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The Distribution of The Invasive Ant *Myrmica rubra* L. in Southern New England: Population Structure, Habitat Suitability, and Spatial Prediction

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The Distribution of The Invasive Ant *Myrmica rubra* L. in Southern New England: Population Structure, Habitat Suitability, and Spatial Prediction

Wen Chen, Ph.D.

University of Connecticut, 2016

The Eurasian ant *Myrmica rubra* L. was first discovered in North America in the early 1900s in Boston and Woods Hole, Massachusetts, and is now established across large parts of the northern United States and Canada. Its aggressive behavior, painful sting and high local population densities make it a potentially serious pest to local residents and a threat to biodiversity. I studied the distribution, habitat affinities, and population structure of this species across southern New England.

A systematic search showed that *M. rubra* is widespread in the Greater Boston area and the Berkshires range, with smaller populations elsewhere in Massachusetts and Rhode Island. The ant was not detected in Connecticut or across large expanses of central Massachusetts and northern Rhode Island, despite the presence of suitable habitat. This distribution suggests a combination of long-distance dispersal mediated by humans coupled with slow local spread. Analysis of mitochondrial DNA revealed multiple haplotypes that are shared with populations in central Europe.

Invasive species distribution modeling is complicated by the fact that many introduced species are not yet in equilibrium with their environment. To address this challenge, a Bayesian hierarchical approach was employed that combines two components: a spatial model, allowing estimation of the geographic extent of the invasion, and a habitat suitability model, quantifying the association between several environmental predictors and the presence of the ant. The spatial component describes uncertainty in where the ant has arrived and can account for spatial dependence in outcomes at nearby locations. This approach discounts the contribution of sites where the species has not arrived to estimation of the habitat suitability model. *M. rubra* was more likely to be found at sites with high amounts of wetland, water, and developed habitat within 300 m, at sites with wetland plants and stands of the invasive *Fallopia japonica* Houtt (= *Reynoutria japonica* Houtt or *Polygonum cusidatum*; Japanese knotweed), and at sites with low

maximum temperatures in the hottest month of the year. The ant was most prevalent in marshes, wet meadows, and wet forests and was rarely found in drier forests or in open areas. These results suggest that, within southern New England, *M. rubra* is limited to places where the soil does not dry out during the summer. Its restriction to these habitats may have contributed to its slow dispersal during the last 115 years.

Several widespread and damaging species of invasive ants have populations that lack colony boundaries, or that are characterized by large supercolonies with many nests and queens, such as *Linepithma humile* (Argentine ant), *Wasmania auropunctata* (little fire ant) and *Anoplepis gracilipes* (yellow crazy ant; Holway et al. 2002, Lach and Hooper-Bui 2010). Bioassays of aggression were used to determine colony and population structure of *M. rubra* at eight sites spanning two major invasions in Massachusetts. Populations at all sites consisted of multiple colonies that were mutually aggressive. The largest colonies can be considered supercolonies, expandable networks of nests distributed across more than a hectare. Hierarchical models, accounting for variation in aggressiveness among colonies and variation in outcomes across replicate assays, were used to analyze patterns of aggression. There was no evidence that aggressive responses depended on distance of separation between colonies or whether colonies were neighbors. Supercolonies were constrained by intraspecific competition and habitat variation and did not achieve the vast sizes seen in introduced populations of some other invasive ants.

**The Distribution of The Invasive Ant *Myrmica rubra* L. in Southern New England: Population
Structure, Habitat Suitability, and Spatial Prediction**

Wen Chen

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M.A., Nanjing Agricultural University, 2009

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APPROVAL PAGE

Doctor of Philosophy Dissertation

The Distribution of The Invasive Ant *Myrmica rubra* L. in Southern New England:

Population Structure, Habitat Suitability, and Spatial Prediction

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INTRODUCTION

With the growth of human population size and worldwide commerce, anthropogenic activities have facilitated the introduction of species to novel environments. Both the number of species moved and the geographic scale of transport have reached unprecedented levels. The distributions of both native and introduced species have been redefined due to this accelerated process.

Invasive ants can cause significant destructive impacts in local ecosystems due to their crucial ecological roles at different trophic levels. Despite the phenomenal environmental and economic impacts of invasive ants (Lach et al. 2010), only a handful have been intensively studied, especially the fire ant *Solenopsis invicta* and the Argentine ant, *Linepithma humile*. Some researchers have studied biogeography, phylogeny and behavior ecology to explore the causes and effects of successful invasions and to infer dispersal routes of these two ants (Holway et al. 2002, Suarez et al. 2008, Ascunce et al. 2011). However, the insights accumulated from the study of these species do not necessarily apply to all invasive ants. There is an immediate need to expand the study of invasive ants to include other species, such as *Myrmica rubra* in North America, and other less known non-native species.

The Palearctic ant species *Myrmica rubra* L. is the most important ant invader in northeastern North America, yet limited information about its biology in the invaded range has been published (Grodén et al. 2005, Wetterer and Radchenko 2011). Sometimes called the “European fire ant” or the “ruby ant” (Wetterer and Radchenko 2011), *M. rubra* is widespread in Europe and central Asia (Fig. 1). The first discovery in North America was in Massachusetts in 1900 (Wheeler 1906). Studies since then have focused on its distribution in Maine (Grodén et al. 2005). Its interactions with homopteran communities, plants, and ant species in invaded ecosystems have been studied by McPhee et al. (2012), Prior et al. (2015), and Garnas et al. (2014) respectively. The unbalanced understanding about this species in its native and invaded range gives rise to great opportunities for fundamental ecological and population studies on this ant.

I used a combination of field observations, behavioral experiments, spatial modeling, and genetic analyses to investigate the following aspects of this ant's population biology.

In Chapter 1, I describe the results of a systematic search for *M. rubra* in southern New England, documenting the extent and structure of the invasion, habitat associations that may limit the rate of spread, and analysis of mitochondrial DNA variation that may reveal the number and origins of introductions.

In Chapter 2, I report the development of species distribution models (SDMs) in order to evaluate possible predictors of the ant's occurrence in southern New England, including land use and habitat type, vegetation characteristics, and climate variables. To address the challenge of fitting SDMs to an incomplete invasion, a Bayesian analysis combines a spatial model of where the species has arrived with a model of habitat suitability to distinguish absences that are due to incomplete dispersal and to unsuitable habitat.

In Chapter 3, I consider the colony and population structure of *M. rubra* at multiple sites in southern New England. Some major ant invaders have unicolonial populations, lacking colony boundaries, or immense supercolonies, which are expandable networks of interconnected nests (Holway 1998, Tsutsui et al. 2003). I documented colony structure and tested whether worker aggression depends on the distance between nests or whether colonies are neighbors.

Taken together, these studies advance our understanding of the invasion biology of *M. rubra* in our region.

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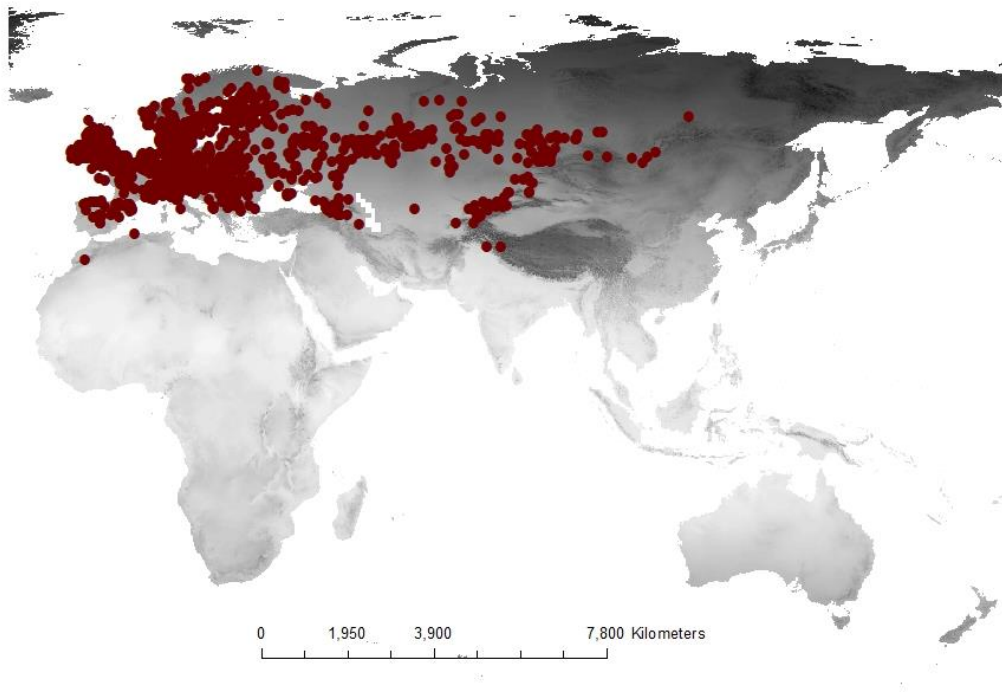


Figure 1. Records of *Myrmica rubra* in its native range (after Wetterer and Radchenko 2011)

Chapter 1

The structure of an ant invasion: *Myrmica rubra* in southern New England

Abstract

The Eurasian ant *Myrmica rubra* was first discovered in North America in the early 1900s in Boston and Woods Hole, Massachusetts, and has since spread to at least seven states within the U.S.A. and six Canadian provinces. However, little is known about the limits of its distribution. We conducted a systematic search for the ant across southern New England, greatly expanding the list of known locations. Populations of *M. rubra* were clustered in widely separated areas, representing multiple distinct invasions. *M. rubra* was widespread in the greater Boston area in eastern Massachusetts and in the Berkshires range of western Massachusetts, with locally high population densities. It was also found in smaller areas on Cape Cod, in the interior of Massachusetts, and in southern Rhode Island. No occurrences were detected anywhere in Connecticut or across large expanses of central Massachusetts and northern Rhode Island. This pattern of distribution suggests a combination of long-distance dispersal by human transport coupled with slow local spread.

The mitochondrial *Cytochrome Oxidase* sub unit I (*COI*) sequences of *M. rubra* collected from 40 different locations revealed seven different haplotypes. We found no clear genetic distinction between the major invasions in eastern and western Massachusetts or between those regions and other sites in the northeast. The most common haplotypes from the invaded range were also found in native populations in France, Germany, and Austria, which suggests that multiple queens or colonies were introduced from northern Europe into New England.

Within the invaded area, *M. rubra* showed strong associations with certain habitat types. The ant was most prevalent in wetlands and in forests near wetland and water; it was uncommon in drier forests

and it was rare in open sites, except for wet meadows. The restriction of *M. rubra* to places where the soil remains wet throughout the year may have contributed to the slow rate of spread over the last 115 years.

Introduction

Because they play important roles at multiple trophic levels, invasive ants can have major impacts on biodiversity (Holway et al. 2002) and can seriously disrupt local communities (e.g., Porter and Savignano 1990, O'Dowd et al. 2003). According to the recently published New England Ant Field Guide (Ellison et al. 2012), there are 132 ant species in New England, of which 14 are non-native. At least one of these, *Myrmica rubra* L., sometimes called the European fire ant or the ruby ant, has become widespread in natural and human-modified environments (Groden et al. 2005, Wetterer and Radchenko 2011). The ecological effects of New England's invasive ants are not well known, but elsewhere in the world abundant ant invaders, such as the fire ant *Solenopsis invicta* Buren, the Argentine ant, *Linepithma humile* (Mayr), the yellow crazy ant *Anoplolepis gracilipes*, and the little fire ant, *Wasmannia auropunctata*, have caused detrimental impacts on native biodiversity in their introduced range (Holway et al. 2002, Lach and Hooper-Bui 2010).

Due to its aggressive behavior, painful sting, and high local population densities, *M. rubra* is a potentially serious pest in New England. However, *M. rubra* has been studied much less than other notorious invasive ant species. *M. rubra* didn't enter public awareness until the early 2000s, when there was a dramatic increase of complaints about this stinging ant from Mt. Desert Island and the Camden-Rockport region in Maine (Groden et al. 2005). Furthermore, little is known about its impacts. McPhee et al. (2012) discovered an association between the density of *M. rubra* and the composition of homopteran communities. Garnas et al. (2014) reported that *M. rubra* aggressively displaced some native ants by forming dense, patchy local infestations. *M. rubra* also aids the dispersal of co-introduced plant species, which alter plant community structure (Prior et al. 2015).

Myrmica is a diverse genus that is native to many temperate regions of the Northern Hemisphere. A Palearctic species, *M. rubra* occurs across a wide range of latitudes, from 39° N to 70° N in its native

range. By 2014, this ant had been found in six eastern U.S. states (Massachusetts, New York, Rhode Island, Vermont, New Hampshire and Maine) and Washington State (Wetterer and Radchenko 2011). The ant is also established in Canada: Quebec, Ontario, New Brunswick, Nova Scotia, Prince Edward Island (Wetterer and Radchenko 2011) and Newfoundland (Hicks et al. 2014). The earliest records of *M. rubra* in North America were from the edges of a rose garden in Woods Hole, Massachusetts, and the Arnold Arboretum in Boston, Massachusetts (Wheeler 1906, 1908; see Fig.1.1). Wheeler (1908) suggested that the species was brought to both places by transportation of plants from Europe. *M. rubra* went through a slow invasion process; it took more than 100 years for the ant to regain researchers' attention. By 2005, only six new locations were added to the list of known occurrences in Massachusetts as well as one in Rhode Island (Grodén et al. 2005). However, no systematic searches had been conducted previously anywhere in the invaded range. It is a fair question to ask if the impression of slow dispersal of *M. rubra* accurately reflects its distribution or whether it has spread more extensively below the myrmecologists' radar.

Documenting the extent of the invasion seems to be the most urgent need. By detecting the limits of the current distribution, we can preserve a snapshot of the species' range for future comparisons. Within the invaded range, we can explore the habitat associations of this ant and formulate a management regime based on prediction of *M. rubra*'s possible distribution in North America. Geographic surveys of *M. rubra*'s distribution also enable us to establish a genetic library to investigate population genetic structure in southern New England.

Genetic data from source and introduced populations of invasive ant species can be used to reconstruct possible dispersal routes and to recreate invasion history. Ascunce et al. (2011) assessed genetic variation of *S. invicta* and proposed that southern U.S. populations are the direct source for all the other invasions except for Taiwan's, which was introduced from California. Similar studies have also been conducted on *Wasmannia auropunctata* and *Linepithema humile* (Mikheyev and Mueller 2007, Drescher et al. 2007). As a widely distributed Palearctic species, *M. rubra* is ideal for phylogeographic studies. Leppänen et al. (2011) used mitochondrial DNA (mtDNA) to reveal that *M. rubra* populations

from the eastern and western parts of its native range belonged to separate haplogroups and they also inferred that the ants survived the last glacial period in several refugia spread over an extensive area in Eurasia. Leppänen et al. (2011) included ants from two sites in North America in their study, Massachusetts and Maine, U.S.A., which contained haplotypes from three common haplogroups in western and central Europe. In a recent study, Hicks et al. (2014) discovered that the most common haplotype of invasive populations of *M. rubra* in Newfoundland is observed only in Dorset, England within the native range. Considering the historical connection between Dorset and Newfoundland and other records, Hicks et al. proposed putative routes of invasion to Newfoundland.

The spread of invasive ant species is often limited by habitat suitability. For example, in pine forests of northern Florida, the fire ant *Solenopsis invicta* is restricted to open, disturbed habitats including roadsides (Tschinkel 1988). In California, the Argentine ant *Linepithema humile*, is associated with riparian woodlands (Ward 1987) and areas with higher vegetation cover (Fitzgerald and Gordon 2012). Given the apparent slow rate of spread of *M. rubra* in North America (Wetterer and Radchenko 2011), we sought to determine the habitat affinities of this ant within our study area.

This study was designed to answer three questions, (1) What is the spatial structure and extent of the *M. rubra* invasion in southern New England? We implemented an e-mail campaign and a systematic search to identify the current distribution of the ant in Massachusetts, Rhode Island, and Connecticut. (2) Where are the likely geographic sources of ants in its invasive range? We compared mitochondrial haplotypes from introduced populations to those in the native range to evaluate whether the separate populations in New England represent different introductions and to determine possible sites of origin. (3) What are *M. rubra*'s habitat associations? We compared the prevalence of the ant in open, forested, and wetland habitats within multiple invaded areas.

Methods

Distribution of *M. rubra* in southern New England

Email campaign. Because there is little published information on *M. rubra* in southern New England, we sought efficient ways of locating populations to gathering preliminary information on occurrences of the ant and its habitat requirements. We decided to initiate an email campaign making use of *M. rubra*'s painful sting to locate possible populations. In the inquiry email, we solicited reports of stinging ants and included a brief description of *M. rubra* as a reddish brown ant a quarter of an inch long (4 – 5.5 mm) and a link to an online photo. Although some native ants in New England can sting, they have much smaller colonies than *M. rubra* and are less aggressive to people. From the winter of 2009 to the summer of 2010, requests were sent to the staff of more than 100 wildlife sanctuaries, parks, and nature centers; 62 golf courses; 40 associations of recreational fisherman; and more than 30 other outdoor organizations in Massachusetts, including land trusts and botanical gardens. We noticed that our original emails were circulated by the recipients to second and third degree contacts including local gardening societies, conservation organizations, professional and amateur entomologists, nurseries, and social media. We gathered possible locations of *M. rubra* based on these replies and visited most of them to determine the species identities of ants.

Systematic search. To spread search effort throughout the region, we divided a map of southern New England (i.e. Massachusetts, Connecticut and Rhode Island) into a grid of 20 x 20 km cells, excluding islands (Fig.1.2). During our first year of work, we visited sites at which *M. rubra* had been previously reported (Grodén et al. 2005) and those identified through the email campaign. It was evident that *M. rubra* was most abundant close to wetlands and riparian habitat with wetland vegetation. Therefore, within each 20 x 20 km cell, we chose publically accessible wetland sites as primary search targets. In the state of Massachusetts, we visited a minimum of two sites in each cell. To improve spatial coverage, we chose sites that were at least 4 km from each other. In Connecticut and Rhode Island, where there have been few reports of *M. rubra*, we visited at least one site in each cell, choosing sites that were at least 9 km apart. As a crude outline of the invaded area was delineated, we added additional sites near the outer extent of each occupied region to provide greater resolution of the limits of the distribution. We also searched for *M. rubra* at all sites in southern New England listed by Grodén et al. (2005).

Sampling methods. We walked paths stopping every 25-40 m to search for workers of *M. rubra* on the ground or vegetation. We also looked beneath stones and fallen branches, at the bases of plants, or in soil, as these are often nesting sites of *M. rubra*. Each such location examined along the trail is considered a site and prevalence is defined here as the proportion of sites in which *M. rubra* was present.

At most sites, ants were collected for genetic analysis. We collected at least ten workers, and often 50-100 per nest; the workers were preserved in 95% ethanol and kept at 4 °C prior to DNA extraction. Laurel Hansen and Barry Hicks provided additional samples from Seattle, Washington, and from Newfoundland, respectively.

Species identification. Ants were tentatively identified to species in the field; identifications were confirmed in the lab using collected samples. Among the 21 species of *Myrmica* in New England (Ellison et al. 2012), *M. rubra* can be distinguished by having a smooth bend at the base of the antenna without pronounced sculpturing, and thin, evenly curved frontal lobes that point upward in full face view (Fig.1.3).

Mitochondrial haplotype analysis

DNA extraction, polymerase chain reaction (PCR), and sequencing. DNA was extracted by crushing an individual ant and used Proteinase K to digest cells at 60 °C overnight, followed by purification with the Nucleo Spin Tissue kit (Macherey-Nagel, manufacture). For the segment of the mitochondrial *Cytochrome Oxidase* sub unit I (*COI*), we used the universal primer LCO 1490 and HCO2198 (Folmer et al. 1994). The 25 µL reactions contained: 1.5X PCR buffer, 1 µM dNTP, 1mM MgCl₂, 0.75 µM of each primer (LCO 1490 and HCO 2198) and 2-18 µg DNA. PCR was carried out on GeneAmp (Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, Connecticut, United States of America) under the following thermocycler conditions: an initial denaturation at 95 °C for two minutes, 30 cycles at 94 °C for 30 seconds, 49 °C for 30 seconds, and 72 °C for two minutes, and a final extension at 72 °C for five minutes.

PCR products were purified for cycle sequencing using EXO-Sap (Fisher Scientific) following the manufacture's protocol. Sequencing was carried out using the BigDye Terminator v3.1 and purified via sepphadex (GE Healthcare). Sequencing products were electrophoresed in an ABI PRISM 3130 XL/ Genetic Analyzer using Sequencing Analysis v.5.4 Software.

Genetic data analysis. We used Sequencher 4.5 (GeneCodes Corporation) to align and edit sequences. Variable site identification was conducted by exporting sequences to MEGA 6. We compared sequences obtained in this study with GenBank accessions referenced by Leppänen et al. (2011). A haplotype network among invaded populations was plotted by using two R packages: haplotypes (Aktas, 2015) and sna (Butts, 2014).

Habitat affinities

The prevalence of *M. rubra* was estimated for three habitat types: open habitat, consisting of old fields and grass, including mown grass; wetlands, consisting of wet meadows dominated by *Phalaris arundinacea* L. (reed canary grass), marshes, and swamps; and forests, which included all types of forests besides wetlands. Habitat type was scored using land use/land cover (LULC) data for 2005 from the Office of Geographic Information of the Commonwealth of Massachusetts (MassGIS). Some LULC designation were corrected by ground truthing; for example, some sites classified as open land proved to have wetland vegetation. *Post hoc*, we subdivided the wetland habitat into forested wetlands (swamps) and non-forested wetlands (marshes and wet meadows), and we also subdivided forests into drier and wetter forests based on observations made on site. Drier forests included mid-slope and hilltop sites away from water or wetland and with relatively thin soils and sparse herbaceous understory vegetation. Wetter forests included riparian habitats along streams and ponds and other sites on lower slopes or with noticeably moister soil and denser herbaceous vegetation.

Because the probability of occurrence of *M. rubra* may vary spatially, we sampled each type of habitat in 20 rectangular blocks in which *M. rubra* was found; 18 of the blocks were spread throughout the greater Boston area, and two were in the Berkshires (Fig. 1.4). To account for large-scale variation in

prevalence, we conducted a hierarchical analysis with sites nested within blocks. The 20 rectangular blocks varied from 3.5 to 35 km² (mean = 13.4) depending on the distribution and accessibility of habitats. As described above, we looked for *M. rubra* workers or nests at sites selected 25–40 m along our search paths. Each habitat type was represented by a minimum of 16 such sites (mean = 30.4; maximum = 54), or approximately 450 m of trails, within each block. Following the *post-hoc* subdivision of forests into wetter and drier sites, and of wetlands into forested and non-forested wetlands, each habitat type was represented in all 20 blocks (mean = 23 sites), with the exception of one block lacking wetter forest. Wetland habitat was considered to include all sites in or within 30m of the forested and non-forested wetland layer boundaries, as well as other sites with wetland vegetation.

The R statistical package version 3.1.2 (R Core Team 2014) was used for all analyses. The lme4 package (Bates et al. 2015) was used to fit hierarchical models; for comparison the glm function from the stats package was used to fit non-hierarchical models. We used generalized linear models (GLM) or generalized linear mixed models (GLMM) with the number of sites with *M. rubra* as the outcome and assuming a binomial distribution and a logit link (Zuur et al. 2009). For GLMM, the rectangular blocks were used as the grouping variable. Akaike's Information Criterion (AIC) scores were used for model selection (Burnham and Anderson 1998). To compare random-effects models differing in which fixed effects were included, AIC scores were based on maximum likelihood (ML), calculated with the glmer function (Zuur et al. 2009). Post hoc comparisons among habitat types were made using Tukey's contrasts, calculated with the multcomp package (Hothorn et al. 2008).

Results

Distribution of *M. rubra* in southern New England

In the early stages of our search, email and social media functioned as important tools for gathering information on possible locations of *M. rubra* populations. We received 37 responses about stinging ants, many with specific locations. It became clear that our messages had been forwarded to other individuals, organizations, lists, and social media sites; these secondary contacts were the source of most

replies and they included locations in Connecticut, Rhode Island, New Hampshire, and Maine, as well as Massachusetts. Based on these responses, we visited 32 locations and found *M. rubra* at 11 new sites in Massachusetts and two new sites in New Hampshire, substantially increasing the known range of the ant. By visiting these locations, we formulated a consistent impression that *M. rubra* was most common in wetlands and riparian habitats.

Many additional populations were discovered by the systematic search across the grid of 20 x 20 km cells and by more intensive searches along the edges of the large invasions. We found *M. rubra* at all locations listed by Groden et al. (2005) in southern New England, including those for which the only information was a town name. For the more specific locations that Groden et al. culled from museum labels, we found *M. rubra* at the Arnold Arboretum (Boston, MA), Shaker Glen (Woburn, MA), Fresh Pond (Cambridge, MA), Rock Meadow (Belmont MA), and Eph's Pond (Williamstown, MA). Fig. 1.2 shows sites where *M. rubra* was and was not discovered. Table 1 lists the towns in which *M. rubra* was found. The invaded range is shown using town boundaries in Fig. 1.5. At some of these sites, there were conspicuous populations, with high densities of workers active along more than 100 m of trail, while at other sites, only a few workers could be found.

M. rubra was found in several regions within southern New England (Figs 1.5 and 1.6). The largest expanse occurred in the Greater Boston area, including sites from Cape Anne in the north to Marshfield in the south and west to Framingham and Sudbury. Within this region, the greatest distance from the site of the original discovery in Boston was 58 km. The second largest expanse was in the Berkshires range of western Massachusetts from Sheffield in the south to Williamstown in the north. The ant was found at scattered locations on Cape Cod, Massachusetts, including one site within 50 m of Wheeler's first discovery (Wheeler 1906). Small and apparently isolated populations were found in Worcester and West Newburyport, Massachusetts. Finally, populations were found in Newport, Rhode Island, and the adjacent town of Middletown. No *M. rubra* were found in Connecticut or throughout large sections of Rhode Island and central Massachusetts.

As we collected samples of *M. rubra*, we spoke to curious onlookers, many of whom were aware of the abundant, stinging ant in their neighborhood. Several of these persons, as well as e-mail correspondents, told us the years of their first contacts with this ant. Based on the most credible reports from persons who correctly identified the ant at present-day locations, *M. rubra* has been in Topsfield, MA, since at least the 1950s; in Brace Cove (Gloucester, MA) since at least 1961; in Rockport, MA, since at least the 1960s; in Chatham, MA, since at least 1965; and on Marblehead Neck, MA, since at least 1974.

Mitochondrial haplotype analysis

A total of 420 base pairs (bp) of COI sequences were obtained for each of 41 individual ants collected at 36 different locations in North America (Table 1.2). Five variable sites at locations 108 (Y), 123 (R), 222 (W), 260 (Y) and 414 (M) defined the seven different haplotypes. There were two common haplotypes, referred to as I and II, and five unique haplotypes detected among the 41 individuals. A total of 15 and 20 individuals belonged to haplotypes I and II respectively; both haplotypes were found within the same population at several locations. Two individuals belonged to haplotype VI and the rest of the four unique haplotypes were found in only one individual. Our data showed no clear differentiation between geographic regions. Haplotype I and II appeared in both the eastern and western invasions in Massachusetts (Fig. 1.7).

Haplotype I matched haplotypes of JF778875, HQ978936 and DQ74370 (Leppänen et al. 2011), and haplotype II matched AY510627 (Germany), DQ074382 (Vienna, Austria), JF779152 (Leppänen et al. 2011), and KM538057 (Not published, Oak Savannah, High Park, Ontario, Canada). Haplotypes III, IV, V, VI, VII are new.

Habitat affinities

The prevalence of *M. rubra* varied substantially among the three habitat types (Fig. 1.8). For hierarchical models with random effects for the 20 geographic blocks (Fig. 1.4), the AIC value was much

lower (indicating greater support) when habitat type was included as a set of fixed effects than when habitat type was omitted ($\Delta\text{AIC} = -145$). The ants were most abundant in wetlands and were almost never seen in open habitats. All post hoc pairwise contrasts between habitat types were significant (Tukey's method, $p < 0.001$). The variance of the random effects was estimated to be 0.57. The prevalence of *M. rubra* in forested and wetland sites was correlated across the 20 blocks (Spearman's rank correlation coefficient = 0.70, $p < 0.001$).

Post-hoc, forests were divided into wetter and drier sites and wetlands were divided into forested and non-forested sites, as described in the methods. For this classification of habitats, the AIC value was also much lower when habitat type was included than when habitat type was omitted ($\Delta\text{AIC} = -227$). *M. rubra* was rarely found in drier forest, and was less prevalent in forested wetlands (swamps) than in non-forested wetlands (marshes and wet meadows; Fig. 1.9).

Discussion

Distribution of *M. rubra* in southern New England

Our sampling greatly extended the known range of *M. rubra* in southern New England and revealed that the ant occurs in two large and widely separated areas, as well as in several smaller populations (Fig. 1.2). By 2005, *M. rubra* had been reported from nine towns in Massachusetts and Rhode Island (Grodén et al. 2005); our survey extended the lists to 74 towns (Table 1.1). The largest invaded area encompasses much of the greater Boston area in eastern Massachusetts, extending 15 to 60 km in all directions from the site of Wheeler's (1906) first discovery in Boston. The Berkshires range of western Massachusetts was the site of the second largest invasion. There, *M. rubra* was found at multiple locations from the southern to the northern border of Massachusetts, from which its range extends into Vermont (Table 1.2) and New York State (M. Morales, pers. comm.).

Several smaller populations were found. *M. rubra* was rediscovered in Woods Hole (Falmouth, MA). The ant was not nearly as common on Cape Cod as in the Boston area, but it was found at three other sites at a maximum distance of 61 km from where Wheeler (1906) first reported it. Isolated populations were found in Worcester (central Massachusetts) and West Newburyport on the banks of the Merrimac River. In Rhode Island, the only previous information was a location label for a museum specimen, indicating that the ant came from a garden in Newport (Grodén et al. 2005). We found colonies in two parks separated by about 3 km in Newport and at another site 6.6 km away in Middletown. Our e-mail campaign also led to confirmed discoveries of *M. rubra* in Rye and Crawford Notch, New Hampshire.

Assuming that Wheeler's (1906) first discoveries of the ant were close to the times and places of introduction, the ant has spread at an average rate of approximately 160 to 580 m per year, depending on the direction, in the greater Boston area and on Cape Cod. However, the limited historical information suggests that the rate of spread is uneven and that *M. rubra* has not moved much beyond several locations that it reached 40 to 50 years ago. For example, one resident in Chatham, Massachusetts, who knows *M. rubra* as a stinging ant infesting his yard, recalls the ant being present in 1965, but we did not find any other populations farther out on Cape Cod 50 years later (Fig. 1.6).

Mitochondrial haplotype analysis

The presence of seven mtDNA haplotypes indicates that the invasive populations in Massachusetts descend from multiple queens. In addition, two other haplotypes were reported from Massachusetts by Leppänen et al. (2011). The two most common haplotypes (I and II) in our samples were found in both eastern and western Massachusetts, as well as at other sites in North America; there was no obvious genetic distinction between the two major invasions in the greater Boston area and the Berkshires (Fig. 1.7). A sample from Seattle, Washington, contained a unique haplotype. Haplotypes I and II match samples from Germany, France, and Austria (Leppänen et al. 2011).

This information is consistent with introduction of multiple queens or colonies from central Europe into eastern Massachusetts, followed by transport to other sites in New England; however, the sparse information from historical records does not allow confident statements about the order of arrival. *M. rubra* has been intercepted in shipments from multiple sites in Europe, including Germany, Belgium, and England (Grodén et al. 2015). A more fine-scaled genetic analysis will be needed to infer dispersal routes (see also Hicks et al. 2014).

Habitat affinities

M. rubra was restricted to particular habitat types (Figs. 1.8 and 1.9). Colonies were nearly absent from non-wetland open habitats, including old fields and mown grass, and from drier forests with well-drained soils. The ant was locally abundant in wetter forests, including riparian habitats along streams and ponds, and forested wetlands. The greatest prevalence was in wet meadows dominated by *P. arundinacea* L. (reed canary grass) and in freshwater marshes.

The prevalence of *M. rubra* in forested and wetland habitats was correlated, underscoring the usefulness of a hierarchical model. The reasons for variation across blocks are unknown, but may reflect unmeasured environmental influences or the length of time since the ant arrived. We had the impression that *M. rubra* was more abundant in forests within 500-800 m of the coast than farther inland. Thus, in southern New England, *M. rubra* appears to be limited to places where the soil does not dry out during the summer. This restriction may contribute to the slow rate of spread, especially if the ant cannot easily disperse across stretches of unsuitable habitat.

Acknowledgements

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Protection, the Department of Environmental Management of Rhode Island, and numerous other organizations and individuals for permission to search for and collect *Myrmica rubra* on their properties. We gratefully acknowledge receiving helpful information on locations of this species from Gary Alpert, Stefan Cover, Frank Drummond, Aaron Ellison, Eleanor Groden, Adam Lazarus, Dave Lubertazzi, Manuel Morales, Thomas Palmer, and Richard Haradon. Laurel Hansen and Barry Hicks provided specimens for genetic analysis from Seattle, Washington, and Newfoundland respectively.

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Figures

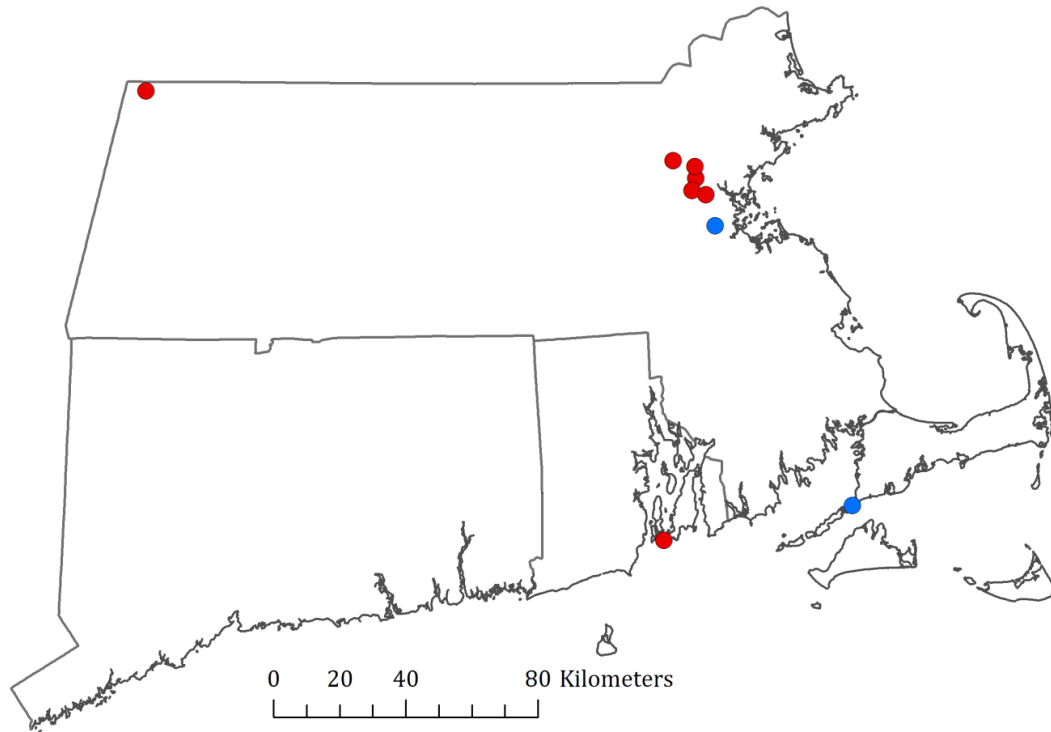


Figure 1.1. Known locations of *Myrmica rubra* in southern New England by 2005. Blue dots represent the locations of the first two reports of *M. rubra* in 1900 and 1902 (Wheeler, 1906) and red dots represent locations compiled by Groden et al. (2005).

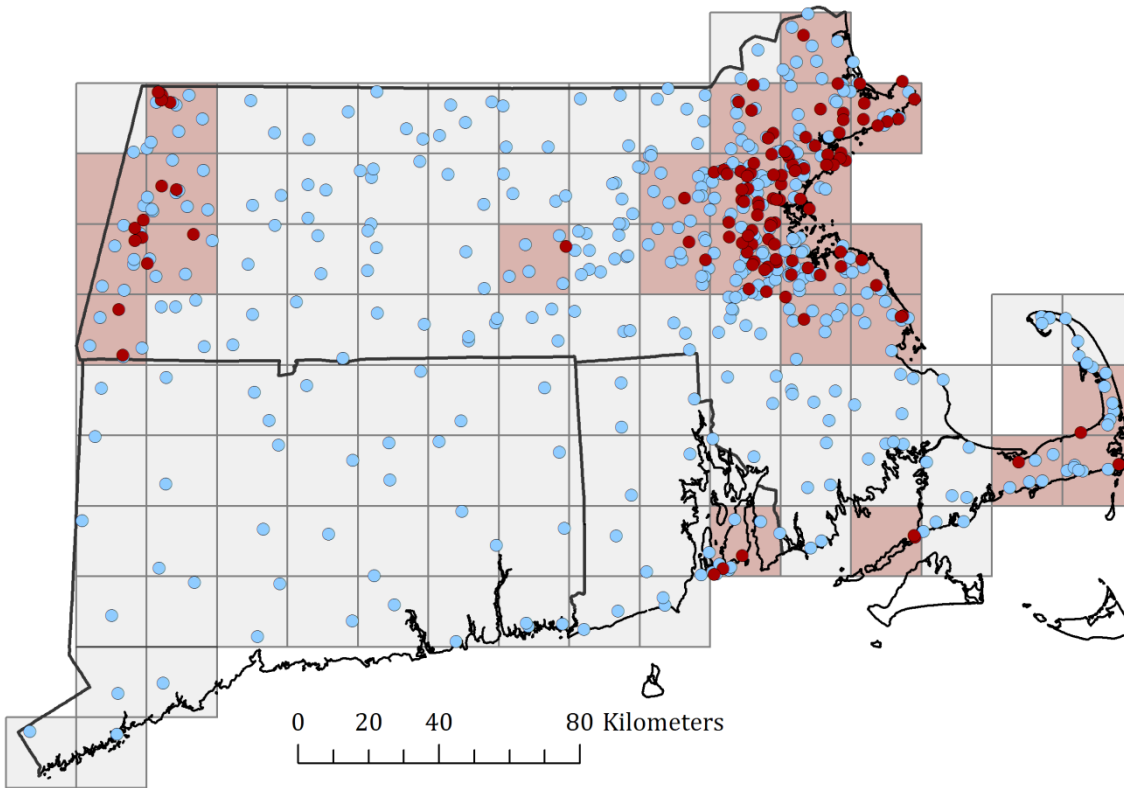


Figure 1.2. Southern New England was divided into a grid of 20 by 20 km cells. Cells in which *M. rubra* was found are shaded red; others in gray. Red circles indicate locations where *M. rubra* was discovered, and blue circles locations where the ant was not found.



Figure 1.3. A frontal face view of *M. rubra* worker ant. Photographer: April Nobile, image from www.AntWeb.org. AntWeb content is licensed under a Creative Commons Attribution License.

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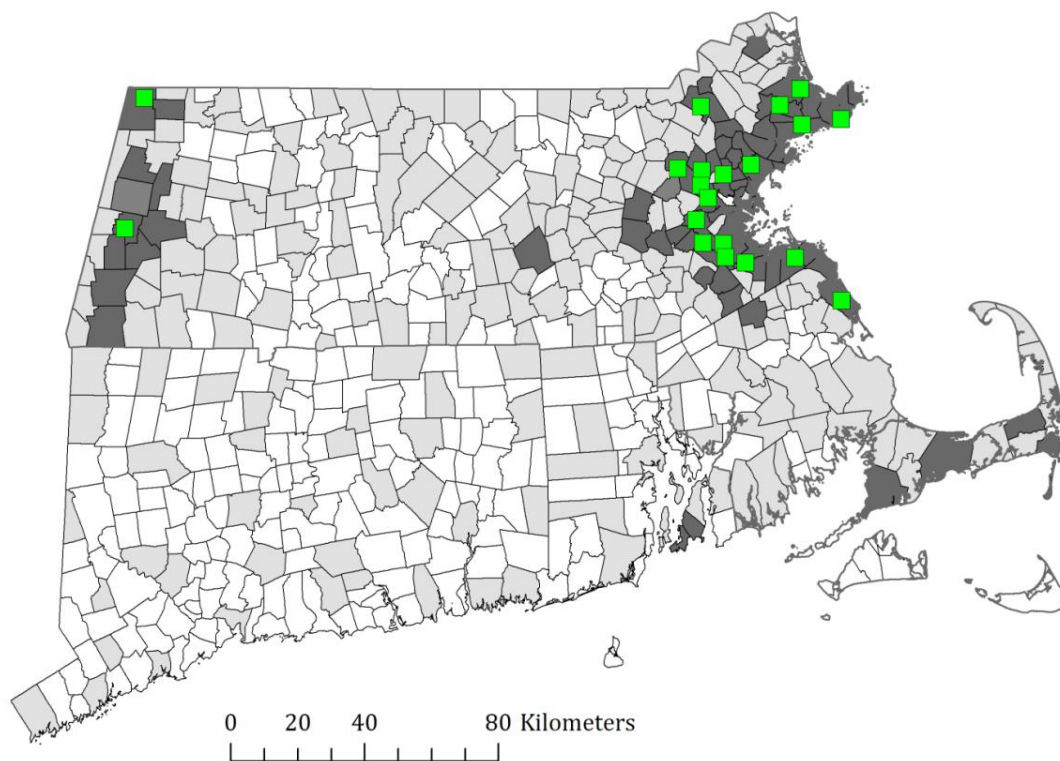


Figure 1.4. Green squares mark the locations of the twenty blocks used for habitat sampling. Towns where *M. rubra* was found are shaded dark gray; towns where we did not find *M. rubra* are shaded with light gray; towns that were not searched are unshaded.

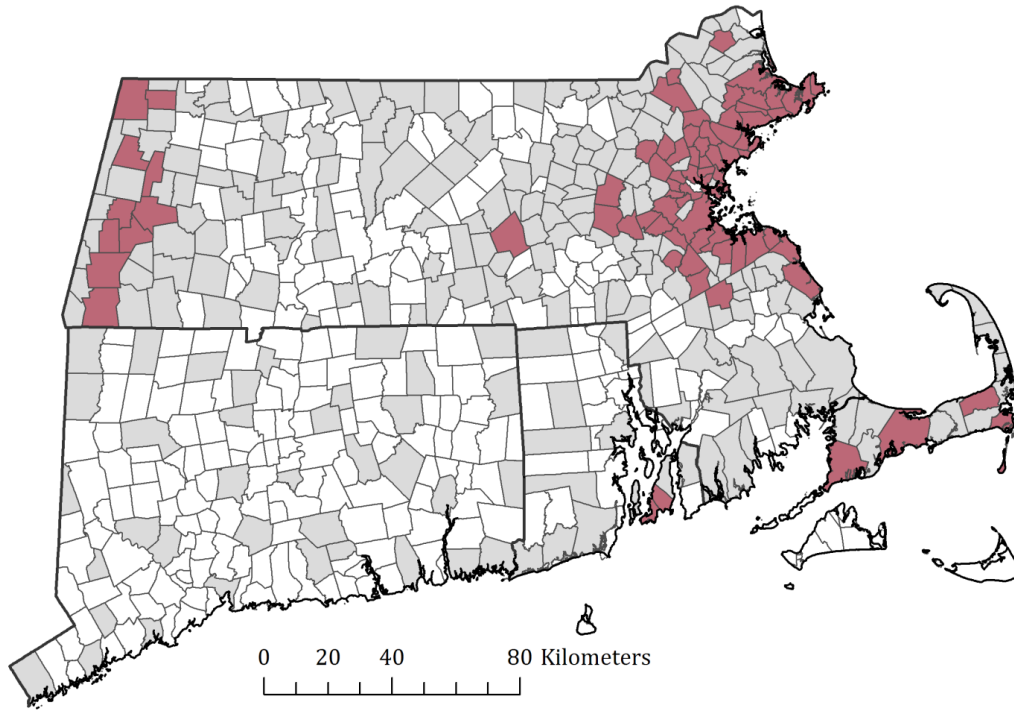


Figure 1.5. Township map of southern New England. Towns where *M. rubra* was found are shaded red; towns where we did not find *M. rubra* are shaded gray; towns that were not searched are unshaded.

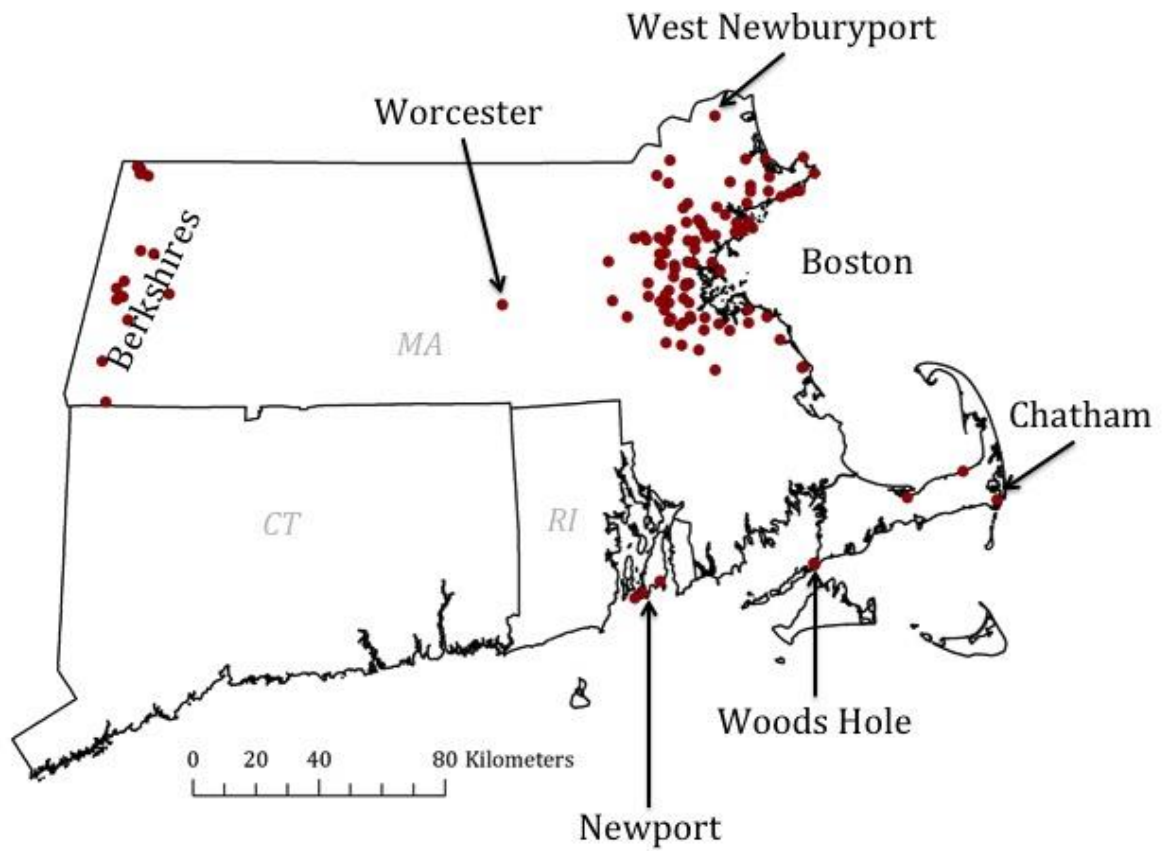


Figure 1.6. Place names for selected locations.

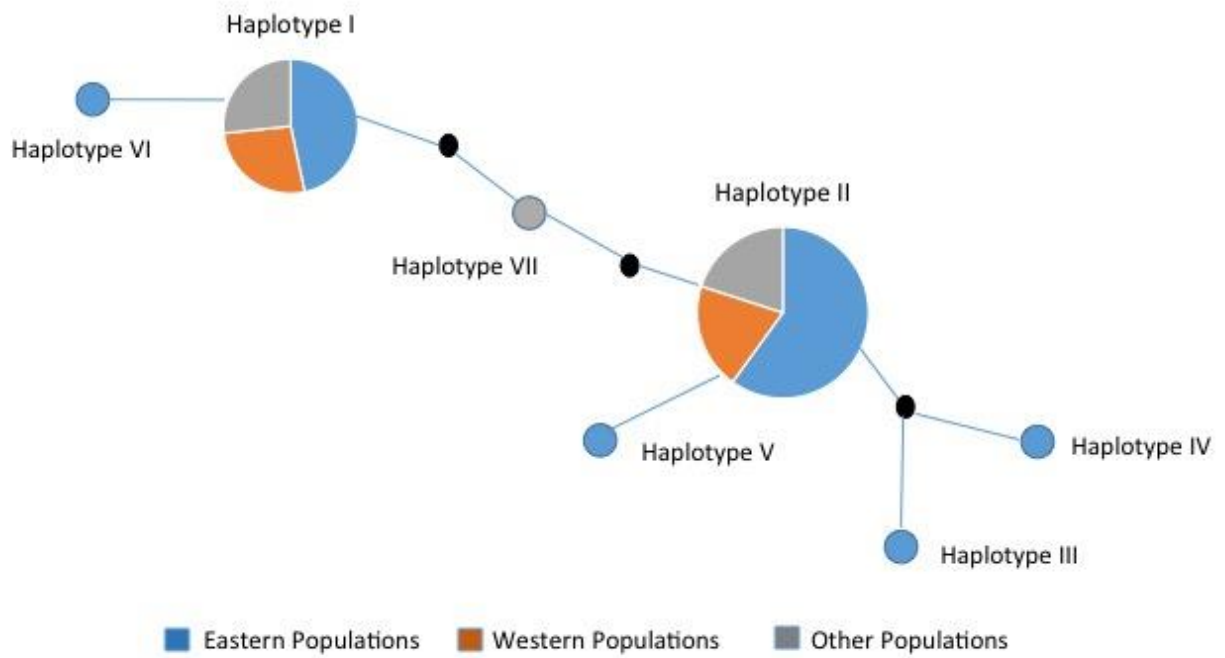


Figure 1.7. Haplotype minimum spanning network for individual worker ants collected in the Greater Boston area (Eastern populations), the Berkshires (Western populations), and some locations outside Massachusetts (other populations). Solid black circles represent inferred intermediate mutations.

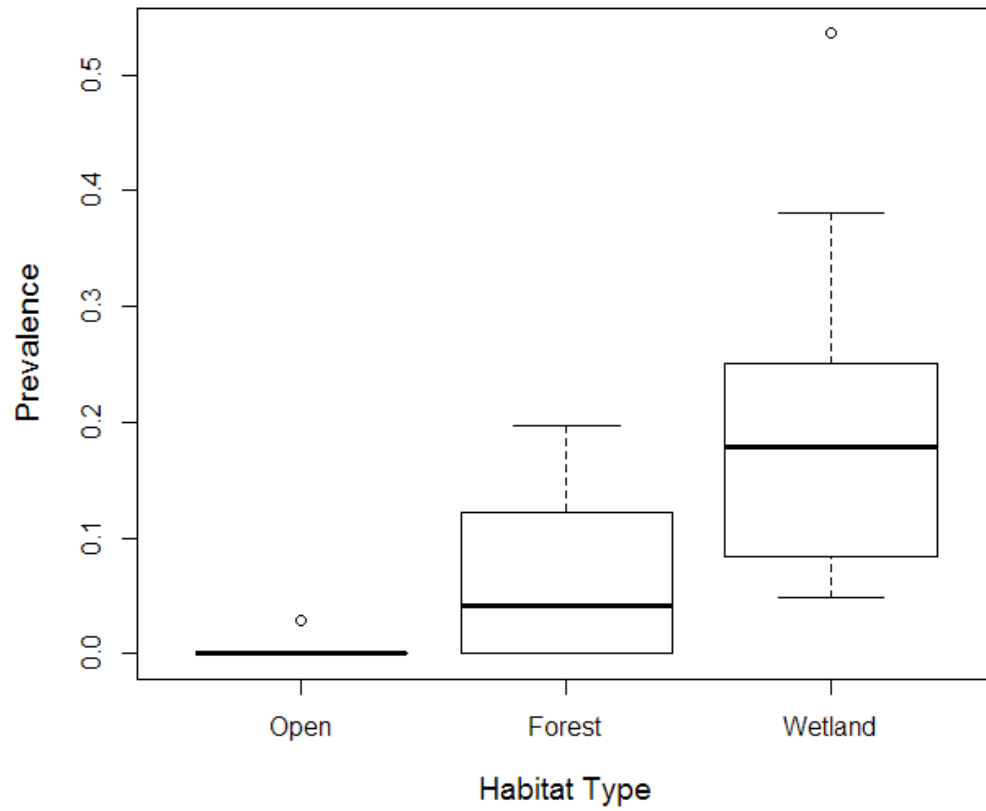


Figure 1.8. Box and whiskers plot for the proportion of sites with *M. rubra*. All pairwise contrasts were statistically significant (Tukey's method; $p < 0.001$).

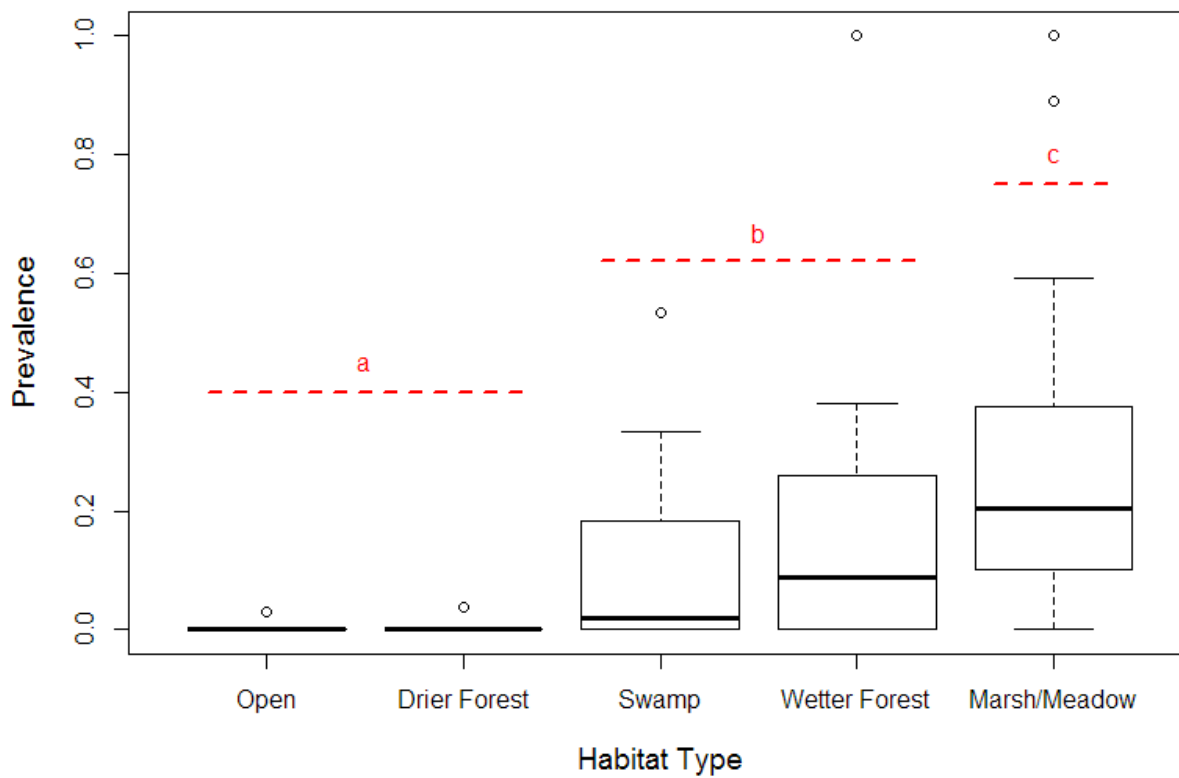


Figure 1.9. Box and whiskers plot for the proportion of sites with *M. rubra*. Forests were divided into drier and wetter sites. Wetlands were divided into forested wetlands (swamp) and non-forested wetland (marshes and wet meadows). Horizontal bars (a, b, c) connect habitat types that did not differ significantly at the 0.05 level (Tukey's method).

Tables

Table 1.1. Towns in which *Mymrica rubra* was found.

STATE OF MASSACHUSETTS

BARNSTABLE COUNTY:

- Barnstable
- Brewster
- Chatham
- Falmouth (Woods Hole)

BERKSHIRE COUNTY:

- Dalton
- Great Barrington
- Lanesborough
- Lee
- Lenox
- North Adams
- Sheffield
- Stockbridge
- Washington
- Williamstown

ESSEX COUNTY:

- Andover
- Beverly
- Danvers
- Essex
- Gloucester
- Hamilton
- Ipswich
- Lawrence
- Lynn
- Lynnfield
- Manchester-by-the-sea
- Marblehead
- Peabody
- Rockport
- Salem
- Saugus
- Swampscot
- Topsfield
- Wenham
- West Newbury

MIDDLESEX COUNTY:

- Arlington
- Bedford
- Belmont
- Cambridge

Everett
Framingham
Lexington
Malden
Medford
Melrose
Natick
Newton
North Reading
Reading
Stoneham
Sudbury
Wakefield
Watertown
Woburn

NORFOLK COUNTY:

Braintree
Canton
Cohasset
Dedham
Milton
Needham
Norwood
Quincy
Stoughton
Wellesley
Weymouth

PLYMOUTH COUNTY:

Brockton
Hingham
Marshfield
Scituate

SUFFOLK COUNTY:

Boston
Revere
Winthrop

WORCESTER COUNTY:

Worcester

STATE OF RHODE ISLAND

NEWPORT COUNTY:

Middletown
Newport

Table 1.2. Haplotypes of mitochondrial COI gene sequenced from worker ants of *Myrmica rubra* from selected sites in eastern and western Massachusetts and locations in the states of Maine, Rhode Island, Washington, and Newfoundland, Canada.

Haplotype	Locality
I	Cohasset, MA
	Crawford Notch, NH
	Essex, MA
	Gloucester, MA
	Ipswich, MA
	Lanesborough, MA
	Lenox, MA
	Newport, RI
	North Pownal, VT
	Weymouth, MA
	Williamstown, MA
	Worcester, MA
	Newfoundland, Canada
II	Beverly, MA
	Buffalo, NY
	Cambridge, MA
	Eastport, MA
	Everett, MA
	Lee, MA
	Lenox, MA
	Lynnwoods, MA
	Marshfield, MA
	Middlesex, MA
	Milton, MA
	Newton, MA
	North Pownal, VT
	Owlshead, ME
	Rockland, MA
	Rockport, MA
	Rye, NH
	Saugus river, MA
	Williamstown, MA
III	Brewster, MA
IV	Topsfield, MA
V	Reading, MA
VI	Bedford, MA
VII	Revere, MA
	Seattle, WA

Chapter 2

Modeling an incomplete invasion: the distribution and habitat affinities of the ant *Myrmica rubra* in Massachusetts

Abstract

Species distribution models (SDMs) enable ecologists to understand potential and future distributions of species; however, naively applying SDMs to invasive species that are still in the process of spreading may produce unreliable predictions. To date, SDMs developed for invasive ants are typically non-spatial and assume that the species is in equilibrium with its environment. We systematically surveyed and recorded occurrences of an invasive ant, *Myrmica rubra* L., in the state of Massachusetts to delineate the extent of its invasion. We applied both classical and Bayesian logistic regressions to model the distribution of *M. rubra* using environmental predictors such as land use land cover, vegetation, and climate data at 484 sites. The Bayesian approach includes a spatial submodel estimating whether the species has arrived within different regions of the state. Both methods yielded similar conclusions as to which sets of covariates predict the occurrence of *M. rubra*, but the Bayesian model quantifies uncertainty in arrival, avoids arbitrary assumptions about the limits of the distribution, and accounts for spatial dependence in outcomes. *M. rubra* was more likely to be found at sites with high amounts of wetland, water, and developed habitat within 300 m and at sites with lower maximum temperatures in the hottest month of the year. The effects of precipitation during the warmest quarter and of the length of the search path were not clear. The ant was more likely to be found at sites with wetland plants and with stands of the invasive weed *Fallopia japonica* Houtt. (= *Reynoutria japonica* or *Polygonum cusidatum*; Japanese knotweed). These results suggest that, within Massachusetts, *M. rubra* is restricted to places where the soil does not dry out during the summer, especially wetlands and riparian habitats, and that human activity promotes its occurrence.

Introduction

Ecologists have developed a variety of statistical models to understand the geographic distributions of species and populations (Franklin 2010). Species distribution models (SDMs) link geo-referenced data on presence and absence or presence-only data to environmental predictors hypothesized to shape the species' occurrence. SDMs have been widely used to predict the potential spread of invasive species by determining the characteristics of the species' distribution in one region and extrapolating to another (e.g., Broennimann and Guisan 2008, Elith et al. 2010, Jiménez-Valverde et al. 2011) or by characterizing the ecological niche of exotic species using data only from invaded regions (e.g., Menke et al. 2009). However, when the invasion is incomplete, commonly used models lack the ability to distinguish sites where the species has not yet arrived from sites with low habitat suitability (Ibáñez et al. 2009). In these circumstances, predictions produced by naively fitting logistic regressions or generalized additive models will likely distort the relationship of a species' distribution to habitat characteristics (Latimer et al. 2006).

Ibáñez et al. (2009) developed a Bayesian modeling approach that is well suited for the analysis of incomplete invasions. Their model simultaneously estimates habitat suitability and the probability of arrival across the sampled area. Regions where the species is unlikely to have arrived carry less weight in estimation of habitat suitability. More generally, Bayesian hierarchical models provide several benefits for spatial ecological prediction (Latimer et al. 2006). Hierarchical models make it easier to quantify uncertainty in ecological processes (Cressie et al. 2009) and inclusion of random effects can account for unexplained variation that is spatially autocorrelated (Gelfand et al. 2006).

Due to their impacts on humans and other species (Lach and Hooper-Bui 2010), considerable effort has been devoted to understanding and predicting the spread of invasive ants. Several global and regional SDMs have been developed for the fire ant *Solenopsis invicta* (Sutherst and Maywald 2005, Fitzpatrick et al. 2007, Peterson and Nakazawa 2007) and the Argentine ant, *Linepithema humile* (Hartley et al. 2006, Menke et al. 2009, Ward 2009, Roura-Pascual et al. 2011, Fitzgerald and Gordon 2012). These are typically non-spatial models, subject to biases caused by spatial autocorrelation in the residuals

(Dale and Fortin 2002, Segurado et al. 2006). None has considered both arrival and habitat suitability as alternative causes for the absence of ants at particular locations.

We developed Bayesian models to estimate both the probability of arrival and the determinants of habitat suitability for the invasive ant *Mymrica rubra* L. in the state of Massachusetts. *M. rubra* originated in Eurasia and was reported to occur in North America by the early 20th century, when it was discovered in Boston and Woods Hole, Massachusetts (Wheeler 1906). *M. rubra* appears to be a slow invader (Grodén et al. 2005, Wetterer and Radchenko 2011, Hicks et al. 2014), but information on its invaded range is limited. Our systematic survey in southern New England (Chapter 1) greatly extended the known range of *M. rubra* in this region. Due to the ant's aggressiveness and painful sting, and its ability to form dense populations, these ants may become a nuisance in residential and agricultural areas, with impacts on other species (Garnas et al. 2014).

We sought to discover environmental characteristics that predict the presence of *M. rubra* in southern New England. Here, we restrict our analysis to mainland Massachusetts, which contains nearly all known occurrences in southern New England, and for which particularly detailed habitat data layers are available. We fit both classical and Bayesian models to data from 484 sites in order to address the following questions: (1) What types of habitats and vegetation are associated with *M. rubra*? (2) How does variation in climate affect the distribution of *M. rubra* on a regional scale? (3) Do classical and Bayesian models yield similar inferences?

Environmental Predictors

Based on the findings of previous studies on invasive ant ecology, we considered three types of environmental variables as possible predictors in the distribution model.

(1) *LULC (land use land cover)*. Like many other species, invasive ants are often associated with particular habitat and land use characteristics. The two best studied invasive ants are the fire ant *Solenopsis invicta* and the Argentine ant, *Linepithema humile*. In northern Florida, *S. invicta* is restricted to disturbed and open habitats (Tschinkel 1988). Controlled experiments have shown that soil disturbance

greatly increases its abundance (King and Tschinkel 2008). The Argentine ant, *Linepithema humile*, shows a strong association with certain soil types in Portugal (Way et al. 1997) and occurs close to water, riparian habitats, and human habitations in California (Ward 1987, Holway 1998, Human et al. 1998, Menke et al. 2007, Fitzgerald et al. 2012). We therefore evaluated whether *M. rubra* is associated with particular LULC types and whether it tends to occur near human development or water.

(2) *Vegetation*. Vegetation can serve as an indicator of soil type, climate, and moisture regimes that can affect ant distributions (e.g., Lubertazzi and Tschinkel 2003, Fitzgerald and Gordon 2012). In our initial work, *M. rubra* appeared to be especially likely to occur near particular kinds of wetland plants. Furthermore, some plants provide direct benefits for ants (Rico-Gray and Oliveira 2007). Ness et al. (2013) showed that when *Fallopia japonica* Houtt. (= *Reynoutria japonica* or *Polygonum cusidatum*; Japanese knotweed) experienced a certain level of leaf damage, both sugar production and *M. rubra* patrolling increased. *F. japonica*, an invasive plant native to East Asia, produces large clones (Hollingsworth and Bailey 2000). In the early 1900s, when *M. rubra* was first discovered in Massachusetts, Rich (1902) reported that the plant was found only in the vicinity of gardens, but it is now common along roadsides and in riparian habitats (Aguilera et al. 2010). During our surveys, we recorded the presence of *F. japonica* and of abundant wetland plants.

(3) *Climate*. At the early stages of SDMs, predictions were based solely on climate data (Guisan and Zimmermann 2000, Guisan and Thuiller 2005). Researchers have developed climate models to forecast future distributions of invasive ant species and how they will be affected by climate change (e.g., Roura-Pascual et al. 2004, Sutherst and Maywald 2005, Chen 2008). Peterson and Nakazawa (2007) showed that models based smaller sets of climate variables may yield more reliable prediction than models based on more complex climate variables. The most important aspects of climate can vary from one end of a species' range to the other. For example, on the edges of arid habitats in California, high summer rainfall is positively associated with increased spread of Argentine ant populations (Heller et al. 2008) but at high elevations in Hawaii, where the climate is colder and wetter, greater rainfall is negatively associated with the rate of spread of the same species (Krushelnycky et al. 2005). Within its

native range, *M. rubra* lives in places that are much colder than Massachusetts (Wetterer and Radchenko 2011). We therefore considered that temperature and precipitation during the warmest parts of the year were more likely to limit local distribution than temperatures or precipitation during the coldest parts of the year, when the ant is inactive.

Methods

Selection of sites. As described in Chapters 1 and 3, the search for *M. rubra* was designed to determine the extent of its invasion and its habitat affinities. All *M. rubra* populations encountered early in the project were in wetlands or riparian habitat. To spread the search out spatially, the state of Massachusetts was divided into a grid of 20 x 20 km cells (Fig. 2.1.). We targeted a minimum of two sites with wetland or riparian habitats in each cell, subject to the constraint that they were at least 4 km from any other site selected for this part of the survey. Within the invaded areas, we added additional sites varying in habitat and land use characteristics. As populations were detected, we surveyed sites around the periphery of invaded areas to determine the limits of the ant's distribution. We also visited all locations in Massachusetts listed by Groden et al. (2005) and others identified by respondents to an e-mail search for stinging ants (Chapter 1).

Sampling methods. At each site, we walked paths stopping every 25-40 m to search for ants. We looked beneath stones and fallen wood, as these are often nesting sites of *M. rubra*. While walking trails, we looked for ants on the ground and vegetation. Worker samples were collected to confirm identities. The lengths of the search paths varied due to constraints of access and time.

Habitat measurements. During surveys at each site, a GPS unit was used to record sample positions along the paths followed while looking for ants. These were corrected in Google Earth when we could see that inaccuracies in GPS readings placed the positions incorrectly with respect to landmarks

visible in Google Earth's satellite photos. For each site, the geometric center was found for the set of locations examined along the trails. These center points were imported into ArcMap 10.1 (Esri, Inc. Redlands, CA). To quantify habitat and land use characteristics, a circle of 300 m radius was drawn around each center point using ArcMap's buffer tool. Circles of this size usually encompassed the entire survey and were used to characterize land use and water in the immediate vicinity.

To quantify land use/land cover (LULC), we used data for 2005 provided by the Office of Geographic Information of the Commonwealth of Massachusetts (<http://www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis/datalayers/lus2005>). Although other land use data sets are available that extend to other states, we found the MassGIS layers to correspond more closely to what we observed in the field. This data layer recognizes 40 different LULC categories. Following preliminary analyses, a reduced list was constructed by omitting some land use categories and merging others, as follows. *Developed land* consists of areas that are largely paved or that include a mix of paved surfaces, buildings, and landscaping; namely, the commercial, industrial, junkyard, marina, nursery, spectator recreational, transitional, transportation, and urban/public institutional categories, as well as the multi-family, very low, low, medium, and high density residential categories. *Wetland* consists of forested and non-forested wetland and cranberry bog, all of which are freshwater wetlands. *Open habitat* consists of the cemetery, cropland, golf course, mining, open land, orchard, participation residential, pasture, power line/utility, waste disposal, and water-based recreation categories. *Forested* habitat consists of the forest and brush land/successional categories. *Water* consists of only the water category, which does not include saltwater. The saltwater/sandy beach and saltwater wetland categories were omitted.

ArcMap 10.1 was used to merge and quantify LULC categories. The center points and land use data layer were imported and projected to a common coordinate system (NAD 1983 UTM 18N). Land use categories were merged as described above and clipped to the 300 m circles. The amount of each type of habitat was transformed by taking the natural logarithm of the quantity in m² plus 0.1.

Vegetation indices. While searching for *M. rubra*, we noted the presence of three particular types of vegetation: two categories of wetland plants (described below), and Japanese knotweed. These were scored as binary predictors, set to 1 if vegetation of that type was present, otherwise to 0. Where present, the plants typically occurred along stretches of at least 10 m; if only a few plants were seen in a smaller area, we set the score to 0.

The National Wetland Plant List (Lichvar 2013) recognizes five categories of wetland status: obligate (OBL; “almost always occur in wetlands”), facultative wetland (FACW; “usually occur in wetlands, but may occur in non-wetlands”), facultative (FAC; “occur in wetlands and non-wetlands”), facultative upland (FACU; “usually occur in non-wetlands, but may occur in wetlands”), and obligate upland (UPL; “almost never occur in wetland”). Reed (1988) subdivides these designations using ‘+’ and ‘-’ signs, to indicate plants towards the higher and lower ends of the category, and lists wetland status for the northeastern United States.

We divided wetland plants into two categories. The first, hereafter called “wetland plants” for brevity, consisted of plants that have a high affinity for wetlands, as indicated by a wetland status of OBL, FACW+, or FACW in the northeastern United States. For these species, at least 83% of occurrences are in wetland (Tiner 2009). Principal examples include *Typha* spp. (cattails; OBL), *Cephalanthus occidentalis* L. (buttonbush; OBL), *Carex stricta* Lam. (tussock sedge; OBL), *Alnus serrulata* Ait. (smooth alder; OBL), *Phalaris arundinacea* L. (reed canary grass; FACW+), *Lythrum salicaria* L. (purple loosestrife; FACW+), *Onoclea sensibilis* L. (sensitive fern; FACW), *Impatiens capensis* Meerb. (jewelweed; FACW) and other sedges and rushes (*Carex* spp., *Cyperus* spp., *Juncus* spp., and *Scirpus* spp.) with OBL, FACW+, or FACW status. We excluded *Symplocarpus foetidus* L. (skunk cabbage) despite its OBL designation because it sometimes grows in places that dry out during the summer and that lack other plants in this category.

The second category, hereafter called “facultative wetland plants” for brevity, consisted of plants with a lower affinity for wetlands, as indicated by a wetland status of FACW-, FAC+, FAC, or FAC-. Between 34 and 83% of sites with these species are in wetlands (Tiner 2009). Principle examples include

Arisaema triphyllum L. (Jack-in-the-pulpit; FACW-), *Clethra alnifolia* L. (sweet pepperbush; FAC+), *Viburnum dentatum* (arrowwood; FAC), and *Rhamnus cathartica* L. (European buckthorn; FAC).

We also recorded the presence of *F. japonica* at each sample site (Japanese knotweed; FACU).

Logistic regressions. Classical (frequentist) logistic regressions were implemented in R version 3.1.2 (<http://www.R-project.org/>) using the glm function. Packages used included AICcmodavg (to produce AIC tables; version 2.0-3; Marc J. Mazerolle), ROCR (to calculate the area under the receiver operating characteristic curve; version 1.0-7; T. Sing, O. Sander, N. Beerenwinkel and T. Lengauer), car (for partial residual plots and calculation of variance inflation factors; version 2.0-25; Fox and Weisberg 2011), and gstat (for variograms; version 1.0-26; Pebesma 2004).

For this part of the analysis, we applied regression only to sites judged to be within areas where *M. rubra* has arrived. Including sites outside the invaded zone would give a misleading view of the habitat affinities of *M. rubra*. We compared three methods for judging the extent of the invaded area. (1) The first estimate was our best subjective judgment of where *M. rubra* has arrived. This includes all places where *M. rubra* has been collected and other sites for which *M. rubra* is within 3-5 kms, using the longer distance if the ant occurs in multiple directions. Our subjective judgment also took into account possible barriers to dispersal, such as the Merrimac River and the mountains of the Berkshires. (2) The second estimate assumes that any point within 5 kms of a known population is within the invaded range. (3) The third estimate uses output from the Bayesian model presented below. Under this method, points were included if the mean posterior probability of arrival under the Bayesian model was ≥ 0.5 .

Akaike's Information Criterion (AIC) scores were used for model selection (Burnham et al. 2010). Models with high support were subjected to several diagnostic tests to evaluate model adequacy. The Hosmer-Lemeshow goodness-of-fit test (Hosmer and Lemeshow 2004) was implemented by dividing the data into ten groups based on the estimate probabilities of the ant being discovered, ranked from lowest to highest. To determine the influence of each sample on estimates of coefficients, the car package (version 2.0-25; Fox and Weisberg 2011) was used to calculate Cook's distances. Partial residual plots, also called component plus residual plots (Fox 2002), were used to look for non-linearities or systematic

patterns in the variances. These problems could indicate that the logit link is inadequate or that one or more variables should be transformed. Multicollinearity was assessed by variance inflation factors. Finally, a variogram was plotted to detect spatial autocorrelation in the residuals.

Bayesian models were implemented in OpenBUGS (Lunn et al. 2009). The code is given in the Appendix. For each model, two chains were run, a burn-in of 1,000,000 samples was discarded and the next 10,000,000 samples were thinned to 1 in 1000. Examination of traceplots showed that the burn-in was of sufficient length, and that the chains converged to the same distribution and mixed well.

We adopted the approach of (Ibáñez et al. 2009) to model the distribution of an invasive species that has not yet spread through the entire area of study. Following their symbolism, h_i , the probability of detecting a species at site i , is the product of the probability of arrival, w_i , and the probability of establishment, p_i . The probability of arrival is described by a spatial model accounting for where the invasive species has spread. If the species has arrived in a region, its probability of establishment at a particular site depends on the site's habitat characteristics.

The spatial model assumes that $\text{logit}(w_i) = v + \phi_i$, where the parameter v accounts for the average probability of arrival and the ϕ_i are site-specific random effects described by a conditional autoregressive (CAR) model. In the CAR model, the value of ϕ at site i is drawn from a normal distribution with a mean equal to the average of the ϕ_i for neighboring units and standard error equal to σ_w . Thus, the estimated probability that the species has arrived at a site is influenced by the occurrence of the species at other nearby sites. We tried several procedures for defining neighborhoods.

The model of habitat suitability is a logistic regression with random effects: $\text{logit}(p_i) = \alpha + \beta X_i + \varepsilon_i$, where α is the intercept on the logit scale, the X_i describe habitat characteristics of site i , the β are unknown coefficients, and the ε_i are normally distributed site-specific errors with a mean of 0 and standard deviation of σ_ε . The α and β terms were given normal prior distributions with means equal to 0 and tolerances set to 0.01 (Appendix).

Ibáñez et al. (2009) provide a more complete description of the model. We made several modifications.

(1) Ibáñez et al. incorporated data from both the native and invaded ranges to model associations between climate and species occurrence. We used data only from southern New England as there are no comparable presence/absence data from the ant's native range or elsewhere in North America.

(2) Ibáñez et al. set w_i to 1 if the species was detected at site i ; otherwise, w_i was estimated by the spatial logistic model described above. In our case, that procedure led to poor estimates of the w_i values; e.g., values of ~ 0.03 throughout regions where the ant was most prevalent. Instead, we used the spatial logistic submodel for w_i consistently without setting w_i to 1 where the ant was known to occur. This choice substantially improved estimates of habitat suitability.

(3) We used more informative priors for the standard deviations of the two random effects. Bayesian posteriors are especially sensitive to the choice of scale parameters for random effects and practitioners are recommended to examine this sensitivity rather than automatically using vague priors (Lambert et al. 2005, Gelman 2006). In preliminary analyses, placing vague inverse gamma priors on σ_w and σ_ε yielded estimates of the probability of arrival that were unreasonably high throughout regions where the ant was never found. More generally, vague priors on σ_ε often produced estimated habitat suitabilities that were very low (< 0.001) in places where the ant was not found but that intuitively seemed to have environments that will support *M. rubra* when it arrives. These problems were solved by placing priors on the standard deviations of the random effects, rather than using inverse gamma priors (Lambert et al. 2005, Gelman 2006), and by using more informative priors for σ_ε . In particular, the prior for σ_ε was normal with $\mu_\varepsilon = 0$ and $\tau_\varepsilon = 0.05$, and the prior for σ_w was normal with $\mu_w = 0$ and $\tau_w = 0.001$.

(4) We used Thiessen polygons (Brassel and Reif 1979), rather than a fixed distance, to define the neighborhood structure for the CAR model. Defining neighbors by a fixed distance was problematic because the density of samples was considerably higher within and around the edges of the invaded area because we sought to find the current limits of the ant's spread and to compare multiple habitats within

invaded areas. A Thiessen polygon encloses the part of a plane that is closer to a particular sample point than to any other sample point. Use of Thiessen polygons, which were calculated in ArcMap, automatically expands the spatial scale of the neighborhood where samples are sparse. This led to more reasonable estimates of the w_i values.

(5) Many of the climate variables were correlated, which can produce problems associated with multicollinearity in regression models. After preliminary analyses, we considered only two climate variables (maximum temperature for the warmest period, and precipitation during the warmest quarter) that were not strongly correlated and that could be expected to affect the distribution of an ant that is sensitive to soil moisture. For faster convergence, we used independent normal prior distributions for the climate predictors, rather than a multivariate normal distribution.

Results

Fig. 2.1 shows sites where *M. rubra* was and was not discovered. Table 2.1 summarizes the characteristics of the sample sites.

Classical logistic regressions

Frequentist logistic regression was applied only to sites assumed to be within the invaded areas. As described in the methods, three methods were used to select these sites. Tables 2.2-2.4 present AIC comparisons for models fitted using the three methods. AIC comparisons were made for models fitted using each of the three methods: (1) subjective judgment (Table 2.2); (2) 5 km radius of known locality (Table 2.3); and (3) Bayesian posterior ≥ 0.5 (Table 2.4). For all three estimates of the invaded area, the model with greatest support includes as predictors three habitat characteristics (the area of wetland, water, and developed land within 300 m; log-transformed), two vegetation characteristics (the presence of wetland plants and the presence of *F. japonica*), and the length of paths followed while searching for the

ant (log-transformed). Under methods (1) or (3), there was no clear support for including any other predictor. The change in AIC caused by adding facultative wetland plants or precipitation in the warmest quarter to the model was approximately what would be expected for adding an uninformative parameter (Arnold 2010). Under method (2), the model with lowest AIC included an additional vegetation index (the presence of facultative wetland plants) and one aspect of climate (the amount of precipitation in the warmest quarter).

We present further information on the model selected under method (3) only, as this corresponds most closely to the Bayesian model described below. Estimates of coefficients are shown in Table 2.5. All coefficients were positive, except for precipitation during the warmest quarter. The area under the receiver operating characteristics curve (AUC) is 0.84, a moderately high value (Franklin 2010).

Diagnostic checks were used to evaluate model adequacy. No significant deviations from the assumptions of the model were revealed by the Hosmer-Lemeshow test for goodness-of-fit ($H = 5.22$; $P = 0.73$). Multicollinearity among the predictors was not important: variance inflation factors were < 1.5 for all variables except for the amount of developed habitat, for which the variance inflation factor was 1.98. AIC comparisons (not shown) did not support including any interactions in the model. Partial residual plots (Fig. 2.2) showed no signs of important non-linearities or unexpected patterns among the residual. A few residuals were notably higher or lower, but there was no indication that any samples exert undue influence on estimates of the coefficients: Cook's distance was < 0.04 for all cases. Fig. 2.2 also shows that using logarithms of several predictors (the habitat measures and the distance traveled while searching for ants) spreads the values out more-or-less evenly along the x axes. The amounts of precipitation during the warmest quarter fall mostly into two groups, corresponding to drier locations closer to the coast and wetter locations in western Massachusetts. In short, these diagnostics revealed no important deviations from the model assumptions.

A variogram for the residuals (Fig. 2.3) is flat to about 50 k, then rises modestly. Thus, residuals for this non-spatial model are more similar for sites within each invaded area than for sites separated by greater distances.

Bayesian models

The Gelfand-Ghosh statistic (Gelfand and Ghosh 1998) was used to compare Bayesian models (Table 2.6). The top ranked model included as predictors the amount of wetland, water, and developed habitat (log-transformed) within 300 m, the presence of wetland and facultative wetland plants, the presence of *F. japonica*, the length of the search path, and the maximum temperature of the hottest month. All of these predictors were positively associated with the presence of *M. rubra* except for two: the maximum temperature of the hottest month was negatively associated with the presence of *M. rubra* and the sign of the coefficient for the length of the search path was uncertain (Table 2.7).

Fig. 2.4 shows the estimated probability of arrival for the Thiessen polygon surrounding each site, and Fig. 2.5 shows the estimated habitat suitability for the survey sites. Figs. 2.6 and 2.7 are maps of developed land and wetland habitats respectively.

Discussion

Modeling an incomplete invasion. After 115 years in Massachusetts, *Myrmica rubra* has apparently not yet arrived in large parts of the state. It was easy to find dense populations in the greater Boston area and in the Berkshires by searching in wetlands and riparian habitats. *M. rubra* was also found at scattered sites on Cape Cod and at an isolated site in Worcester (Fig. 2.1). However, surveys revealed no populations throughout large parts of central and southern Massachusetts, including sites with favorable habitats (Fig. 2.5). Although our study undoubtedly underestimates the species' distribution, these results strongly suggests that the ant has not yet invaded large regions where it will eventually occur. A species distribution model fitted to all sites would likely produce an inaccurate model of habitat or climate associations, as many of the absences are due to dispersal limitation rather than to site characteristics.

We used two approaches to account for the incomplete dispersal of *M. rubra* across the study region. First, we fitted logistic regressions to just those sites that were judged to be within the invaded zones by either subjective or simple objective criteria (Tables 2.2-2.4). This approach is easy to implement and allows rapid model development and checking. Second, we implemented a Bayesian approach that includes a spatial submodel of ant arrival (Ibáñez et al. 2009). The Bayesian model takes into account uncertainty in arrival and avoids arbitrary assumptions about the limits of the distribution. Furthermore, the random effects can describe spatial dependence in outcomes, avoiding problems caused by spatially autocorrelated residuals (Latimer et al. 2006). Output of the spatial component of the Bayesian model matched our subjective estimate fairly closely. The two approaches yielded similar conclusions as to which predictors to include in models of the species' distribution (Tables 2.2- 2.4, 2.6) and about the signs of the coefficients, but the absolute values of coefficients were greater under the Bayesian model (Tables 2.5 and 2.7).

As is often the case with hierarchical models, care was needed in choosing priors for the standard deviations of random effects (Lambert et al. 2005, Gelman 2006). Estimates of the probability of arrival were sensitive to the choice of priors for the standard deviation of errors in the habitat submodel, σ_e , and, to a lesser extent, in the spatial submodel, σ_w . When vague priors were used for σ_e , suitability estimates were too low at the low end, with posterior means well below 0.001 at sites that, to our eyes, should have a reasonable chance of supporting *M. rubra*. Placing moderately informative priors on σ_e solved these problems. The magnitude of the coefficients (Table 2.7) also depended on the priors used for random effects.

Habitat suitability and probabilities of arrival are jointly estimated by the Bayesian model; changes in either submodel affected the other. An accurate spatial submodel discounts the contribution of sites where *M. rubra* has not yet arrived to the fit of the habitat suitability submodel. Conversely, we noticed that as we successively improved the habitat submodel in exploratory stages of development, the predictions of the spatial model came to match the observed distribution more closely.

Predictors of M. rubra occurrence. One way to describe the models is to consider them as tools for finding additional sites in Massachusetts that are occupied by *M. rubra*. The ant is most likely to be found by searching in places with wetland plants and stands of *F. japonica*, with low maximum temperatures during the hottest month, and with large amounts of wetland, water, and developed land in the immediate vicinity (Table 2.7). The model was improved by including the length of the search path, however, the sign of the effect could not be identified confidently. The spatial submodel predicts that new discoveries are more likely where *M. rubra* is present at multiple neighboring sites (Fig. 2.4).

These results indicate that, in Massachusetts, *M. rubra* lives principally in places that do not dry out during the summer, including areas in and near marshes, swamps, and riparian habitats. Several of the predictors reflect these conditions: the presence of wetland plants, and the amount of wetland habitat and water within 300 m. The spread of Argentine ants, *L. humile*, is also limited by drying of soil during the summer in arid regions (reviewed by Krushelnycky et al. 2010); in southern California, experimental irrigation allows its populations to spread (Menke et al. 2007). However, Massachusetts is much wetter than California, implying that *M. rubra* requires even more moisture than *L. humile* to thrive.

The association of the ant with developed habitats, which include paved surfaces and commercial and residential development, was not anticipated but is strongly supported by model comparisons (Tables 2.2-2.4, 2.6). There are several possible explanations for the association between development and *M. rubra*: (1) Development may modify the environment in ways that favor the ant. For example, disturbance of the soil and irrigation promote the spread of *S. invicta* and *L. humile*, respectively (Menke et al. 2007, King et al. 2009). Such direct impacts do not seem especially likely because *M. rubra* was rarely found within the developed parts of the 300 m circles; instead, it was limited to wetlands, riparian habitat, or forested areas next to them. (2) Development may be associated with human activities that transport ants, including movement of plants, wood, and soil (e.g., Walsh et al. 2004, King et al. 2009). Invasive ants typically arrive first in places modified by human activity and may be slow to spread into more natural habitats (Krushelnycky et al. 2010). (3) Areas with more developed habitat may be closer to the original

points of entry of the ant from Eurasia. In particular, Boston and surrounding towns are highly developed (Fig. 2.6). However, the degree of development within the 300 m circles was not simply a function of distance from the earliest known locations.

M. rubra was more likely to occur where stands of the invasive plant *F. japonica* were present. *F. japonica* is most common in areas disturbed by human activity and along river banks (Beerling et al. 1994), so the plant serves as an indicator for those environments. Furthermore, *F. japonica* provides direct benefits for the ants in the form of nectar (Ness et al. 2013) and the ants sometimes nest in its hollow dead stems (pers. obs.). The association was not obligate. Unlike Ness et al. (2013), we found large populations of *M. rubra* at sites where *F. japonica* was completely absent.

For wetland plants, there was support for including as predictors species that occur in wetland at least 83% of the time and species that occur in wetlands 33-83% of the time. Our search image for *M. rubra* habitat includes especially *Phragmites australis* Cav. (common reed; except in salt marshes), *Alnus* spp. (alders), and *Phalaris arundinacea* L. (reed canary grass). We also found *M. rubra* near stands of *Typha* (cattails) and other wetland plants that grow in water or saturated soil. *Onoclea sensibilis* L. (sensitive fern) and *Impatiens capensis* Meerb. (jewelweed) were common in some moist forests that supported large populations of *M. rubra*, but both species also occur in places that appear to be too dry for the ant during the summer. We were less likely to find *M. rubra* in wetlands dominated by *Clethra alnifolia* L. (sweet pepperbush) and *Rhamnus cathartica* L. (European buckthorn), two common plants in the facultative wetland category.

The effect of search effort (distance walked) was uncertain. Including this predictor led to a lower Gelfand and Ghosh criterion and the posterior mean of the coefficient was positive, but the 95% CI straddled zero. Longer searches provide more opportunities to find nests, but it is also true that we walked shorter distances at sites with limited suitable habitat. Hence, there was a negative correlation (-0.48) between the length of the search and the amount of developed habitat (with both variables log-transformed). The model's predictive ability was not improved by including the amount of forested or

open habitat (Tables 2.2-2.4, 2.6). *M. rubra* was absent from mown grass and open fields. In forests, the ant was restricted to places near water and wetland.

Climate doesn't vary much across the study area, which may be why support for including measures of climate was equivocal (Tables 2.2-2.4, 2.6). High temperatures during the warmest month were negatively associated with the presence of *M. rubra* (Table 2.6 and 2.7). Because the ant is associated with habitats that stay wet, low summer temperatures could potentially preserve conditions favorable to *M. rubra*. Rainfall during the warmest quarter mostly distinguishes sites in western Massachusetts (> 300 mm rain) from those in the eastern part of the state (< 265 mm; Fig. 2d), so this variable may stand in for any differences between these regions that affect the ant. The influence of climate on ant distributions becomes more obvious on larger spatial scales or where there is a steeper cline in precipitation or temperature (e.g., Roura-Pascual et al. 2004, Menke et al. 2009).

Low or biased detectability can distort inferences from species distribution modeling (Gu and Swihart 2004, Royle et al. 2005). Although we did not design the study to measure detection rates, we revisited 28 sites where we found *M. rubra* within 1 month to 5 years (two to five repeated visits per site), and we found the ants again on every occasion. The density of workers foraging on the ground and vegetation was noticeably lower during hot dry periods; nevertheless, ants could be found along the same stretches of trail as on other days. This was true in marshes, swamps, and riparian habitats, including places where the ants were highly localized (e.g., present along just 3 m of trail) and present in low numbers. Furthermore, we found *M. rubra* at all Massachusetts sites listed by Groden et al. (2005) and at specific locations identified by other scientists and members of the public. Thus, the probability of detection appears to be high, and we have no evidence that it varies by habitat.

The habitat specificity of *M. rubra* in southern New England may contribute to its slow rate of spread. Little is known about mating flights in *M. rubra* (Hicks 2012), but if the gynes are poor fliers, it may be difficult for the ant to jump from one wetland to another. The large gaps between the major invaded areas (Fig. 2.1) strongly suggest that human transport is responsible for long distance dispersal, as in other invasive ants (Suarez et al. 2001). Wetterer and Radchenko (2011) hypothesized that the slow

rate of spread may be because the colonies of *M. rubra* initially introduced to Cape Cod were from a different climate in Eurasia and are poorly adapted to southern New England. An alternative possibility is that the lower prevalence of wetland in Cape Cod (Fig. 2.1) and the absence of major river systems reduce opportunities for *M. rubra* to spread and establish.

Acknowledgments

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Appendix

```
model{
  for (i in 1:N){
    Y[i]~dbern(p[i])
  }

  # Probability of establishment (habitat suitability)
  for (i in 1:N){
    logit(s[i]) <- alpha + beta[1]*logwetland[i]
    + beta[2]*logdev[i] + beta[3]*logwater[i]
    + beta[4]*knotweed[i] + beta[5]*WetlandVeg[i]
    + beta[6]*FacultativeVeg[i] + beta[7]*logmeters[i] + beta[8]*MaxTemp[i] + err[i]
    est[i]<-max(0,s[i])
    # probability that the ant is present = probability of arrival x probability of establishment
    p[i]<-arr[i]*est[i]
  }

  # Probability of arrival (spatial model)
  for (i in 1:N){
    logit(k[i])<-nu+phi[i]
    arr[i]<-max(0,k[i])
  }
  #Priors
  for (i in 1:N){err[i]~dnorm(0,tau.err)}
  alpha ~ dnorm(0,0.01)
  for (i in 1:8) { beta[i]~dnorm(0,0.01) }
  phi[1:N]~car.normal(adj[],weights[],num[],tau.a)
  for (j in 1:NNN) { weights[j]<-1 }
  nu~dnorm(0, 0.01)
  tau.a <- pow(sigma.a, -2)
  sigma.a ~ dnorm(0, 0.001) I(0, 100000)
  tau.err <- pow(sigma.err, -2)
  sigma.err ~ dnorm(0, 0.05) I(0, 100000)
}
```

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Figures

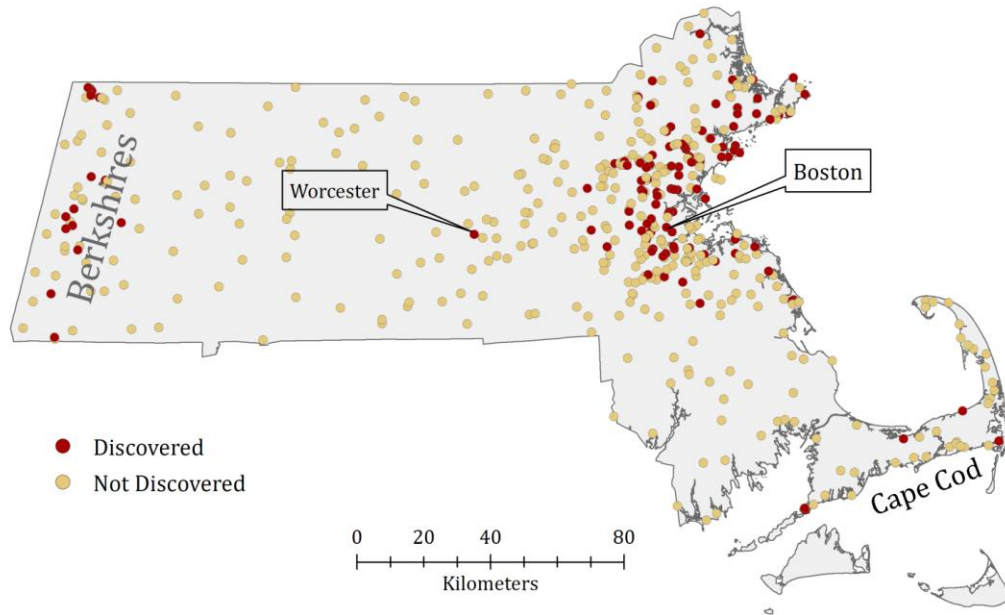


Figure 2.1. Sites where *M. rubra* was and was not discovered in Massachusetts. Invaded areas include a large region around Boston in eastern Massachusetts and the Berkshires range in western Massachusetts. *M. rubra* had a spotty distribution on Cape Cod and was found at an isolated location in Worcester.

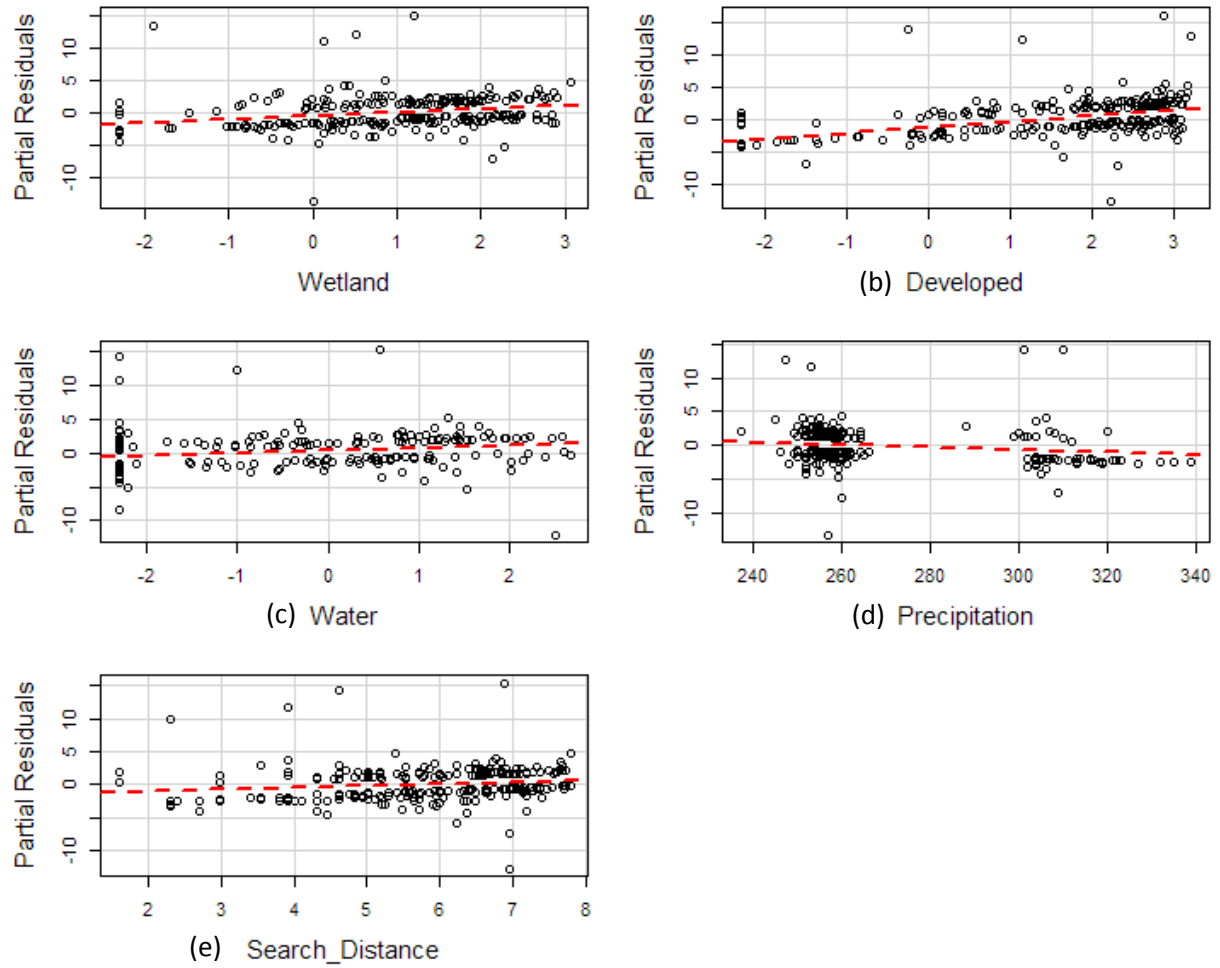


Figure 2.2. Partial residual plots for the five continuous predictors show no signs of non-linearity

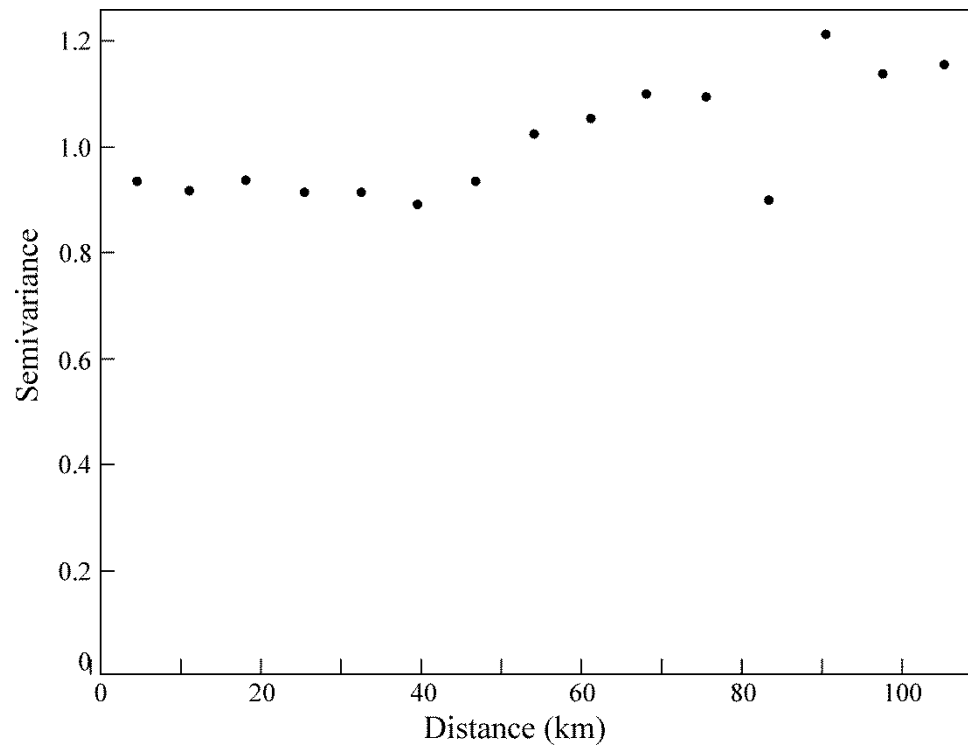


Figure 2.3. Variogram for residuals from the logistic model with greatest support.

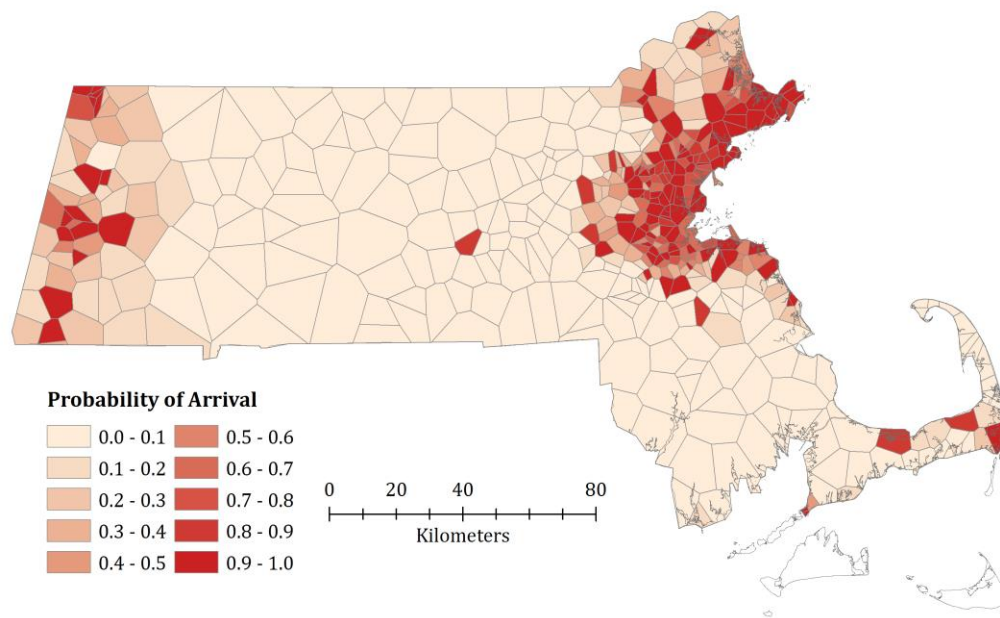


Figure 2.4. The estimated probability of arrival of *M. rubra* in each cell is indicated by the intensity of the color.

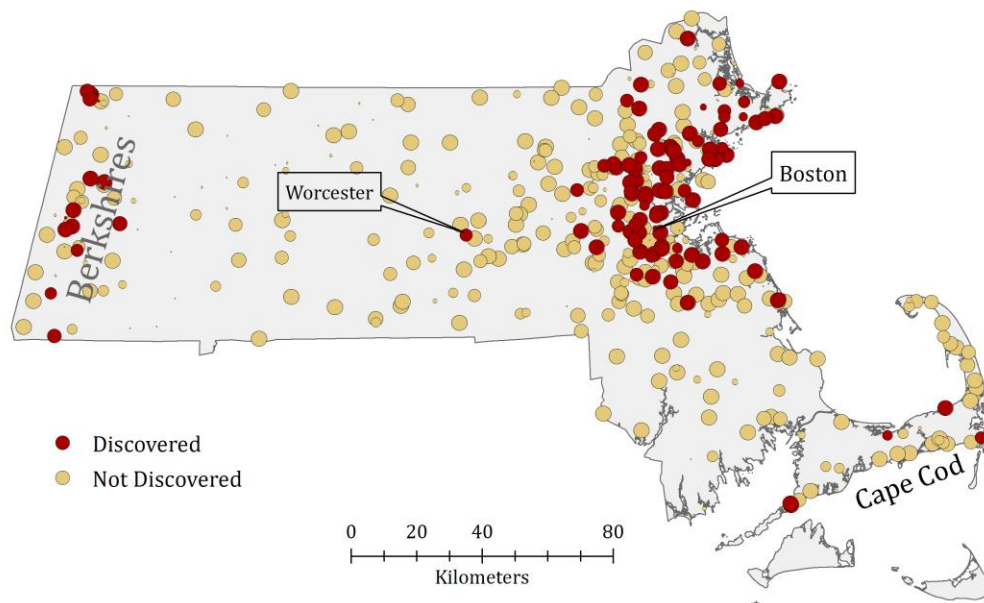


Figure 2.5. The area of the circle is proportional to the estimated habitat suitability at the survey site. Values ranged from < 0.001 to 0.995, representing the probability of detecting the ant if it has arrived at the site. Many sites outside the invaded range have high habitat suitability.

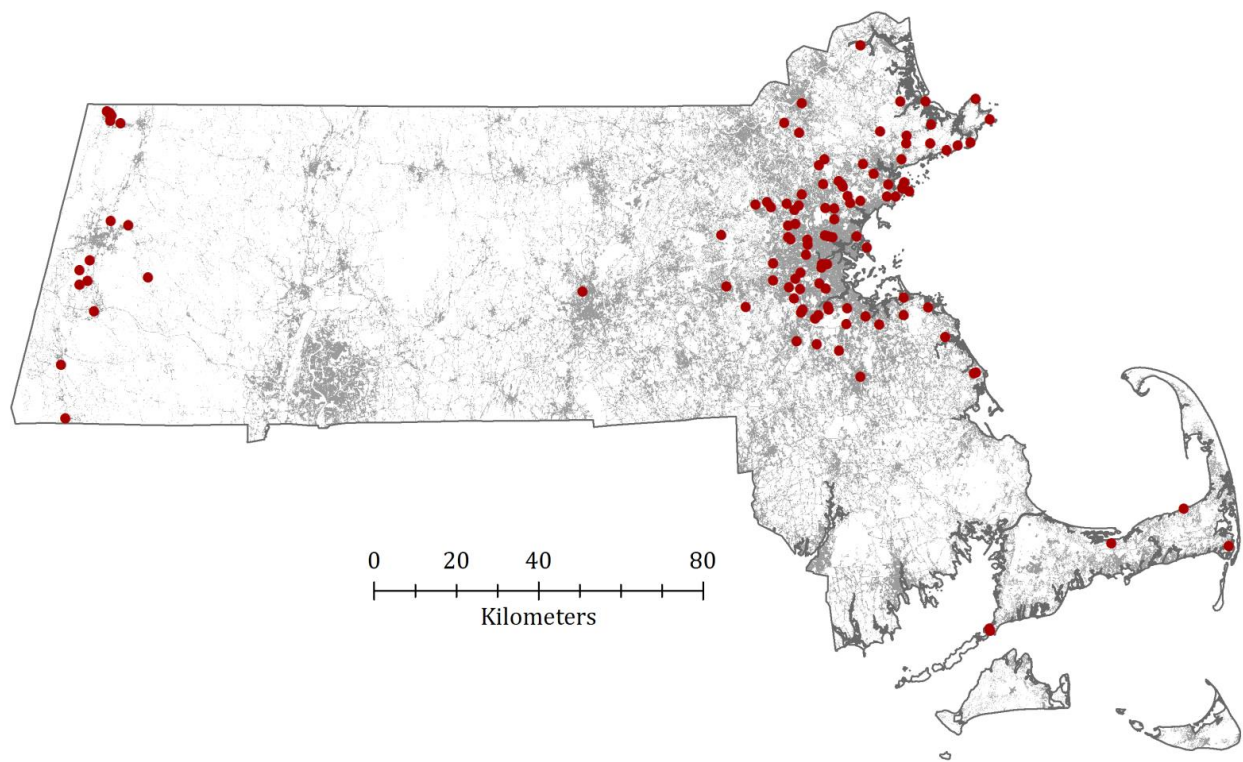


Figure 2.6. Developed land is shown in gray. Locations where *M. rubra* was discovered are indicated by red circles.

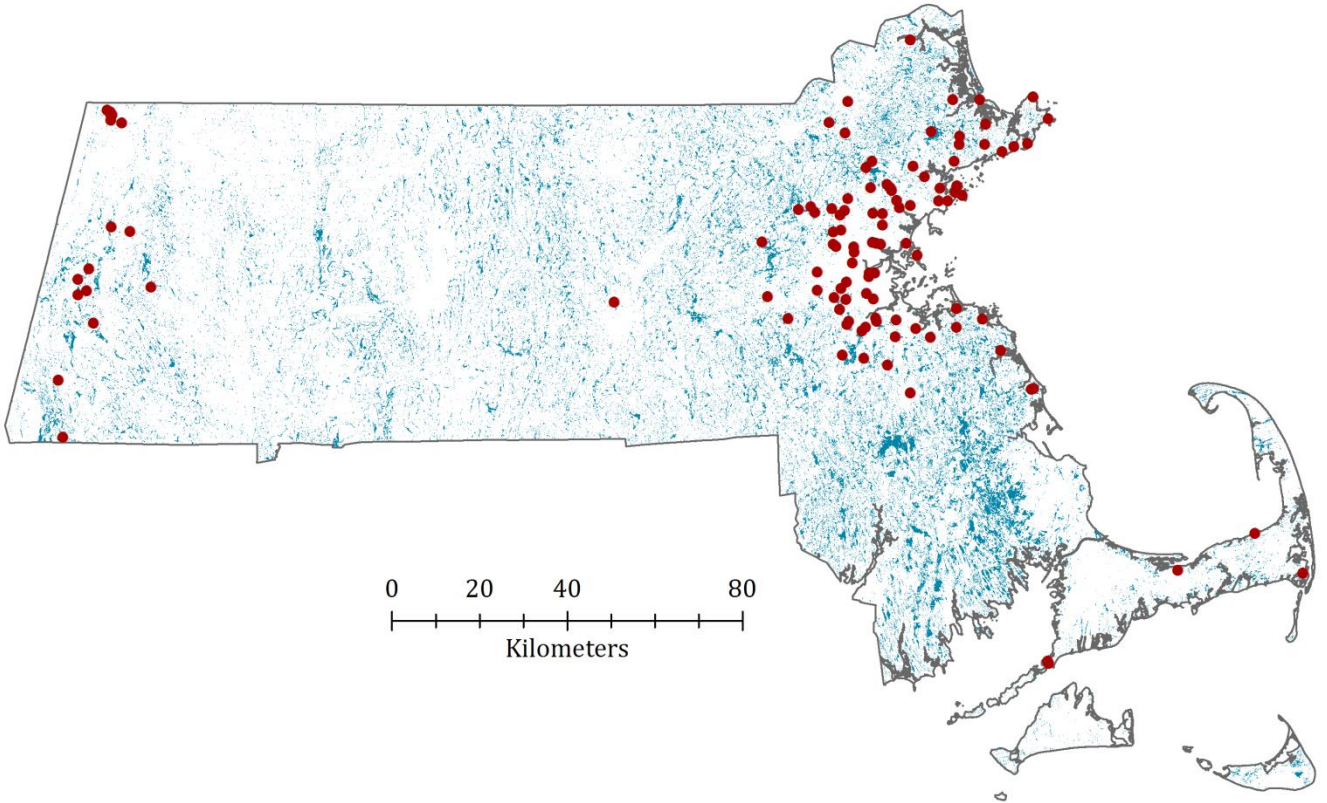


Figure 2.7. Wetland habitats are shown in blue. Locations where *M. rubra* was discovered are indicated by red circles.

Tables

Table 2.1. Characteristics of sample sites in Massachusetts. The amounts of water and of four habitat types were quantified within a circle of 300 m radius drawn around the geographic center of each survey.

	Minimum	1 st quartile	Median	3 rd quartile	Maximum
Wetland habitat (ha)	0	1.51	4.32	7.85	27.7
Developed habitat (ha)	0	1.10	5.25	11.94	25.01
Water (ha)	0	0	0.31	2.06	14.26
Forested habitat (ha)	0	4.87	9.05	14.08	28.26
Open habitat (ha)	0	0.16	1.61	4.89	22.90
Search distance (m)	5	150	350	705	2400
Precipitation in warmest quarter (mm)	233	255	260	280	339
Maximum temperature (degrees C)	23.7	27.2	27.8	28.1	29.4

	Number of sites	
	Present	Not present
<i>Fallopia japonica</i>	88	396
Wetland vegetation	367	117
Facultative wetland vegetation	177	307

Table 2.2. Ranking of models to describe the relationship between site characteristics and discovery of *M. rubra*. Identification of the invaded zone is based on subjective judgment about where the ant has arrived. For brevity, models are identified by addition or subtraction of predictors from a base model that includes as predictors ln(wetland), ln(water), ln(developed land), ln(meters surveyed), presence of *F. japonica*, presence of obligate wetland plants, and presence of facultative wetland plants. For each model, the table shows the number of estimated parameters (K), the AIC value, the difference between the AIC value and the lowest AIC value (Δ), and the AIC model weight.

Predictors included or excluded	K	AIC	Δ	weight
Base model	8	295.42	0.00	0.29
- facultative plants	7	295.74	0.33	0.25
+ precipitation in warmest quarter	9	297.43	2.02	0.11
- facultative plants, + precipitation in warmest quarter	8	297.84	2.43	0.09
+ precipitation in warmest quarter, + maximum temperature	10	298.44	3.03	0.06
- facultative plants, - ln(meters)	6	298.88	3.46	0.05
+ precipitation in warmest quarter, + ln(forest)	10	299.60	4.18	0.04
+ precipitation in warmest quarter, + ln(open)	10	299.60	4.19	0.04
- ln(meters), + precipitation in warmest quarter	8	300.05	4.63	0.03
- facultative plants, -ln(water)	6	300.77	5.35	0.02
- ln(water), + precipitation in warmest quarter	8	301.91	6.50	0.01
- facultative plants, - <i>F. japonica</i>	6	302.54	7.12	0.01
- <i>F. japonica</i> , + precipitation in warmest quarter	8	302.75	7.34	0.01
- ln(wetland), + precipitation in warmest quarter	8	303.67	8.25	0.00
- obligate plants, - facultative plants	6	303.86	8.44	0.00
- ln(wetland), - facultative plants	6	304.63	9.21	0.00
- obligate plants, + precipitation in warmest quarter	8	305.32	9.90	0.00
- facultative plants, - ln(developed)	6	310.91	15.49	0.00
- ln(developed), + precipitation in warmest quarter	8	314.88	19.46	0.00

Table 2.3. Ranking of models to describe the relationship between site characteristics and discovery of *M. rubra*. The invaded zone was assumed to include all locations within 5 km of any place where the ant has been found. For brevity, models are identified by addition or subtraction of predictors from a base model that includes as predictors ln(wetland), ln(water), ln(developed land), ln(meters surveyed), presence of *F. japonica*, presence of obligate wetland plants, and presence of facultative wetland plants. For each model, the table shows the number of estimated parameters (K), the AIC value, the difference between the AIC value and the lowest AIC value (Δ), and the AIC model weight.

Predictors included	K	AIC	Δ	weight
- facultative plants	7	316.00	0.00	0.29
Base model	8	316.66	0.67	0.21
- facultative plants, + precipitation in warmest quarter	8	318.11	2.11	0.10
+ precipitation in warmest quarter, + maximum temperature	10	318.23	2.23	0.10
+ precipitation in warmest quarter	9	318.74	2.75	0.07
- facultative plants, - ln(meters)	6	319.78	3.79	0.04
- facultative plants, - ln(water)	6	320.54	4.54	0.03
+ precipitation in warmest quarter, + ln(open)	10	320.82	4.83	0.03
+ precipitation in warmest quarter, + ln(forest)	10	320.86	4.86	0.03
- ln(wetland), - facultative plants	6	320.93	4.93	0.02
- facultative plants, - <i>F. japonica</i>	6	321.12	5.12	0.02
- ln(meters), + precipitation in warmest quarter	8	321.71	5.72	0.02
- ln(wetland), + precipitation in warmest quarter	8	321.88	5.88	0.02
- ln(water), + precipitation in warmest quarter	8	322.88	6.88	0.01
- <i>F. japonica</i> , + precipitation in warmest quarter	8	323.02	7.02	0.01
- obligate plants, - facultative plants	6	325.56	9.56	0.00
- obligate plants, + precipitation in warmest quarter	8	328.03	12.04	0.00
- facultative plants, - ln(developed)	6	333.11	17.12	0.00
- ln(developed), + precipitation in warmest quarter	8	337.26	21.26	0.00

Table 2.4. Ranking of models to describe the relationship between site characteristics and discovery of *M. rubra*. The invaded zone was assumed to include all locations for which the estimated probability of arrival under Bayesian analysis was > 0.5 . For brevity, models are identified by addition or subtraction of predictors from a base model that includes as predictors $\ln(\text{wetland})$, $\ln(\text{water})$, $\ln(\text{developed land})$, $\ln(\text{meters surveyed})$, presence of *F. japonica*, presence of obligate wetland plants, and presence of facultative wetland plants. For each model, the table shows the number of estimated parameters (K), the AIC value, the difference between the AIC value and the lowest AIC value (Δ), and the AIC model weight.

Predictors included	K	AIC	Δ	weight
+ precipitation in warmest quarter	9	267.30	0.00	0.34
+ precipitation in warmest quarter, + maximum temperature	10	267.59	0.29	0.29
+ precipitation in warmest quarter , + $\ln(\text{forest})$	10	269.09	1.79	0.14
+ precipitation in warmest quarter , + $\ln(\text{open})$	10	269.29	1.99	0.12
- obligate plants, + precipitation in warmest quarter	8	271.82	4.51	0.04
- <i>F. japonica</i> , + precipitation in warmest quarter	8	272.27	4.96	0.03
Base model	8	272.67	5.36	0.02
- $\ln(\text{meters})$, + precipitation in warmest quarter	8	274.23	6.93	0.01
- facultative plants, + precipitation in warmest quarter	8	274.41	7.11	0.01
- $\ln(\text{water})$, + precipitation in warmest quarter	8	278.99	11.69	0.00
- facultative plants	7	280.89	13.59	0.00
- $\ln(\text{wetland})$ + precipitation in warmest quarter	8	281.74	14.44	0.00
- obligate plants, - facultative plants	6	284.20	16.90	0.00
- facultative plants, - <i>F. japonica</i>	6	287.14	19.84	0.00
- facultative plants, - $\ln(\text{meters})$	6	289.88	22.58	0.00
- facultative plants, - $\ln(\text{water})$	6	290.27	22.97	0.00
- $\ln(\text{wetland})$, - facultative plants	6	297.03	29.72	0.00
- $\ln(\text{developed})$, + precipitation in warmest quarter	8	300.55	33.25	0.00
- facultative plants, - $\ln(\text{developed})$	6	311.11	43.81	0.00

Table 2.5. Estimated coefficients for the model from Table 2.4 with highest support. *Z* scores and *P* values are also shown, but model selection was based on AIC comparisons.

Parameter	mean \pm s.e.	<i>Z</i> score	<i>P</i>
Intercept	0.02 \pm 2.14	0.011	0.99
ln(wetland habitat + 0.1)	0.54 \pm 0.14	3.838	< 0.001
ln(developed habitat + 0.1)	0.75 \pm 0.14	5.192	< 0.000001
ln(water + 0.1)	0.37 \pm 0.11	3.568	< 0.001
<i>F. japonica</i>	1.07 \pm 0.41	2.612	< 0.01
wetland vegetation	1.00 \pm 0.40	2.514	< 0.05
facultative wetland vegetation	1.14 \pm 0.39	2.961	< 0.01
ln(length of search path in meters)	0.40 \pm 0.14	2.942	< 0.01
precipitation in warmest quarter	-0.019 \pm 0.007	-2.648	< 0.01

Table 2.6.

Summary of Bayesian models. The Gelfand-Ghosh statistic (D) is the sum of a goodness-of-fit component (G) and a penalty (P). Columns represent covariates (see text). For each model, included predictors are indicated by + if the 95% credibility intervals for the coefficient was entirely above 0, - if the 95% credibility interval was entirely below 0, or NS if the 95% credibility interval included 0; if blank, the covariate was not included in the model.

Rank	D	G	P	Wetland	Developed land	Water	Forest	Open	meters	Wetland plants	Facultative plants	<i>F. japonica</i>	Rain	Temp
1	28.6	19.2	9.4	+	+	+			NS	+	+	+		-
2	29.6	19.9	9.7	+	+	+			NS	+	+	+	NS	
3	29.7	20.0	9.7	+	+	+		NS	+	+		+	-	
4	30.8	20.7	10.1	+	+	+				+	+			
5	30.9	20.7	10.2	+	+	+			+	+		+		
6	31.1	20.9	10.2	+	+	+	NS		+	+		+	NS	
7	31.2	21.0	10.3	+	+	+			NS	+	+			-
8	31.5	21.1	10.4		+	+			NS	+	+	+		NS
9	31.5	21.2	10.4		+	+		NS	+	+		+	-	
10	31.8	21.3	10.5	+	+	+			NS	+				NS
11	32.4	21.8	10.7	+	+				NS	+	+	NS		NS
12	33.3	22.3	11.0	+	+	+			+	+		+	-	
13	33.4	22.4	11.0	+		+			NS	+	NS	+		NS
14	33.6	22.6	11.1	+	+	+			+	+				NS
15	33.7	22.7	11.0	+	+	+			NS	+		NS	NS	NS
16	34.0	22.8	11.2	+	+	+		NS	+			+		NS

Table 2.7. Estimated coefficients for the top-ranked model from Table 2.6.

Parameter	mean	s.d.	95% credibility interval
α (Intercept)	-2.14	5.30	-13.2 to 7.7
ln(wetland habitat)	4.73	2.27	1.46 to 9.91
ln(developed habitat)	6.29	2.26	2.86 to 11.48
ln(water)	2.91	1.55	0.65 to 6.38
<i>F. japonica</i>	8.03	4.72	0.04 to 18.67
obligate wetland vegetation	7.33	4.08	0.09 to 16.08
facultative wetland vegetation	9.00	5.06	0.70 to 20.54
ln(length of search path in meters)	2.48	1.61	-0.43 to 5.92
max temperature of hottest month	-1.17	0.60	-2.38 to -0.06

Chapter 3

Colony and population structure of the invasive ant *Myrmica rubra* in southern New England revealed by bioassays of aggression

Abstract

Several notorious invasive ants have unicolonial populations, which lack colony boundaries, or are characterized by large supercolonies with many mutually non-aggressive nests; these characteristics may facilitate the spread of exotic ants. We used bioassays of aggression to determine the colony and population structure of the invasive ant *Myrmica rubra* L. at eight sites in southern New England. Aggressive responses showed that populations at all sites were multicolonial, with little to no aggression among different nests within colonies, but with high rates of fighting when ants from different colonies were mixed. The largest colonies can be considered supercolonies, i.e. expandable networks of nests distributed across areas exceeding a hectare. To analyze patterns of aggression, we used Bayesian hierarchical models accounting for variation in aggressiveness among colonies and variation in outcome across replicate assays using the same pair of colonies. We found no evidence that aggressive responses depended on distance of separation between colonies or whether colonies were neighbors. Supercolonies were constrained by intraspecific competition and habitat variation and did not achieve the vast sizes seen in introduced populations of some other invasive species.

Introduction

Nestmate discrimination in ants is profoundly intertwined with colony and population structure. Ant biologists have traditionally distinguished between “multicolonial” populations, with strict colony boundaries reinforced by aggressive behavior among the workers, and “unicolonial” populations, in which workers readily accept unfamiliar non-nestmates and conspecific workers and reproductives move freely among nests (Hölldobler and Wilson 1990, Bourke and Franks 1995). In recent years, this view has

been modified by the discovery of colony limits within populations previously thought to be unicolonial. When examined over a large enough spatial scale, some of these ants turn out to have genetically distinct “supercolonies,” some spanning vast distances, whose workers are aggressive towards other supercolonies of the same species (Vogel et al. 2009). Instead of having only two dichotomous structures, ant populations are now seen as lying along a continuous spectrum based on the degree of exchange of workers and queens among nests (Krushelnycky et al. 2010). At one extreme, each colony lives in a single nest that is defended intraspecifically, possibly with a single queen. At the other extreme, colony boundaries are completely lacking, but that structure may be rare (Helanterä et al. 2009). In between are populations with multiple supercolonies, each of which has multiple nests and queens and an indefinite potential to spread by budding (Helanterä et al. 2009, Moffett 2012).

Unicoloniality and large supercolonies are said to be over-represented among invasive ants, although formal comparative studies have not been conducted (Holway et al. 2002). Supercolonies have many queens, which can often mate in or near the nest, and they reproduce by budding; therefore, colony fragments that are accidentally transported are likely to contain reproductives, do not need to find mates from other nests, and can readily spread into new habitats. The Argentine ant, *Linepithema humile*, forms supercolonies in both native and introduced populations, but they are orders of magnitude larger where the ant has been introduced than within Argentina (Suarez et al. 2008). The reasons for this contrast are a matter of debate (Suarez et al. 2008, Helanterä et al. 2009, Vogel et al. 2009, Moffett 2012). Among other major ant invaders, the polygyne (multiple queen) form of the fire ant *Solenopsis invicta* is unicolonial, and *Wasmannia auropunctata*, *Anoplolepis gracilipes*, and *Lasius neglectus* have supercolonies, but the monogyne (single-queen) form of *S. invicta* is multicolonial and territorial (Holway et al. 2002, Helanterä et al. 2009). The lack of territorial boundaries within large supercolonies has been hypothesized to promote the invasiveness of exotic ants because release from the costs of fighting could allow the ants to achieve higher worker densities (Holway et al. 1998, Tsutsui and Suarez 2003). This hypothesis remains speculative, due to a lack of supporting evidence (Adams 2016).

The process by which ants decide whether to accept nestmates is well understood: ants compare odors on the surface of another individual to a learned template of their own colony odor and choose whether to attack based on the degree of similarity (Hölldobler and Wilson 1990, Vander Meer and Morel 1998, Sturgis and Gordon 2012). Ant nestmate recognition is not as simple as a binary outcome; it varies along a gradient depending on factors such as distance and familiarity (Sturgis and Gordon 2012). Within colonies, individuals differ in their propensity to attack non-nestmates due to individual variation in odor (Newey 2011) and attack thresholds (Reeve 1989), task group (Sturgis and Gordon 2013), and previous experience (van Wilgenburg et al. 2010).

Assays of aggression between workers from different nests are key methods for determining colony and population structure (Breed 2003). The level of aggression can be used to determine which nests are part of the same colony (e.g., Levings and Adams 1984, Buczkowski 2012), to find the limits of large supercolonies (e.g., Giraud et al. 2002), and to compare the population structure of invasive ants in native and introduced populations (e.g., Suarez et al. 1999). But it has become clear that interpretation of the results requires knowing whether the two nests used in the assay are members of the same colony. For example, aggression assays for *L. humile* showed that workers taken from nests that are at least several hundred meters apart are much less likely to fight in introduced populations than in native populations, prompting some authors to postulate that the ant underwent a change in social structure during the invasion process (Giraud et al. 2002, Tsutsui and Suarez 2003). But later studies suggested that the lower aggression in introduced populations of this species is because most of the tests were done between nests that were members of the same colony (Vogel et al. 2009).

We used assays of aggression as a window on the colony and population structure of the invasive ant *Myrmica rubra* L. within its invaded range. Some authors have considered *M. rubra* to be an invasive species that forms unicolonial populations (van der Hammen et al. 2002). However, Garnas et al. (2007) found that, within an invaded part of Maine, workers from nests separated by 10 m or more often showed high levels of aggression towards one another, indicating a multicolonial structure. Furthermore, the degree of aggression within a site increased linearly with the distance between nests. There are at least

two possible causes of such a pattern. First, an association between distance and aggression could arise simply because the probability of that two nests are members of different colonies is higher for nests that are farther apart; there may be no other effect of distance on aggression. Second, it could be that colonies that are farther apart are more aggressive to each other because they are more dissimilar genetically (e.g., Beye et al. 1998) or because neighbors become less hostile to each other after repeated contact (Langen et al. 2000, Tanner and Keller 2012). We attempted to separate these two possibilities. We first asked whether assays of aggression can be used to determine colony identities in *M. rubra* and whether populations are multicolonial at eight locations in Massachusetts. The state of Massachusetts includes the sites where this ant was first discovered in North America (Wheeler 1906, Groden et al. 2005, Wetterer and Radchenko 2011). We then asked whether aggression between colonies is an increasing function of the distance of separation.

Aggression towards non-nestmates can also vary as a result of familiarity. Behavioral responses to neighboring colonies can be categorized into two main types. Once the colony boundaries are settled, neighbors may lower their aggression to reduce fighting costs, a pattern known as the “dear enemy” phenomenon (Thomas et al. 1999, Langen et al. 2000). Alternatively, neighbors may be treated more aggressively than strangers, since they are more likely to pose substantial threats (e.g., Gordon 1989, Thomas et al. 2007, Newey et al. 2010). Aside from strategic explanations, there are also functional and mechanistic reasons for ants being able to distinguish neighbors from strangers. Neighbors may be more closely related (Beye et al. 1998) or may share odors due to the use of similar nesting materials (Heinze et al. 1996). With repeated exposure, neighbors may habituate to each other’s odors (Langen et al. 2000) or may become even more hostile (Thomas et al. 2006, van Wilgenburg et al. 2010). We asked whether the degree of aggression between ants from different nests depended on whether the nests were from neighboring or non-neighboring colonies.

In studies on social insect recognition, it is common to use particular colonies in multiple tests with other colonies (e.g., Pirk et al. 2001, Katzerke et al. 2006, van Wilgenburg 2007). Because colonies vary in aggressiveness, replicates using the same colony are likely to have correlated outcomes and failing

to take this into account during the analysis can result in pseudoreplication. Hierarchical models that match the nested structure of the data (Zuur et al. 2009) are well suited for this kind of analysis but are not often used in studies on social insect behavior (but see Zinck et al. 2008, Gill et al. 2012). We used Bayesian hierarchical models, estimating random effects at the level of the nest and the assay to account for variation in aggression and overdispersion in counts of fighting ants (Gelman and Hill 2007).

Methods

Study species.

Originating in Eurasia, *Myrmica rubra* L. is a Palearctic temperate ant species that was introduced to North America by the early 20th Century (Wheeler, 1906). By 2011, *M. rubra* had been found in five southeastern Canadian provinces (New Brunswick, Nova Scotia, Ontario, Prince Edward Island, and Quebec), six northeastern U.S. states (Maine, Massachusetts, New Hampshire, New York, Rhode Island, and Vermont), and one northwestern state, Washington (Wetterer and Radchenko 2011). *M. rubra* has reddish-brown monomorphic workers that are 4 - 5 mm long. Due to their preferred nest locations, aggressiveness, and painful sting, these ants may become a nuisance in residential and agricultural areas, as well as in natural settings (Grodén et al. 2005).

This species has been well studied in Europe; however, it has attracted limited attention from researchers in the invaded range. *M. rubra* may reach high densities in parts of North America, such as 1.24 nests/m² at Acadia National Park in Maine, exceeding those observed in its native range (Grodén et al. 2005). Colonies of *M. rubra* are highly polygynous (with multiple queens) and polydomous (with multiple nests; Seppa and Pamilot 1995, Walin et al. 2001, Garnas 2004). The number of queens per nest changes dynamically, which may have effects on population ecology and seasonal fluctuation of density (Elmes 1980). Two main reproductive mechanisms have been recorded: mating flights (Boomsma and Leusink 1981) and budding (Elmes 1980). Little is known about reproduction of this species in its invaded range, but Hicks (2012) reported male dispersal swarms in Newfoundland. Limited research in

North America using bioassays of aggression has revealed that *M. rubra* population is polygynous with a multicolonial organization.

Study Sites

During a survey of *M. rubra* in southern New England (Chapter 1), we selected eight sites in which workers were seen across a span of at least 200 m. The two first reports of *M. rubra* in North America were from Forest Hills (Boston) and Woods Hole (Falmouth), Massachusetts. We selected one site, the Boston Nature Center, immediately next to Forest Hills. *M. rubra* was rediscovered in Woods Hole, but did not occupy a large enough area for use in this study. Other sites were selected spanning the greater Boston invasion area (Daniel Webster Sanctuary, Marshfield; Neponset River Reservation, Milton; Pope's Pond, Milton; Marblehead Neck Wildlife Sanctuary, Marblehead) and much of the Berkshires invasion area (War Memorial Park, Lenox; Williams College, Williamstown). Study sites were each separated by a minimum of 2.5 km.

Assay of aggression.

Preliminary trials established that workers collected from different nests of *M. rubra* often fight when mixed in small containers. Combining 50 workers from each nest produced results that were highly repeatable; however, the proportion of ants fighting was sometimes significantly lower when the ants were brought to the lab and tested over the next few days than when assays were conducted in the field on the day of collection. Behaviors observed included immediate investigation without aggression, lunging after brief investigation, and use of the mandibles to grasp the legs, antennae, or petiole of another ant.

Based on these preliminary trials, we chose the following protocol. All assays were conducted in the field on the day the ants were collected. Nests were located and marked with flags showing unique identifiers. In order to collect workers that respond actively to alarm, and to avoid inhaling spores or soil, an index card was placed next to the nest and a mouth aspirator was used to remove workers that climbed onto the card. The collected workers were kept in the shade in ventilated containers (17 cm x 12 cm x 6

cm) with moist paper towels. For each assay, 50 workers from each of two nests were removed with an aspirator and placed in a plastic container (11 cm x 11 cm x 3.5 cm) at the same time. The walls of the container were coated with Fluon (polytetrafluoroethylene; Northern Products Inc., Woonsocket RI), which forms a slippery surface that the ants cannot climb. The ants were observed continuously during a five-minute test period and the number fighting was recorded. Ants typically fought in pairs or trios, recognized by grappling and holding with the mandibles. Because individual ants may fight more than once, which could inflate the estimate of the proportion fighting, as soon as it was clear that any ants were fighting, they were counted and removed by aspiration. Thus, we can report the proportion of ants out of 100 that fought during the 5 min assay. All tests were blind: one person counted out 50 workers from each of two nests and another person, who did not know the identity of the nests, conducted the assay. After each assay, containers were cleaned with 90% ethanol and then with distilled water to eliminate residual chemical cues. No workers were used in more than one assay. Assays were conducted in the shade.

Delineating colony boundaries.

We used assays of aggression among many pairs of nests to delineate colony boundaries in detail at two locations, in Framingham and Milton, Massachusetts. At each site, selected nests with large worker populations were flagged with identifying numbers in a region spanning approximately 250 by 100 m. For this part of the study, the number of workers used per assay varied from approximately 40 to 200. Pairs of nests for which no ants fought during 5 minutes of continuous observation were judged to be members of the same colony. Many of these inferences were tested further with standardized numbers through the assays described in the next section. As information accumulated on the extent of each colony's territory, additional nests were selected to identify the approximate positions of boundaries. A Delorme GPS unit (Earthmate PN-40) was used to record the GPS coordinates of each nest.

Experimental design.

The eight field sites are shown in Fig. 3.1. At each location, a focal nest was selected from which hundreds of ants could be collected. Ants were also collected from many other nests at various distances from the focal nest. We conducted preliminary assays of aggression between the focal nest and other nests, and among pairs of the other nests. This allowed us to identify neighboring and non-neighboring colonies as well as to locate nests that were in the same colony as the focal nest.

We then conducted a standardized series of assays of aggression between workers from each focal nest and several categories of ants: (a) a second set of ants from the same nest; (b) ants from a second nest within the same colony as the focal nest; (c) ants from a neighboring colony; (d) ants from a non-neighboring colony in the same habitat patch; (e) ants from a distant colony (5 km to 25 km) in a separate habitat patch. We used two tactics to improve the ability to detect the effects of distance or other predictors on the degree of aggression. (1) We used a repeated measures design, using each focal colony in assays with multiple other nests. (2) We conducted replicate assays (usually two) for each pair of nests. This design improves the capacity to determine whether variation in aggression is due to the predictors of interest or is simply due to inherent differences in aggression among colonies or to background variability in outcomes among replicate assays.

To avoid pseudoreplication, we used a statistical model (described below) that matched the nested structure of the data. To avoid possible problems in interpretation associated with the use of ordinal categories of behavior (Michell 1986), our tests were based on the proportion of ants fighting. To evaluate model adequacy, residuals were plotted against other variables, such as day of the year and temperature.

We used multi-level logistic regression to analyze variation in aggression. Because the number of focal nests was small, classical hierarchical regressions are not reliable (Gelman and Hill 1995); therefore, Bayesian methods were used. We fitted all models in OpenBUGS, an open source package for Bayesian statistical analysis that uses Markov chain Monte Carlo (MCMC) methods to sample from the posterior probability distribution (Lunn et al. 2009).

Statistical models.

We carried out three analyses all of which aim to disentangle the relationships between aggression level, distance and colony identity.

(1) Effects of distance between colonies. For this analysis, we used only assays ($n = 50$) for which the ants came from two different colonies. As reported below, colony identities could usually be determined unambiguously. We asked whether the proportion of ants fighting depended on distance (m) between the colonies or on the temperature (degrees C) at the time of the assay. Distances were log-transformed to spread them more evenly, avoiding samples that would have high leverage.

We modeled f_i , the number of fighting ants in assay i , as binomially distributed given the probability p_i that an individual fights and the sample size of 100 ants:

$$f_i \sim \text{binomial}(p_i, 100)$$

The logit of p_i , which is equal to $\ln(p_i/(1-p_i))$, is the sum of several influences.

$$\text{logit}(p_i) = \alpha_0 + \alpha\text{Focal}_{j[i]} + \alpha\text{Opponent}_{k[i]} + \beta_1 \times \text{Temperature}_i \quad (1)$$

where $\alpha\text{Focal}_{j[i]}$ and $\alpha\text{Opponent}_{k[i]}$ are random effects at the level of the focal nest $j[i]$ and the second (opponent) nest $k[i]$ used in the i th assay, and Temperature_i is the temperature in degrees C at the time of the assay. The random effects, which account for differences among nests in the propensity to fight, are modeled as normally distributed:

$$\alpha\text{Focal}_{j[i]} \sim N(0, \sigma_{\text{Focal}}^2) \text{ for the 8 focal nests indexed by } j = 1, 2, \dots, 8 \quad (2)$$

$$\alpha\text{Opponent}_{k[i]} \sim N(0, \sigma_{\text{Opponent}}^2) \text{ for the opponent nests, indexed by } k$$

Distance between the nests was included as a predictor in some models. This distance is the same for all replicate assays performed with a particular pair of nests, so it enters the model at the level of the opponent nest k .

$$\alpha\text{Opponent}_{k[i]} \sim N(\beta_2 \times \text{Distance}_{k[i]}, \sigma_{\text{Opponent}}^2) \quad (3)$$

As is typical for logistic-binomial models, this model assumes that the probability of fighting for each ant is identical and independent of the probability of fighting for the other ants. However, it is

entirely possible that some ants are more aggressive than others or that fighting by one ant influences fighting by other ants. Either possibility can cause overdispersion; that is, higher than expected variation in the number of ants fighting across replicate assays for the same nest pair. To test for overdispersion, we used posterior predictive checks (Gelman and Hill 1995). In particular, we compared the average standardized residual for the observed data set to the average standardized residual for a large number of data sets simulated using random draws from the posterior distributions of the model parameters. The standardized residual (Dobson 2002) is:

$$\frac{f_i - 100\hat{p}_i}{\sqrt{100\hat{p}_i(1-\hat{p}_i)}} \quad (4)$$

where \hat{p}_i is the estimated probability that an ant fights in the i th assay. We monitored the posterior distribution of the mean of the residuals for the data using \hat{p}_i values estimated by the model during 200,000 MCMC steps. Overdispersion is indicated if p_{MCMC} is small; that is, if the average residual for the data is greater than all but a small proportion of the average residuals for simulated data. When overdispersion was detected, we added random effects, ε_i , at the level of the assay (Gelman and Hill 1995):

$$\text{logit}(p_i) = \alpha_0 + \alpha \text{Focal}_{j[i]} + \alpha \text{Opponent}_{k[i]} + \beta_1 \times \text{Temperature}_i + \varepsilon_i \quad (5)$$

$$\varepsilon_i \sim N(0, \sigma_{\text{Assay}}^2)$$

Inferences were based on 200,000 samples after discarding a burn-in of 50,000 steps. Diagnostic plots consistently confirmed that quantiles stabilized within the first 50,000 steps of the Markov chain and that multiple chains converged.

We fitted models with and without assay-level random effects, and with and without distance between nests or temperature as predictors.

(2) Effects of opponent type. The second analysis asks whether the degree of aggression tended to vary among five categories of assays (total $n = 96$). The two groups of ants were either (a) from the same nest,

(b) from different nests in the same colony, (c) from neighboring colonies, (d) from non-neighboring colonies in the same habitat patch, or (e) from colonies in different habitat patches.

Two models were fitted. For both models, random effects are included for focal nest, opponent nest, and assay. The first model treats the five categories as fixed effects. The proportion of ants fighting is estimated separately for each of the five types of assays by

$$\text{logit}(p_i) = \alpha\text{Category}_{c[i]} + \alpha\text{Focal}_{j[i]} + \alpha\text{Opponent}_{k[i]} \quad (6)$$

where $\alpha\text{Category}_{c[i]}$ is the logit transformation of the mean proportion of ants fighting for each of the five categories of assays, indexed by c , and the other terms are as previously defined. The second model pools the five categories; the mean proportion of ants fighting is the same for all types of assays.

$$\text{logit}(p_i) = \alpha 0 + \alpha\text{Focal}_{j[i]} + \alpha\text{Opponent}_{k[i]} \quad (7)$$

(3) Effects of distance between nests, ignoring colony identities. The third analysis avoids inferences about which nests belong to the same colony and asks whether the degree of aggression among workers from different nests varies with distance between the nests. The model is the same as in expressions (1) to (3) above, but the data set included assays in which both nests were inferred to be from the same colony. Temperature was not included as a predictor.

For all models, we used diffuse normal priors for coefficients, with means set to 0 and tolerances set to 0.001 (Appendix). A weakly informative prior was used for the standard deviations of random effects; specifically, half-normal distributions with means equal to 0 and standard deviations equal to 1. The half-normal distribution prevents negative values for the standard deviation. Adopting a larger standard deviation on the logit scale would imply that the proportion of ants fighting commonly varies from less than 1% to more than 99% among replicate assays for the same pair of nests, which is improbable for most ants. However, we used priors that allow high estimates if they are indicated by the data. Priors that are too diffuse result in overestimating the magnitude of random effects (Gelman and Hill 1995).

For comparison, we also fitted a standard (classical) logistic regression of the proportion of ants fighting versus distance, omitting random effects, using the glm function of the R statistical package, version 3.1.2 (<http://www.R-project.org/>).

Results

Delineating colony boundaries.

At each site, sets of nests were located among which ants were not aggressive. For example, Fig. 3.2 shows a network diagram for assays conducted at a site in Milton, Massachusetts. There were two groups of nests (shown in blue and red) within which ants were non-aggressive (solid lines; no more than one pair of ants fought per assay). When ants from “blue” and “red” nests were mixed, a minimum of 6 ants fought (dashed lines). The mutually non-aggressive nests were spatially clustered (Fig. 3.3). Ants from these nests fought with ants from nest X, which was farther away in the same habitat patch, and with ants collected at more distant sites in Milton (3 km), Dedham (6.7 km), and Framingham (25 km). With few exceptions, aggressive relationships were transitive: if ants from two nests did not fight (e.g., D and O), both groups fought with the same set of other nests (e.g., E, N, and M). The same criteria of consistent, transitive patterns of aggression and spatial clustering were used to decide which nests were members of the same colony at other field sites.

At the Framingham field site, three colonies were identified (Fig. 3.4). Of the nests used in bioassays, ten belonged to colony A, eight to colony B, and 19 to colony C. There were additional nests in each colony that were not used in our assays. The spatial extent of territories varied from 1,630 m² (colony B) to 280 m² (colony C). Ant density varied noticeably within the colony complex. Colonies all bordered areas lacking *M. rubra*. At the Milton study site, only one of three territories was completely delineated (Fig. 3.3). The colony occupied an area of approximately 11,200 m², spanning 150 m along the longest axis. Twelve nests from that colony were confirmed to be mutually non-aggressive. There were a few exceptions. The first occurred when two nests that were close together (9 m) were non aggressive, but

only one of these nests was aggressive towards a distant nest (480 m) in replicate trials. The other occurred when aggression was seen (18 of 100 ants fighting) in only one of three trials for a pair of nests judged to be members of the same colony (90 m separation). More commonly, a single pair of ants fought in tests using ants from different nests that were judged to be members of the same colony.

Statistical models

For all models, it was necessary to include random effects at the level of the assay to account for overdispersion. In other words, the proportion of ants fighting differed more among replicate assays than expected for a binomial distribution. When assay-level random effects were not included, the average standardized residual was much larger than expected under the model (well beyond the 95% c.i.). When random effects were added as in eqn. (5), the standardized residuals for the data were within the 95% c.i. for simulated data. Hereafter, we summarize only models that include random effects at the assay level.

(1) Effects of distance between colonies.

The model was not improved (the DIC value was not reduced) by adding either temperature or distance between nests as a predictor (Table 3.1). The mean and 95% CI (credibility interval) for the coefficients were -0.10 (-0.25 to 0.05) for temperature and -0.03 (-0.24 to 0.19) for log (distance). Plotting numbers of fighting ants with respect to temperature or distance or day of year showed no pattern (Fig. 3.5). Thus, there was no evidence that the amount of aggression depends on the distance between colonies or the temperature at which the assay was conducted. The accepted model includes assay, focal nest, and opponent nest random effects (Table 3.2). For different focal colonies, the pattern of aggression with distance varied greatly (Fig. 3.6). For one colony, the proportion of ants fighting was much lower when challenged by a neighboring colony (16% fighting) than when challenged by ants from a colony that was 7.3 km away (53% fighting). Another colony showed the opposite pattern: 71% fought when mixed with a neighboring colony but only 27% fought when mixed with a colony that was 6.9 km away.

(2) Effects of opponent type.

There was strong support for the model treating the five categories of assays as fixed effects over the pooled model (Table 3.3). When two groups of 50 ants from the same nest were mixed, none fought (mean = 0). When ants from different nests of the same colony were mixed, 1.15% fought (mean distance of separation = 56 m). By contrast, when ants from different colonies were mixed, 46% fought on average, but there was no evidence that the proportion fighting differed for neighboring colonies, non-neighboring colonies, and colonies that were from different habitat patches (combined mean distance = 4.5 km; Fig. 3.7, Table 3.4).

(3) Effects of distance between nests, ignoring colony identities.

The estimated coefficient for the effect of log-transformed distance on the proportion of ants fighting is 0.72 (95% c.i.: 0.43 to 1.04). This suggests that aggression increases with distance. However, including distance as a predictor lowered the DIC value by only 0.9 (Table 3.5). By contrast, a classical model without assay-level random effects suggested strong support for including distance in the model ($\Delta AIC = 16.3$).

Discussion

Bioassays of aggression proved to be a useful tool for delineating colony boundaries in the field. Lack of aggression does not necessarily signify lack of nestmate recognition (Breed 2003). However, when aggressive responses are observed in *M. rubra* populations, they are direct evidence for the existence of distinct colonies. Especially when both non-aggressive and aggressive responses are detected among a set of spatially clustered nests, the most plausible explanation is that some nests share similar colony odors, while other nests do not. We carried out multiple bioassays with different combinations of nests with different colony identities to make sure aggression patterns were transitive. Our results show that this method is easy to adopt in the field and produces consistent results for delineating colony boundaries.

At all sites, the *M. rubra* population consisted of multiple distinct colonies that were mutually hostile. By several criteria, these colonies can be considered “supercolonies”: they contain many nests and are large enough that ants from opposite ends are unlikely to interact directly (Helanterä et al. 2009); the larger ones are likely to contain more than one million workers and they have an indefinite capacity for growth by budding in suitable habitat (Moffett 2012). However, the limits of the term “supercolony” are somewhat arbitrary. The colonies were limited in size by intraspecific competitors and by habitat variation (Figs. 3.3 and 3.4). The ants were abundant in moist woods and wetlands, uncommon in drier forest, and absent from open areas. Even though we did not attempt to locate all nests, the largest colony included more than 40 nests, while the smallest colony contained fewer than ten. Spatial extent also varied greatly, with some territories in isolated habitat patches occupying only a few m², while one colony occupied more than 11,000 m² (Fig. 3.4). Even if they can be called supercolonies, *M. rubra* colonies in Massachusetts have not undergone the vast expansion of colony size seen in introduced populations of some other invasive ants, in which colonies can span hundreds of hectares or kilometers. Examples of the latter include the Argentine ant, *L. humile*, in the United States, Europe, and New Zealand (Suarez et al. 2008); the little fire ant, *Wasmannia auropunctata*, in New Caledonia (Le Breton et al. 2004); or the yellow crazy ant *Anoplolepis gracilipes*, on Christmas Island (Abbott 2006).

We realize that our reasoning about colony identities could be subject to circularity: little aggression was seen among nests judged to be from the same colony, but those judgments were based on lack of aggression. The question then is whether it’s safe to conclude that nests are members of the same colony if no aggression is observed. The sets of non-aggressive nests were spatially clustered and worker traffic was ubiquitous, suggesting the presence of active connections. Furthermore, the presence or absence of aggression was almost always consistent across repeated assays. However, we did occasionally see aggressive responses among workers from nests thought to be part of the same colony. This may indicate that there is imperfect sharing of odors among the many nests of a large colony, so there are occasional recognition errors when ants are artificially transported over distance longer than they

normally move. Alternatively, stress or injury caused by aspirating ants may stimulate some aggression. Yet bioassays of aggression generally produced a coherent and stable view of colony membership.

We found no evidence that aggression depends on the distance between colonies. Superficially, this appears to contradict the findings of Garnas et al. (2007), who reported that aggression increased linearly with distance of separation within sites in Maine. However, Garnas et al. did not determine colony identities and their analysis likely mixed pairs of nests that were members of the same colony with pairs that were members of different colonies. When we also ignored colony identities and random effects, our analysis appeared to show that the proportion of ants fighting increased with the distance of separation between nests. But when the analysis was restricted to assays involving members of different colonies and a hierarchical model was used, there was no support for including distance as a predictor (Table 3.1); indeed, the distance patterns varied greatly for different focal colonies (Fig. 3.6). Thus, the apparent increase in aggression with distance arises simply because nests that are closer together are likely to be members of the same colony. We note one other differences from the methods used by Garnas et al. (2007). We used standardized numbers of ants in our bioassays. Garnas et al (2007) transported colonies in artificial nests, observing fights in a more natural setting, but that method confounds encounter rates with aggressiveness.

We also found no evidence that aggression depended on whether colonies were neighbors (Fig. 3.7). In some species, ants respond differently to conspecifics from neighboring and non-neighboring territories. There are several possible reasons. First, colonies that are closer together may be more closely related genetically, producing more similar odors and hence lower aggression (e.g., Beye et al. 1998, Pirk et al. 2001). Second, ants may habituate to the odor of neighbors after repeated contact, so that neighbors become less aggressive than strangers (Langen et al. 2000, Dimarco et al. 2010, Tanner and Keller 2012). Third, the opposite pattern may arise when ants become sensitized to enemies after repeated contact, with the result that they are more aggressive to familiar neighbors than to unfamiliar strangers (Knaden and Wehner 2003, Thomas et al. 2007, van Wilgenburg et al. 2010, Gill et al. 2012). Other species of territorial ants and termites show no significant contrast (e.g., Adams et al. 2007, Boulay et al. 2007). In

M. rubra, ants from some particular colonies were more aggressive to those from an adjacent territory (e.g., the Framingham colony, Fig. 3.6), while others were less aggressive to their neighbor (e.g., the colony at Pope's Pond, Fig. 3.6). In general, the pattern was highly variable, indicating that colony identity mattered much more than familiarity. During our field studies, we did not see battles between colonies. If the level of aggression needed to maintain territory boundaries is low, ants may not learn to distinguish familiar from unfamiliar competitors.

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Figures

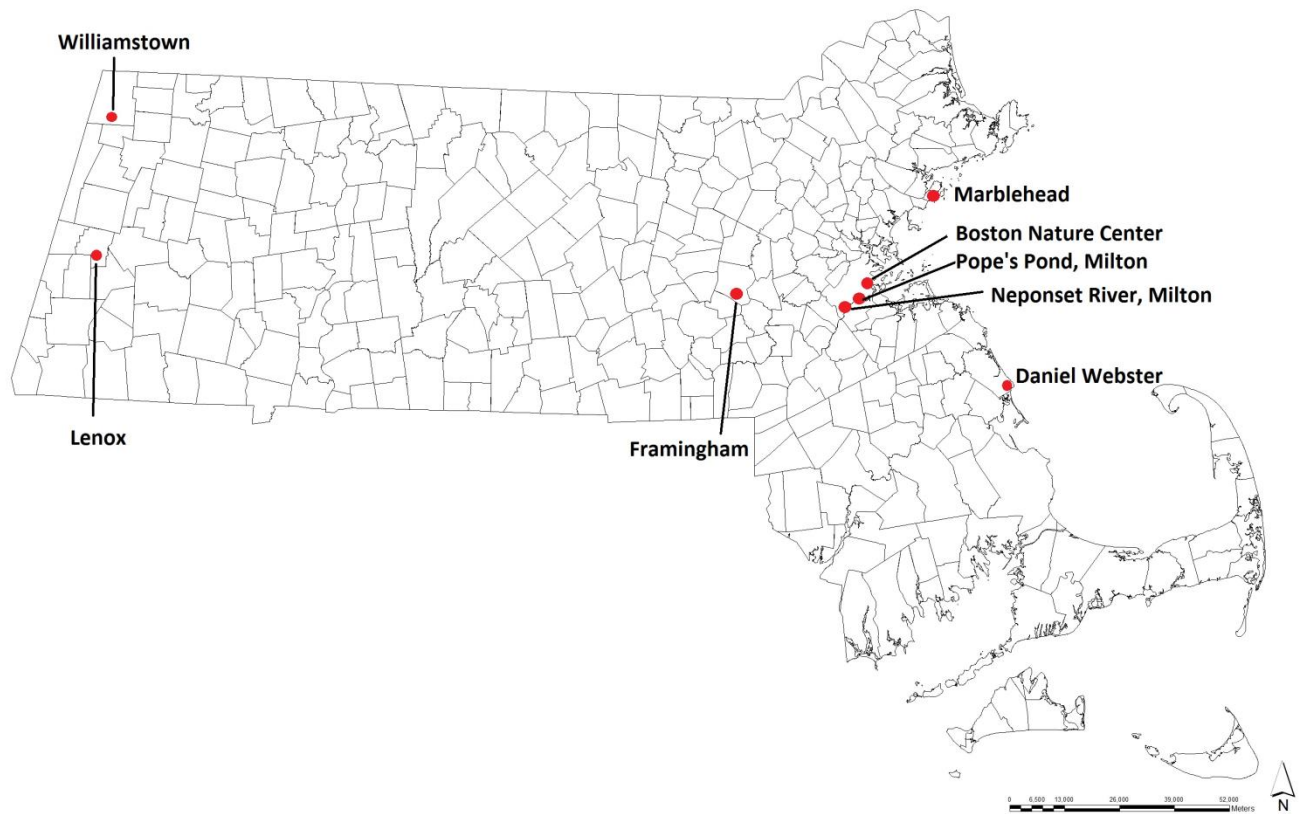


Figure 3.1. Red circles indicate the locations of eight study sites in Massachusetts.

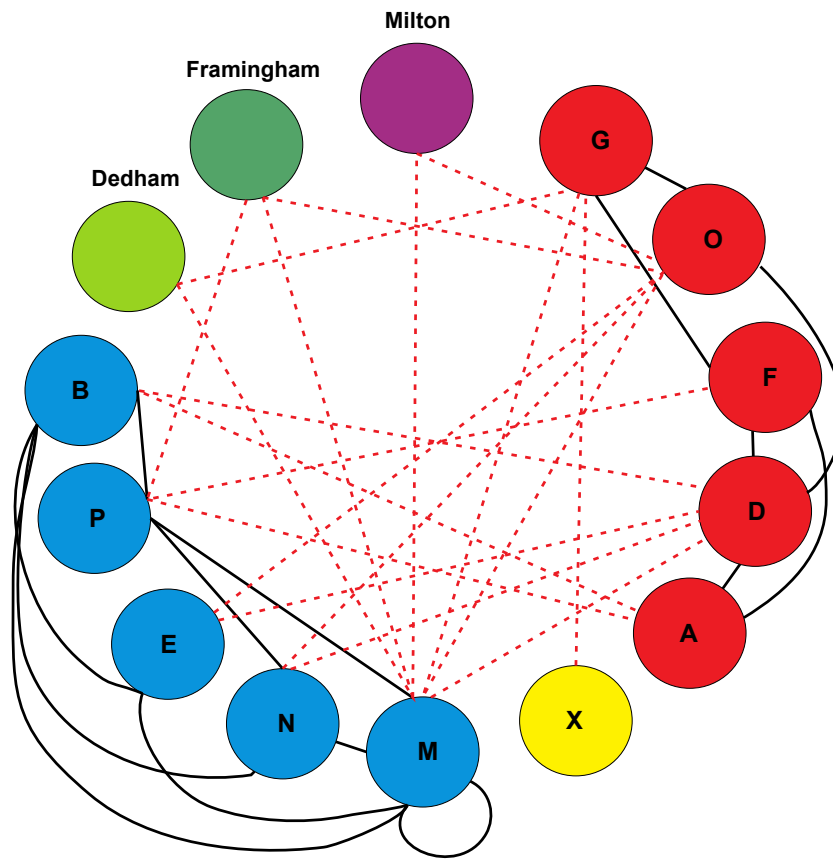


Figure 3.2. Network diagram of aggression among nests at Pope's Pond, Milton, Massachusetts. Each circle represents a separate nest. Solid black lines indicate a maximum of one fighting pair (out of 100 ants) during bioassays of aggression; dashed red lines represent assays in which at least 6 ants fought. Nests at the Milton site are represented by single letters, with blue and red colors indicating two colonies and yellow a single nest from a third colony. Ants were also collected from sites in Dedham, Framingham, and Milton, Massachusetts, which were between 3.2 and 25.1 km away.

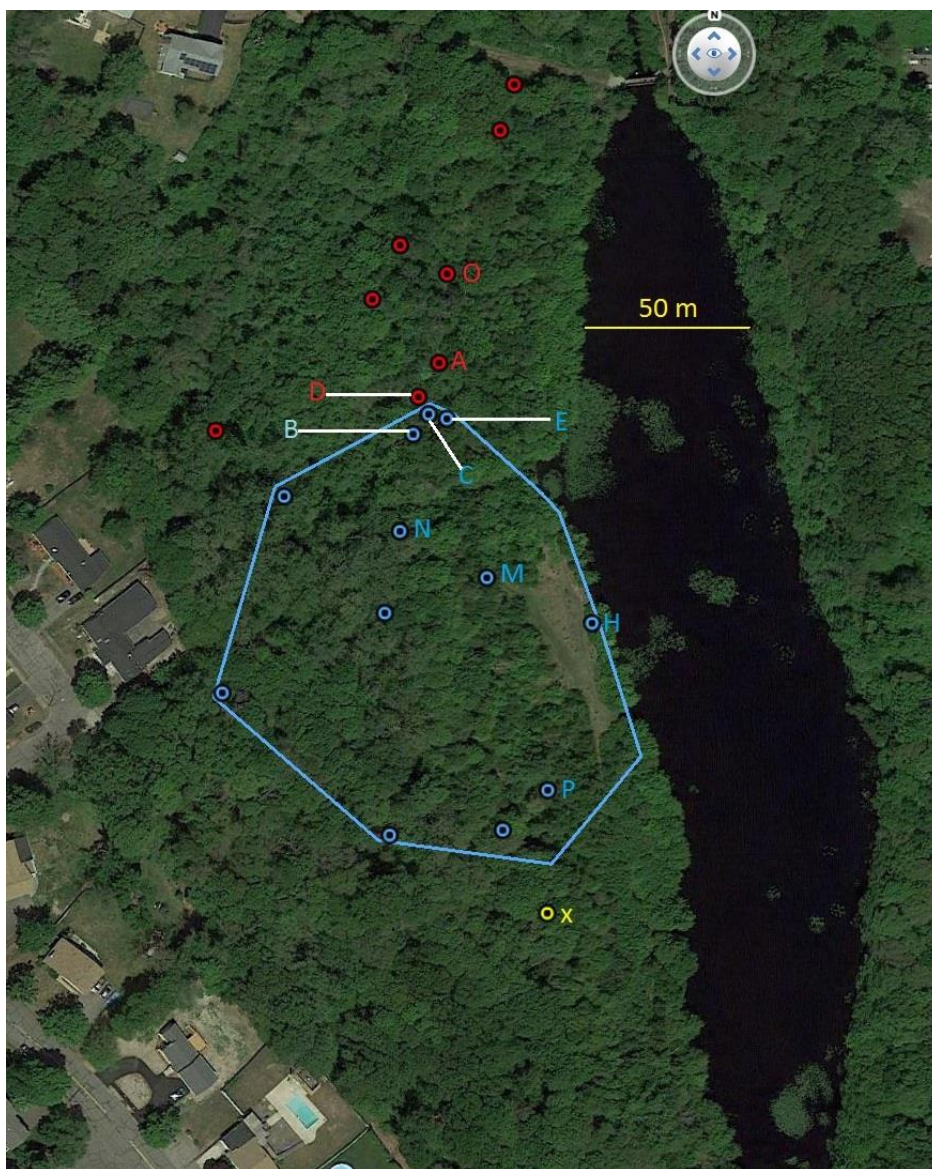


Figure 3.3. Colony boundaries based on aggression assays at Pope's Pond, Milton, Massachusetts. Nests (circles) shown in the same color are mutually non-aggressive. The blue polygons enclose nests inferred to be members of the same colony. The nest labels correspond to those in Fig. 3. 2.

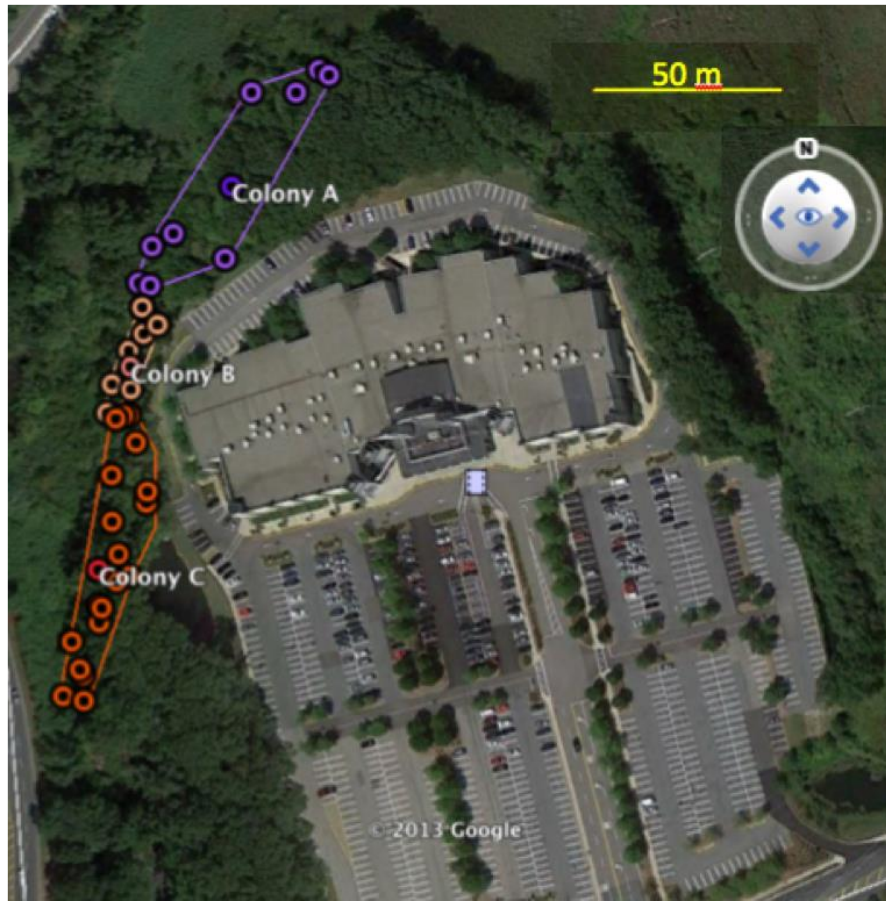


Figure 3.4. Colony boundaries based on aggression assays at the Framingham, Massachusetts study site. Nests (circles) shown in the same color are mutually non-aggressive. Polygons enclose nests inferred to be members of the same colony (Google Image).

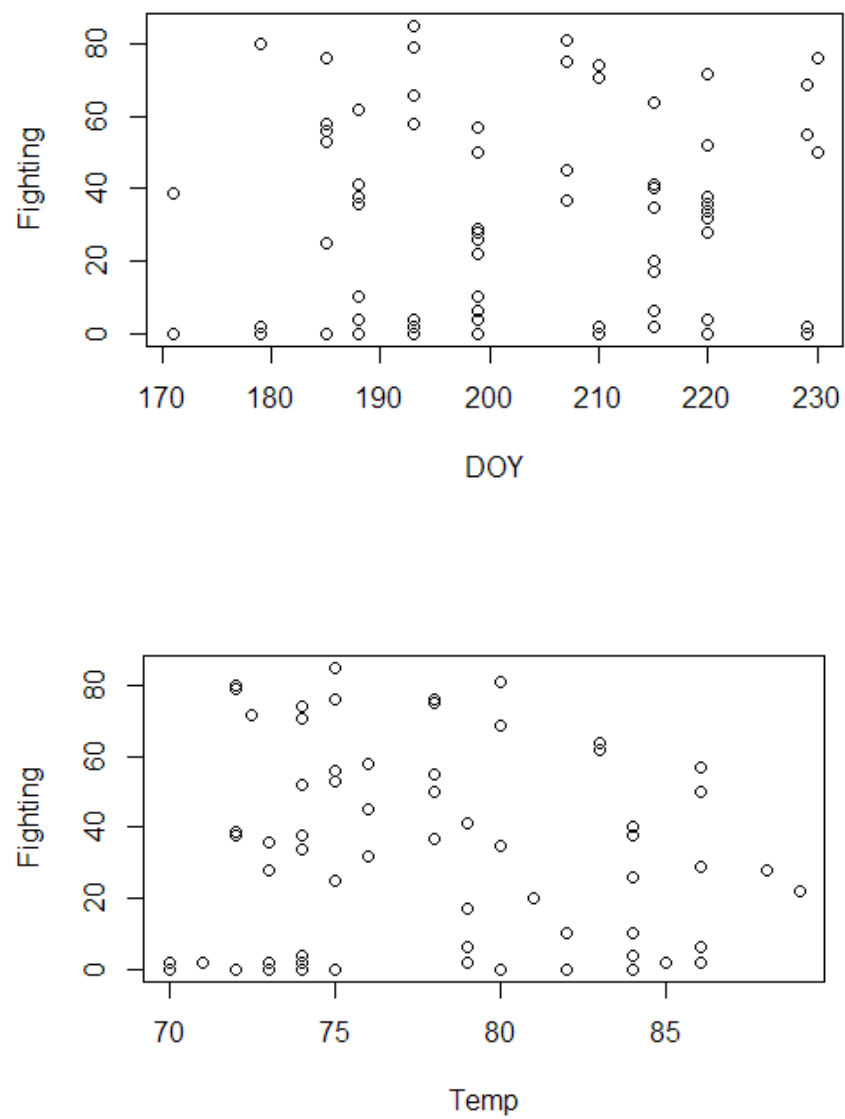


Figure. 3.5. The number of fighting individual plotted against date of the year (top) and temperature (bottom).

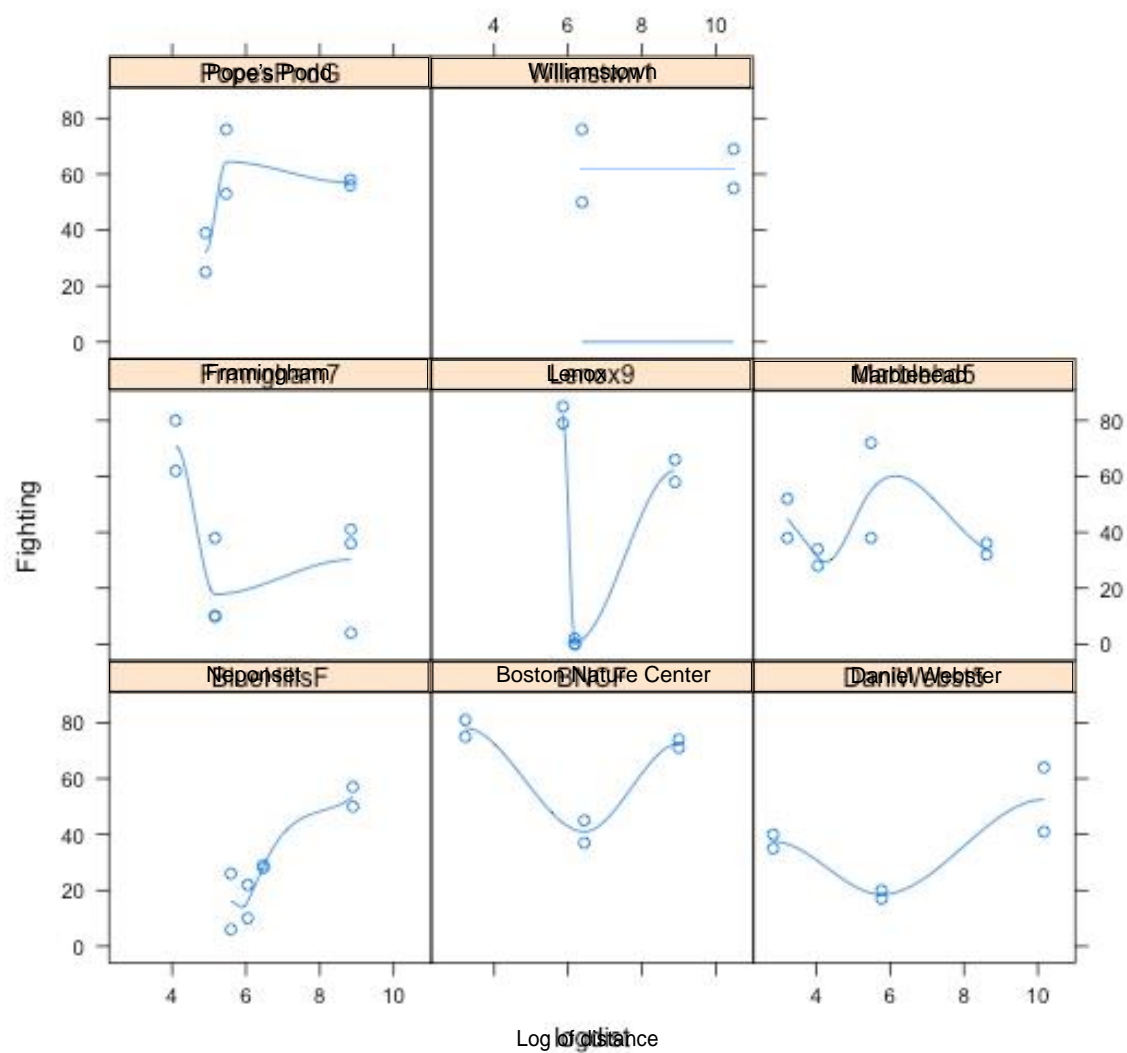


Figure 3.6. The number of fighting ants as a function of distance between nests for the eight study sites.

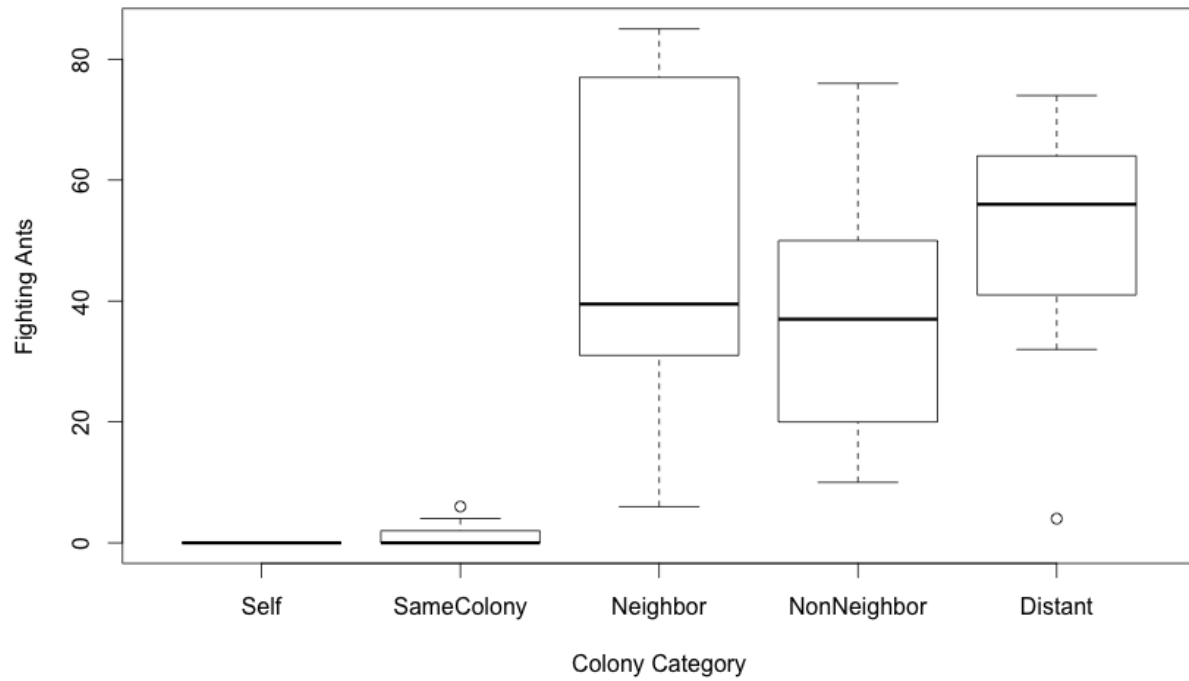


Figure 3.7. Box and whiskers diagram of the number of fighting ants for each colony category.

Tables

Table 3.1. Deviance Information Criterion (DIC) and pD (model dimension) values for three models of the proportion of ants fighting in assays of aggression between ants from different colonies.

Model	pD	DIC	Δ DIC
1. No predictors	41.1	302.4	0
2. Log (Distance between nests)	41.2	302.4	0
3. Temperature	41.7	303.1	0.7

Table 3.2. Summary of the posterior probability distributions for parameters of the accepted model.

	mean	sd	95% c.i.
α_0	-0.14	0.29	-0.70 to 0.45
σ_{Assay}	0.64	0.11	0.46 to 0.90
σ_{Focal}	0.53	0.31	0.04 to 1.24
σ_{Opponent}	0.75	0.20	0.36 to 1.17

Table 3.3. Deviance Information Criterion (DIC) and pD (model dimension) values for two models of the proportion of ants fighting in standardized bioassays.

Model	pD	DIC	Δ DIC
1. Fixed effects for the 5 categories	60.7	424.5	0
2. With the 5 categories pooled	67.5	431.4	6.9

Table 3.4. Summary of the posterior distributions of the probability of fighting in five types of bioassays.

Probability that an ant fights	Median	95% c.i.
1. Ants from the same nest	0	0 to 0.00006
2. Ants from different nests of the same colony	0.006	0.002 to 0.012
3. Ants from neighboring colonies	0.489	0.299 to 0.679
4. Ants from non-neighboring colonies in the same patch	0.366	0.207 to 0.561
5. Ants from different habitat patches	0.518	0.328 to 0.701

Table 3.5. Deviance Information Criterion (DIC) and pD (model dimension) values for two models of the proportion of ants fighting in standardized bioassays

Model	pD	DIC	Δ DIC
Distance between nests	65.1	431.9	0
No predictor	65.6	432.8	0.9