Intercolony Aggression Within and Among Local Populations of the Invasive Ant, *Myrmica rubra* (Hymenoptera: Formicidae), in Coastal Maine

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ABSTRACT *Myrmica rubra* L. was introduced into New England in the early 20th century, and at present, has a patchy distribution in parts of northeastern North America, including records from 31 communities in Maine. *M. rubra* is highly polygynous, and colonies reproduce vegetatively, forming dense local populations where conditions are favorable. Using mobile nests and baited arenas in a series of field aggression bioassays, we tested patterns of internest tolerance within and among local populations on Mt. Desert Island, ME. We found that foragers originating from fragments of the same colony or from neighboring nests retained a high level of intraspecific tolerance over several months, whereas significant intercolony aggression among workers was present between colonies within the same local patch separated by ≈10 m. Within populations, aggression score values were found to increase linearly with internest distance within a site. Aggression was highest between colonies from spatially different populations on the island and was higher still when nests were assayed against colonies at an off-island site 70 km away in Castine, ME. These data strongly suggest a multicolonial organization within and among local populations of *M. rubra* in parts of its introduced range. These findings contradict the loss of intraspecific aggression and unicolonial social structure over large geographic areas that have previously been observed in other invasive ant species, particularly *Linepithema humile* Mayr.

KEY WORDS *Myrmica rubra*, invasive ants, social organization, unicoloniality, intercolony aggression

One of the major mechanisms cited in recent years to account for the widespread dominance of invasive ant species worldwide is the apparent switch to unicoloniality, a population structure characterized by a breakdown of colony boundaries and loss of intraspecific aggression (Wilson 1971, Chen and Nonacs 2000, Tsutsui and Case 2001, Giraud et al. 2002, Holway et al. 2002). Under conditions of unicoloniality, individual nests or colonies that would otherwise divert workers and energetic resources to the task of defending territorial borders from conspecific encroachment or attack are able to function together as a single cooperative unit over a large geographical area (Wilson 1971, Hölldobler and Lumson 1980, Hölldobler and Wilson 1990, Holway and Case 2001). Invasive populations of *Linepithema humile* Mayr have been intensely studied for their apparent loss of intercolony aggression in their introduced range, mediated by the homogenization of recognition cues and nestmate recognition abilities (Holway et al. 1998). Similar changes in social organization have been found in other introduced ant species (e.g., *Wasmannia aurapunctata* Roger and *Pheidole megacephala* F.), as well as in the polygyne form of *Solenopsis invicta* Buren (Ross and Keller 1995, Ross et al. 2003), although perhaps over a more limited geographic scale. Although the mechanisms driving such a change are variable or unknown, they may include ecological release from pathogens and predators and subsequent habitat saturation (Ross and Keller 1995); greatly reduced genetic diversity compromising nestmate discrimination abilities (Holway et al. 1998, 2002); the loss of independent colony-founding behavior (Ross et al. 2003); genetic bottlenecks and the evolution of green beard genes (Ross et al. 2003); or selection for uniformity in the absence of density-dependent regulation by pathogens or parasites (Starks 2003).

Myrmica rubra, a Myrmicine ant of Holarctic origin and distribution (Elmes 1975, Collingwood 1979), has invaded numerous sites in the northeastern United States and Canada during the past several decades (Groden et al. 2005). Although the full history of the ant's introduction and spread is not known, *M. rubra* has been present in Boston since at least 1906 and in a number of Maine communities since the 1950s or earlier (Wheeler 1908, Groden et al. 2005). Many sites of infestation in coastal Maine are characterized by an

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Table 1. Caste composition of mobile nests used in aggression assays

Excavation site	Latitude/longitude	Nest no.	Established	Workers	Larvae	Queens
Bear Brook Pond	44.36218 N	1	29 May	3,400	850	27
Bear Brook Pond	68.19676 W	2	2 July	1,380	335	11
Old Farm Road	44.37341 N	1	27 May	3,585	2,230	10
Old Farm Road	68.19546 W	2	1 July	1,742	1,600	37
Sand Beach House	44.33188 N	1	27 May	2,950	1,175	15
Sand Beach House	68.18094 W	2	3 July	2,170	1,250	16
Visitors' Center	44.40980 N	1	29 May	2,400	1,300	30
Visitors' Center	68.24649 W	2	3 July	2,300	1,685	11
Woodchip Pile	44.37677 N	1	26 May	5,170	2,200	11
Woodchip Pile	68.25679 W	2	3 July	5,100	3,500	19
Mean ± SEM				3,020 ± 413	1,613 ± 278	18.7 ± 3.0

All sites are within Acadia National Park, Mt. Desert Island, ME. Nests were collected during the spring and summer of 2003. No males or pupae were collected from any nests.

extremely high density of both nests and workers. *M. rubra* nests in a variety of substrates, including excavated cavities in the soil, under and within rotting wood, at the base of trees and herbaceous vegetation, under stones, in moist hummocks, and in and among leaf litter and coarse woody debris. Populations have been shown to virtually saturate a habitat, reaching a mean density of 1.4 nests/m^2 in local populations (Groden et al. 2005). Workers are highly aggressive toward intruders or prey (vertebrate and invertebrate alike), occurring in great numbers and readily and repeatedly deploying a painful sting at the slightest provocation (Garnas 2004). Before these studies, little overt aggression had been observed among *M. rubra* foragers under natural conditions in its introduced range, despite the close proximity of nests and corresponding overlap in foraging areas. Given *M. rubra's* highly polygynous life history (Elmes 1975, Groden et al. 2005) (Table 1), apparent forfeiture of mating flights in favor of reproduction through colony budding (in Maine and in parts of their native range) (Elmes 1975, Elmes and Petal 1990, Seppä and Pamilo 1995, Seppä and Walin 1996; also see Tsuji and Tsuji 1996), and low incidence of intraspecific aggression among foragers within heavily infested sites under natural conditions (J.R.G., unpublished data), introduced populations superficially resemble supercolonies of *L. humile* and other invasive ants cited in the literature (Passera 1994, Tsutsui and Suarez 2003). Presumed native populations of *M. rubra* exhibiting elevated levels of intercolony tolerance have been described in parts of northern Europe (Seppä and Pamilo 1995, Seppä and Walin 1996, van der Hammen et al. 2002). Such populations are characterized by localized patch dominance, high (while variable) levels of polygyny and polydomy (single colonies often span multiple nest sites), low intercolony relatedness, reproduction by colony budding, and restricted gene flow between local patches. No direct assessments of intercolony aggression or tolerance were reported in studies of these populations. In England and other parts of Europe, local patch dominance by *M. rubra* is rarely cited, although the ant is a common and widely distributed component of a more diverse community including *Myrmica*, *Formica*, and *Lasius* species, with which it coexists as a generally subordinate competitor across much of its range (Brian 1952, 1964, Pontin 1969, Czechowski 1985, Vepsäläinen and Savolainen 1990). Whether the mechanisms or conditions that give rise to patch saturation are similar in northern Europe and in Maine or whether the evolution of this highly successful social structure is linked with the introduction to novel habitat remains unknown.

Extensive sampling and observation have shown that *M. rubra* achieves patch dominance in infested sites in Maine (Groden et al. 2005); these populations seem to exhibit social dynamics similar to that reported in Scandinavia. Genetic assessments of kin structure and relatedness have not yet been performed in North American populations. The goal of this research was to study the hypothesis that introduced populations of *M. rubra* in Maine are "unicolonial." We define unicoloniality as it is most often applied in the invasive ant literature as the abandonment of intraspecific aggression and a breakdown of colony boundaries within local patches and/or across populations (Passera 1994, Human and Gordon 1996, Holway et al. 1998). With this definition in mind, we experimentally tested for aggression between groups of workers and colony fragments in the field.

Materials and Methods

Field Bioassays. Two bioassays were conducted in the field to assess internest aggression between workers within and among local populations. The first bioassay was conducted in 2003 and involved the use of mobile nests that could be transported within and between populations. Workers in the mobile ant nests were allowed to interact at baits in open arenas, with workers naturally foraging in various locations. A second bioassay, conducted in 2004, was used to assess levels of aggression within a population at varying distances along a linear transect from a supposed source nest. This assay involved setting out glass slides with a sugar bait, allowing foragers to recruit to the bait, and transporting the glass slide to a new location a given distance from the source. Interactions between the recruited foragers on the glass slide with new recruits in the new location were observed and recorded. Descriptions of the bioassays and methods of analysis follow.

Mobile Nest Bioassays. To test colony-level aggression between nests both within and among *M. rubra* populations, we established 10 mobile nests that we repeatedly used in internest aggression assays. We excavated nests from five sites colonized by *M. rubra* on Mount Desert Island, ME, in late May and early July 2003, taking two nests from each site. Care was taken to excavate each nest in its entirety, although given the highly polydomous habit of this species (Elmes 1975, Walin et al. 2001), each mobile nest likely comprised a fragment or satellite of an original colony. All castes were counted in the field and placed (along with several liters of original nest soil) in 9.8-liter Rubbermaid tubs. Tub sides were coated with Fluon to prevent climbing. Each mobile nest contained a representative mix of queens, brood, and workers (Table 1). We held mobile nests for 2 wk before use in aggression assays, feeding them a standard diet of 25% sucrose (by volume) and live insects. Food insects were captured locally from typical *M. rubra* habitat with a sweep net, thus providing colonies with a varied source of protein and minimizing the potential effects of acquired cuticular hydrocarbons from a single prey item, shown to influence nestmate recognition in some ant species (Silverman and Laing 2001). We misted nest soil weekly and provided the colonies with a constant source of water through a piece of saturated gauze.

We assayed each mobile *M. rubra* nest against conspecific foragers in the field, selecting assay sites to correspond to each of four experimental treatments. Treatments, defined as assay locations relative to the original site of excavation of each of the mobile nests, were as follows: (1) the site of excavation (testing captive colonies against former close neighbors or ex-colony fragments), (2) 10 m from the excavation site (within the same local population), (3) in the interior (center) of a noncontiguous *M. rubra* patch elsewhere on Mt. Desert Island (between 1.2 and 10.2 km from the excavation site), and (4) along the perimeter of the same noncontiguous patch (edge). The infestation edge proved difficult to pinpoint, however, and did not differ statistically from the center treatment in an analysis of variance (ANOVA; $P = 0.97$, Tukey's multiple comparison), although the overall ANOVA model was significant ($F = 13.01$; df = 3,45; $P < 0.0001$). Thus, data from treatments 3 and 4 were pooled for all analyses. We tested each mobile nest at each of the treatment locations within a window of a few hours to minimize behavioral variability potentially correlated with temperature or time of day. Treatment order was randomized within each block. We performed a total of 49 assays in July and August 2003. Additionally, two assay trials were performed against an off-island coastal population in Castine, ME.

Protocol for Mobile Nest Bioassays. We transported mobile nests among local *M. rubra* populations with as little disturbance as possible, leaving them undisturbed in a shady location for 20–30 min before beginning an aggression trial. We attached the mobile nest to a plastic (14 by 14 cm) assay arena with Tygon tubing (Fig. 1). Assay arenas had two entrances, the second opposite the entrance for the captive foragers,

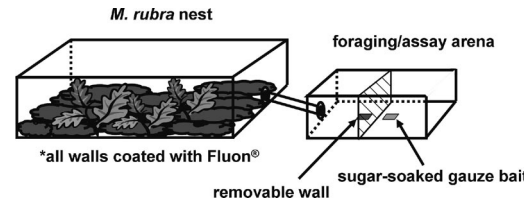

Fig. 1. Schematic of a mobile nest. Removable wall separated captive from local foragers until sufficient numbers of each (10–25) recruited to the arena.

thus allowing access to *M. rubra* foragers from the habitat. A removable wall of Fluon-coated plastic was fit across the center of the arena, and a small (1 cm^2) piece of gauze soaked in 50% sucrose solution was placed on either side as bait. This allowed both the mobile nest foragers and local foragers ("local foragers" refers to those ants recruiting from the habitat, most likely from one or more neighboring colonies or satellite nests) to recruit to the arena without mixing before the start of a timed trial. Once 10 foragers had arrived on each side of the central wall (≈2–5 min), the wall was gently removed, and the assay observations were begun. Assays were conducted for a total of 10 min, and a suite of behaviors associated with aggression (Table 2) along with feeding and recruitment rates were quantified during each of five consecutive 2-min intervals.

The number of mobile nest foragers and local foragers were counted at the beginning of the assay, and the total number of ants entering the arena through the Tygon tube was recorded for each 2-min period by a second observer. We subtracted this number from the total number of ants in the arena (also counted per 2-min period) to later calculate local forager recruitment per interval. Mobile nest and local forager recruitment was comparable across trials. We quantified behaviors in the aggression assays in categories modeled after protocols defined by de Vroey and Pasteels (1978) and de Vroey (1980) for the assessment of *M. rubra's* behavior during interspecific conflict during laboratory studies (Table 2). Some behaviors were difficult to observe in the field and were dropped from the protocol (e.g., mandible opening, gaster dragging, or vibration).

Glass Slide Distance Bioassays. In 2004, a sugar bait technique was used to describe the spatial pattern of intraspecific aggression within an infested area. This bioassay was designed to determine the aggressive response between worker ants recruited to and dominating a food source from a given foraging territory and workers from another foraging territory encountering food and "foreign" defending ants.

The bioassay was carried out on three dates (1 July, 12 July, and 10 August 2004) in Acadia National Park at the "Woodchip Pile" site (see Table 1). The method consisted of deploying a sugar bait on the ground at various flagged distances along a linear transect across the infested area. The bait was a 2-cm-square, triple-layer cotton gauze soaked in a 25% (by volume) sugar solution placed on a 6 by 10-cm glass plate. Ant re-

Table 2. Behavioral classification of aggression between foragers in arena bioassays

Behavior[a]	Weighting factor	Description
Antennation	NA	Nonaggressive[b] behavior scored each time one ant passed its antennae over the cuticle of another
Attack/threat	1	Lunging, mandibles open, irrespective of physical contact
Grasping	2	Biting, grasping with mandibles; often for extended periods
Carrying	2	Lifting with mandibles, carrying about arena[c]
Fighting/stinging	3	Grappling, gaster flexion, stinging, rolling about arena

Weighting factor used in the calculation of total aggression score (see text for details).

[a] Escape/avoidance behavior and trophallaxis (social feeding) were also quantified but were exceedingly rare and therefore were not included in analyses.

[b] Prolonged antennation can be considered a low-level aggressive behavior, indicative of non-nest odor recognition (Holway et al. 1998, Tsutsui et al. 2000). In *M. rubra* trials, behavior was characteristically brief and occurred between nestmates and non-nestmates with approximately equal frequency.

[c] Characteristic posture in social versus agonistic context (e.g., nest moving, tandem running; see Abraham and Pasteels 1980) was not observed. Carrying was unambiguously aggressive in our assays.

NA, not applicable.

cruitment to the bait was allowed until 20–25 workers settled to feed. A cardboard box top (5.5 by 5.5 cm) was placed over the glass plate with minimal disturbance to the feeding ants. The plate, with ants shielded from visual stimuli, was moved to a new location along the transect, ranging from <1 (control) to 144 m away from the point of initial recruitment. At the new location, the glass plate containing the bait was placed on the ground and the cover was removed. The bait was observed with a ×4 magnifying glass until the first worker from the new location encountered the bait with the feeding ants from the original location. At this point, a stop watch was started and for 5 min the following behavioral interactions were recorded: number of antennations, attacks/threats, grasping, carrying, and fighting/stinging (Table 1). After the 5-min bioassay, the ants and bait were disposed of in soapy water, and a new bait with ants from another location was deployed along the transect. Glass slides that resulted in apparent disturbance to the occupying foragers (rapid movement and threat displays) when moved were discarded from use in an aggression assay. At the completion of the bioassays, the flagged transects were measured so that the distances between all bioassay locations were ascertained. On each date, a different linear transect direction was randomly selected for bioassay. The number of paired aggression bioassays conducted along a transect on each date was 25, 14, and 27, respectively.

Scoring Aggression. Aggression assays were scored as the maximum level of aggression reached by any two ants during the trial (de Vroey 1980, Obin 1986, Obin and Vander Meer 1988, Tsutsui et al. 2000). We calculated an index of whole colony response from weighted counts of each behavior using the following formula:

$$\text{Aggression score} = (1 \times \text{Attacks}) + (2 \times \text{Grasping}) + (2 \times \text{Carrying}) + (3 \times \text{Fights})$$

This hierarchy of behaviors is similar to that used by de Vroey (1980). Attack/threat behaviors represented the lowest level of aggression, occurring occasionally between nestmates as well as non-nestmates. Grasping and carrying behaviors were also classified as aggressive, falling above threat behavior on the continuum. Because carrying and grasping could (and did) evolve into one another during the course of the assay trials, both were considered equivalent in terms of aggressive level. Fight behavior was the most aggressive and was weighted accordingly. It was noted during assay trials that the number of interactions between ants in the arenas was generally higher when overall aggression was highest; for this reason, we chose not to standardize aggression score by the overall number of interactions (which would tend to under-represent whole colony aggression). Instead, we tested all treatment effects using the number of antennations (a proxy for interaction frequency) as a covariate in our early analyses to test whether this variable had relevant explanatory power. However, antennation was found to be uncorrelated with aggression score (Pearson $r = -0.04$, $P = 0.78$) and was subsequently dropped from the analyses in favor of the more parsimonious ANOVA.

Data Treatment and Analyses. ANOVA was used in hypothesis testing for the mobile nest bioassays. A square-root transformation of the dependent variable (aggression scores or behavioral counts) was used to meet the assumptions of normality and homogeneity of error variance. Where appropriate, Tukey's adjustments for multiple comparisons were made (Miller 1985). Owing to the somewhat unpredictable nature of recruitment among foragers from the mobile nests and to the difficulty in locating edge habitat, it was not always possible to execute all treatments in a single day using a given mobile nest. Tests were initially run using an incomplete block design with each mobile nest and site as a block, but because no significant block effects were detected, the remainder of ANOVA testing was run using only assay location (treatment) as a factor in a completely randomized design.

Preliminary model exploration using a variety of temperature and weather parameters—site of assay, mobile nest, date, and time of day—revealed no significant trends, suggesting that the treatments themselves were the major cause of variation. Analysis of

Table 3. Results (mean ± SEM) of mobile nest bioassays

Behaviors	Treatment location relative to ES of mobile nest				
	ES ($n = 12$)	10 m from ES ($n = 13$)	Different population on island ($n = 24$)	Off-island site ($n = 2$)	Test statistic
Attack/threat	4.4 ± 1.7^{A}	22.2 ± 8.2^{B}	35.3 ± 3.4^{C}	88.5 ± 4.5	$F_{2,46} = 19.4, P < 0.0001$
Grasping	0.6 ± 0.3^{A}	5.4 ± 1.9^{A}	9.0 ± 1.8^{B}	19.5 ± 1.5	$F_{2,46} = 12.8, P < 0.0001$
Carrying	3.6 ± 1.4^{A}	11.6 ± 5.1^{B}	21.5 ± 3.2^{B}	43.5 ± 2.5	$F_{2,46} = 11.8, P < 0.0001$
Fighting/stinging	0.0 ± 0.0^{A}	0.2 ± 0.1^{AB}	0.5 ± 0.2^{B}	5.5 ± 0.5	$F_{2,46} = 4.5, P = 0.016$
Aggression score	12.8 ± 5.0^{A}	56.7 ± 20.6^{B}	97.9 ± 10.2^{C}	231.0 ± 14.0	$F_{2,46} = 19.8, P < 0.0001$
Maximum aggression	1.2 ± 0.3	2.0 ± 0.2	2.4 ± 0.1	3.0 ± 0.0	$R_S = +0.66, P < 0.0001$

Superscripted letters indicate statistical differences between groups (Tukey's multiple comparisons, $P = 0.05$). Data from the off-island site are presented but are not included in statistical analyses because of low sample size.

ES, excavation site.

covariance (ANCOVA) was also used, with the number of antennations and recruitment rate as covariates, to test for the contribution of overall interaction frequency in the observed aggression score treatment differences, but these covariates were not significant and were dropped. We also determined the maximum aggression value for each of the assays and performed a Spearman's rank correlation analysis between maximum aggression and the ranked distance from the site of excavation for each corresponding treatment. All means are reported ±SE. Models and tests were performed using Systat for Windows, version 11.00.01 (Systat Software 2004).

We analyzed data from the glass slide distance bioassays using a randomization Monte Carlo Mantel test (Mantel 1967) between the paired aggression score matrix and the distance matrix (distances between locations involving aggression bioassays) to determine whether a significant linear correlation (r) existed between aggression index and distance among locations. The Mantel test is robust to nonindependence among replicates; because forager aggression at each point along each linear transect cannot be considered to be independent, a key assumption of linear regression is violated, and traditional parametric tests cannot be used. The tests were computed using PC-ORD software (PC-ORD; MjM Software Design 1999) using 1000 permutations.

Results

Aggression Scores and Behavioral Interactions. Aggression scores between mobile *M. rubra* nest foragers and undisturbed natural foragers differed significantly by treatment location relative to the excavation site of each mobile nest (Table 3). At the excavation site, where mobile colonies were tested against their former close neighbors or ex-colony fragments, there was very low aggression, although aggression scores did vary somewhat across trials. Aggression score increased at 10 m from the site of excavation and was highest when nests were assayed against different populations.

The frequency of specific aggressive behaviors also varied by treatment (Table 3). Escape/avoidance behavior (data not shown), while viewed as low-level aggression by some researchers (Holway et al. 1998, Suarez et al. 1999, Tsutsui et al. 2000), was extremely rare (two instances in 51 assays) and so was not included in the analysis. Carrying behavior was nearly absent at the excavation site, although it was more common at both the 10-m and pooled distinct population treatments. Aggression, when present, was generally dominated by grasping behavior, and no fighting/stinging was observed at the site of excavation. In contrast, attacks, grasping, and carrying were common in the 10-m and distinct population treatments, with fighting/stinging behavior occurring at relatively low levels.

The data from the two trials conducted in August 2003 testing mobile nests against a coastal "off-island" *M. rubra* population are treated separately in the analysis because the small sample size limits our ability to make statistical comparisons. Nonetheless, results from these two assays were quite distinct from those obtained when testing mobile *M. rubra* nests anywhere on Mt. Desert Island. Both were characterized by extremely high levels of aggression; compared with the Mt. Desert Island treatments, counts of aggressive behavior are higher for all categories. While fighting/stinging was nearly absent on the island within a site and at low levels during assays from the different population treatments, the mean count of fight behaviors during the two off-island trials was statistically distinct from all other treatment using Tukey's multiple comparisons during exploratory analysis. Grasping, carrying, and attacks were also considerably more frequent during offsite trials

Maximum Aggression by Assay Location. The mean maximum aggression score between any two individual ants during an assay was lowest in the excavation site trials and increased with distance corresponding to each treatment. A significant Spearman's correlation exists between maximum aggression value and treatment number ranked by distance from the site of excavation (Table 3). Given that both off-island trials were skewed toward fight behavior, the maximum aggression mean of 3 falls substantially above any of the on-island treatments, although, again, the $n = 2$ sample size did not warrant pairwise analyses.

Aggression by Distance Within a Local Population. Glass slide assays of aggression between individuals along a transect within a local population revealed significant positive correlations between aggression

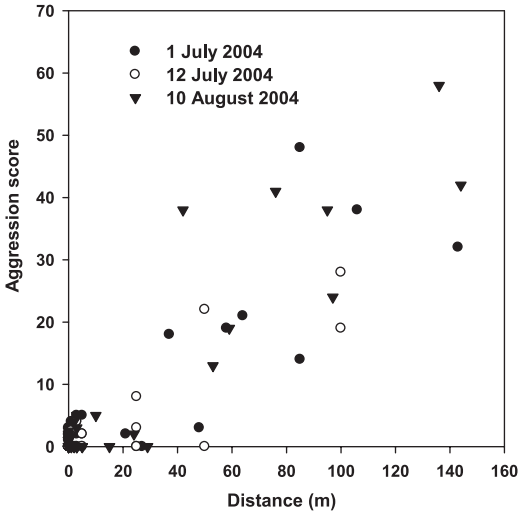

Fig. 2. Aggression scores at baits as a function of distance between foragers' colony of origin in the glass slide experiment, 2004. Data analyzed using Mantel test; 1 July: $r = +0.781$, $P < 0.001$; 12 July: $r = +0.727$, $P = 0.007$; 10 August: $r = +0.0.864$, $P < 0.001$. When data are pooled over the three dates, there is a significant correlation coefficient of $r = +0.808$ ($P < 0.001$).

index and distance on each of the three assay dates (1 July: $r = +0.781$, $P < 0.001$; 12 July: $r = +0.727$, $P = 0.007$; 10 August: $r = +0.864$, $P < 0.001$) and for data pooled over all dates ($r = +0.808$, $P < 0.001$), with the aggression index increasing over the transect distances (Fig. 2). As with the mobile nest trials, aggression between foragers from the same and/or closely neighboring colonies was characteristically low. Aggression began to increase between foragers separated by ≈20 m and increased linearly with distance up to 144 m.

Discussion

The results of this study show that *M. rubra* populations on Mt. Desert Island, ME, are not unicolonial. Despite its complex polydomous colony structure, high nest densities, and low levels of observable conflict between foragers within sites of infestation under natural conditions, intercolony aggression is present both within and among local *M. rubra* populations. Furthermore, aggression between colony pairings is positively correlated with the distance that separates them within a population. Whether such patterns arise out of genetic differences between colonies within a site, variability in hydrocarbon signature mediated by environmental or resource heterogeneity, or a combination of these and other factors awaits further research. Strong relationships between physical and genetic distance have been shown to exist in some ant species (Bourke and Franks 1995, Pirk et al. 2001), although as an introduced species, it is likely that some of *M. rubra's* allelic diversity has been lost through the founder effect (F.A.D. and K.S. Schonrogge, unpublished data).

The presence of aggression within a local patch differs from other invasive ant systems, including invasive populations of *L. humile* and the polygyne form of *S. invicta* (Porter and Savignano 1990, Holway et al. 1998). In contrast, we found strong support for the existence of a multicolonial structure within *M. rubra* populations in coastal Maine. Mobile *M. rubra* nests were found to be tolerant of close neighbors and/or to fragments of their former colony. Tolerance was maintained throughout the season, despite the fact that mobile nests were reared separately from their undisturbed counterparts for several months. The longevity of this response does not preclude environmental regulation of colony recognition cues but does suggest the importance of endogenous factors. Stuart and Herbers (2000) compared colony aggression in monogynous/monodomous versus primarily polygynous/polydomous populations of *Temnothorax longispinosis* and found that genetically controlled cues for nestmate recognition were more important when colonies occupied multiple nests. The authors concluded that heavy reliance on exogenous regulation of colony odor would result in a high error rate/misdirected aggression between polydomous satellite nests, because even adjacent nest sites are likely to experience subtle variations in the environment. Similarly, research on *S. invicta* and *L. humile* has confirmed that nestmate recognition cues are heritable but that exogenous factors such as diet can alter the hydrocarbon profile of a nest or colony, and with it, behavior (Obin and Vander Meer 1988, Vander Meer et al. 1990, Silverman and Laing 2001, Suarez et al. 2002). Further research on kin structure and the genetics of nestmate recognition, along with the potential regulatory contribution of subtle variations in diet composition or microhabitat characteristics, is necessary in invasive *M. rubra* populations to help explain these complex dynamics.

Much of the current theory surrounding the ecological and genetic preconditions for the appearance of unicoloniality has been formed in the context of ant invasions, because this social structure seems to be integral to the widespread ecological success of such species. A loss of genetic diversity at nestmate recognition loci subsequent to introduction and the selection for common alleles under relaxed ecological constraints (e.g., the shedding of pathogens and/or parasites from the native range, allowing for greater proximity of nests) have been suggested as important factors driving observed patterns in *L. humile* (Passera 1994, Ross et al. 1996, Starks et al. 1998, Tsutsui et al. 2000, Giraud et al. 2002). Unicolonial populations have been described as an alternative social form in *M. rubra*, particularly in the northern regions of its native range, although the mechanisms leading to its expression are potentially distinct as an introduced invader (Seppä and Pamilo 1995, Seppä and Walin 1996, Pedersen and Boomsma 1999, Walin et al. 2001). Van der Hammen et al. (2002) describe a linear succession in *Myrmica* ants in a stable habitat toward "low-relatedness supercolonies" that are stable in time and capable of saturating a local habitat. The authors presented a

hypothetical progression from a single-queen foundation in a high-quality, novel patch to a stage characterized by excessive inbreeding and the production of sterile, diploid males. Next, they predicted an intermediate stage of rapid reproduction through budding, moderate relatedness, and reduced inbreeding caused by rare immigration of males (or gynes) from different populations. This is followed by effective unicoloniality, with near-zero relatedness and accompanied by high-density habitat saturation. While as yet circumstantial, indigenous *Myrmica* populations with predicted kin and colony structure have been identified in northern Europe and corroborate this progression (Seppä 1996, van der Hammen et al. 2002). The authors speculate that the process of convergent selection (for similar colony odor/identifying cues) within long-lived, uniform sites could drive populations toward conditions with very low relatedness and a breakdown of colony borders within a site. Although low relatedness and high nest and colony density may correspond to habitat saturation and ecological dominance and/or success, no direct assessments of intercolony aggression are provided in this body of research, and it remains untested (or unreported) whether such populations conform to the definition of unicoloniality as defined by the true loss of intraspecific aggression.

Many of the characteristics of *M. rubra* colony and kin structure in such "low-relatedness supercolonies" are in evidence within invasive populations in Maine. The significantly higher aggression exhibited by colonies from different local populations in our study is apparently consistent with the discovery of *Myrmica* aggregations with comparatively high levels of genetic substructure between patches (Seppä and Pamilo 1995, van der Hammen et al. 2002). Although we have no direct estimates of genetic divergence between infestations, our observation of reproduction by colony budding and the limited dispersal of reproductives between sites is consistent with such observations. Interestingly, the starting point of the creation of a unicolonial population or patch, according to van der Hammen et al. (2002), is an introduction to a novel patch, although in this case, still within the native distributional range of the insect.

Several possibilities exist to reconcile the existence of a certain level of aggression within a site with *M. rubra's* observed ecological dominance and habitat saturation in Maine. First of all, aggression at the 10-m site was moderate and highly variable, suggesting that an infestation may comprise a few large colonies or that now distinct colonies originated as fragments of the same large colony and retain some mutual tolerance/recognition. Also, food may not be limiting in the habitat (at least during the years in which the ant has been actively studied), which could potentially facilitate passive coexistence. Abundant homopterans provide food to the worker force (Garnas 2004) and may encourage vertical foraging, also reducing spatial overlap of territories (Davidson 1998). Czechowski (1984) noted a seasonal expansion of satellite nests in the spring and a concomitant increase of overt aggression in the habitat. In Maine, aggression was apparently temporally stable under seminatural experimental conditions, although it is likely that there are times during the year when the natural encounter rate would increase, such as during mating or during the seasonal expansion of satellite nests (Czechowski 1985, Garnas 2004). Also, because our research documented relatively low-intensity aggression on the whole (resulting in few, if any, casualties), it could be that moderate to low levels of aggression have little impact on colony spatial dynamics. While aggressive behaviors undoubtedly represent some cost in terms of foraging efficiency (if not worker mortality), a carbohydrate surplus (from nectar or homopteran exudates) could potentially subsidize the workers with energy beyond what is necessary for brood production. As such, minimal levels of aggression may have little measurable impact on population dynamics (Davidson 1998, Holway 1998). It is not difficult to imagine that the benefits of effective territoriality and virtual exclusion of native ant competitors from entire patches of habitat could far outweigh any costs of limited intercolony aggression among wandering foragers. Although not devoid of intraspecific aggression, each *M. rubra* infestation may function as a cohesive, essentially cooperating unit. By whatever mechanism this is maintained, it seems to be a highly successful strategy, at least in the short term.

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