through mating flights performed by

individual queens (Bhatkar 1992). High mortality associated with these flights is likely exacerbated by a lack of available nest space in invaded habitats. Those species that the Japanese pavement ant cannot outcompete for food resources may be unable to successfully disperse and local extinction occurs after the mortality of existing colonies. This study demonstrates that the Japanese pavement ant has a significant effect on native ant communities that is on par with the most destructive invasive ants in the world (Holway et al. 2002).

These three studies demonstrate not only the serious nature of this recently discovered invasion, but provide evidence for potential mechanisms driving its success, patterns of historical spread, and avenues of conservation. The Japanese pavement ant in North America is unicolonial, omnivorous, and a generalist attracted to disturbance and human settlement – characteristics common among the world's most widespread and destructive invasive ants (Holway et al. 2002). Rapid range expansion due to anthropogenic dispersal could eventually lead to this invasion spreading throughout the U.S. Midwest and significantly reducing native ant diversity, particularly in open, disturbed habitats (Steiner et al. 2008).

As a model system to study the early evolution of a successful ant invasion, the Japanese pavement ant demonstrates that after a single introduction event, a population can evolve into a unicolonial, ecologically successful invader capable of rapid range expansion. Few known ant invasions have well documented native and invaded ranges; this system lacks many of the complexities associated with more cosmopolitan ant invasions, making it a prime natural experiment to study the evolution and characteristics of a successful invader (Sax et al. 2005, Steiner et al. 2008). To the best of my

knowledge, the Japanese pavement ant is the only system where the site unicolonialism initially evolved is known. Further study of behavior, population genetic structure, community ecology, and chemical communication in both the native and invaded ranges can shed light on the factors that lead to the evolution and maintenance of unicolonialism and ecological success. Current conservation policy has proven unable to slow the destructive advance of invasive ant species; only greater knowledge of their biology and the roots of their ecological success can shed light on potentially effective methods of control.

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Vita Auctoris

Keefe Reuther was born in San Francisco, CA on October 13, 1980. He spent his childhood attending school near Ocean Beach in the Sunset neighborhood, culminating with his graduation from Saint Ignatius College Preparatory in 1998. Keefe attended university in nearby Berkeley, CA at the University of California, Berkeley. Over five years he earned dual B.A. degrees in Integrative Biology and Geology in 2003. During his undergraduate tenure, Keefe conducted research in a fly biomechanics, codling moth sexual behavior, aggressive and sexual behavior in fishes, as well as plant pathology. He owes much of his science training to the efforts and experience of Dr. George Barlow, an acclaimed expert on fishes and animal behavior. Their research on the East African cichlid genus *Julidochromis* led to a continued interest in the study of aggression. He spent one semester on the island of Moorea, French Polynesia studying territorial behavior in amphidromous gobies. After graduation, Keefe spend one year as a researcher studying Sudden Oak Death in the laboratory of Dr. Matteo Garbelotto. He has since spent the past five years working on his Ph.D. at Saint Louis University. During his tenure at SLU, he earned the first National Science Foundation graduate research fellowship in school history. He was a Monsanto Fellow, and earned a National Science Foundation Doctoral Dissertation Improvement Grant on his first attempt. He has also earned funding from the Webster Groves Nature Study Society and the Litzsinger Road Ecology Center. He currently resides in San Diego where he teaches introductory Ecology and Evolution at the University of California, San Diego and his wife is a Head and Neck Surgeon at the University of California, San Diego Medical Center.