



Intraspecific aggression and colony fusion in the Argentine ant

GISSELLA M. VÁSQUEZ & JULES SILVERMAN

Department of Entomology, North Carolina State University, Raleigh

(Received 22 September 2006; initial acceptance 12 January 2007;
final acceptance 5 June 2007; published online 4 December 2007; MS. number: A10567R)

Unicolonial ants possess an unusual social system characterized by the absence of internest aggression resulting in expansive networks where individuals move freely among distant nests. The Argentine ant, *Linepithema humile* (Mayr), can form geographically vast and numerically large unicolonial populations, or supercolonies, a trait that has been linked to its ecological success in the introduced range, and is one of the few invasive ants in which native and introduced populations have been examined to elucidate the origins and maintenance of unicoloniality. Supercolony formation may result from mixing of genetically homogenous and nonaggressive colonies, or initially aggressive colonies harbouring the most common recognition alleles. In this study, we examined interactions between mutually aggressive *L. humile* colonies in the absence of barriers limiting intercolony encounters to determine whether aggressive interactions result in either colony elimination or fusion into new nonaggressive colonies. By pairing experimental laboratory and field colonies displaying varying levels of intraspecific aggression, we determined that pairs that did not fuse had higher numbers of workers fighting and killed than colony pairs that fused and that genetic and cuticular hydrocarbon similarity between colony pairs was correlated with both levels of intraspecific aggression and colony fusion. We suggest that selective fusion of initially aggressive colonies sharing certain recognition cues may be a proximate mechanism shaping *L. humile* social structure, leading ultimately to extreme unicoloniality in introduced populations when ecological conditions are favourable.

© 2007 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Keywords: aggression; Argentine ant; colony fusion; cuticular hydrocarbons; genetic similarity; invasive species; *Linepithema humile* (Mayr); nestmate recognition; unicoloniality

Most social insect populations contain colonies that recognize nest boundaries and maintain colony integrity by exclusion of both heterospecific and conspecific intruders (Hölldobler & Wilson 1990). In this multicolonial social structure, individuals probably distinguish nestmates from intruders by means of a nestmate recognition system consisting of genetically determined and/or environmentally derived olfactory cues (recognition label) and a genetically determined or learned sensory template continuously updated as to follow the dynamically changing cues (Lacy & Sherman 1983; Gamboa et al. 1986; Crozier & Pamilo 1996). A proposed model for nestmate recognition involves matching the label of the encountered conspecific to the individual's inner template so that it can take action (accept or reject) according to the

degree of template-cue dissimilarity (Sherman & Holmes 1985; Reeve 1989). High intranest relatedness may explain the well-developed recognition system in multicolonial ant species (Breed & Bennett 1987) allowing individuals in these 'closed' societies to aggressively defend territories (Sudd & Franks 1987).

In contrast to these closed societies, some ant species form unicolonial populations, whereby individuals move freely among distant nests (Hölldobler & Wilson 1977, 1990; Bourke & Franks 1995). Limited genetic differentiation among nests in unicolonial populations produces colony odour homogeneity and, therefore, no within-colony aggression (Hölldobler & Wilson 1990), although individuals from other unicolonial populations can be recognized and attacked (Tsutsui et al. 2000; Giraud et al. 2002). Unicoloniality has been observed in some species of *Anoplolepis*, *Formica*, *Lasius*, *Linepithema*, *Monomorium*, *Pheidole*, and *Wasmannia* (Hölldobler & Wilson 1990; Holway et al. 2002). Interestingly, most of these species thrive in human-altered habitats, and share characteristics

Correspondence: J. Silverman, Department of Entomology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695, U.S.A. (email: jules_silverman@ncsu.edu).

including polygyny, colony reproduction by budding, general nesting and dietary requirements, high worker and nest densities, and superior competitive abilities (Passera 1994; Moller 1996). Moreover, unicoloniality seems to play a key role in the ecological success of invasive ants (Chapman & Bourke 2001; Holway et al. 2002).

Studies comparing native and introduced populations of two highly successful invaders, *Solenopsis invicta* and *Linepithema humile*, have shed light on the origins of unicoloniality and led to the emergence of ecological and genetic hypotheses to explain its evolution in invasive ant species (Ross & Keller 1995; Chapman & Bourke 2001; Holway et al. 2002). *Solenopsis invicta* social organization is genetically regulated, with workers bearing the 'green beard' allele (*b*) at the *Gp-9* locus accepting only multiple queens sharing the same allele (Keller & Ross 1998), a condition that has been found in polygynous and dominant *S. invicta* colonies in introduced areas in U.S.A. In *L. humile*, the formation of unicolonial populations may be explained by release from ecological constraints, increased non-nestmate encounters, and selection against diverse recognition loci by favouring colonies possessing the most common recognition alleles (Giraud et al. 2002), and lack of nestmate discrimination because of a genetic bottleneck followed by selection against genetic diversity (Tsutsui et al. 2000, 2003).

Ecological factors shaping the social organization of ant colonies, such as habitat structure, climate, interspecific competition and more efficient exploitation of plant and hemipteran exudates (Hölldobler & Wilson 1977, 1990; Herbers 1993; Davidson 1997), may not only regulate colony size and abundance in invasive ants but also the variation in the expression of unicoloniality. It seems that loss of intraspecific aggression and colony and nest structure flexibility play a major role in the expression of unicoloniality and invasion success of *L. humile* (Holway et al. 1998; Tsutsui et al. 2000; Tsutsui & Case 2001; Ingram 2002a, b; but see Heller 2004). In the absence of intraspecific aggression, high worker numbers are more likely to monopolize resources (Human & Gordon 1996; Holway & Case 2001) while flexibility in nest size, queen number, frequency of colony budding and movement between nests facilitates adaptation to new environments (Ingram 2002a, b). Therefore, ecological context may influence *L. humile* unicoloniality, with abiotic (temperature, humidity, resource availability) and biotic factors (intra- and interspecific competition) affecting colony survival, rates of colony expansion, and competitive ability (Tsutsui et al. 2000; Holway et al. 2002; Suarez et al. 2002; Walters & Mackay 2003; Holway & Suarez 2004). For example, regional differences in *L. humile* social structure in U.S.A., that is, small, patchily distributed, highly aggressive colonies with high genotypic variability in the southeast (Buczkowski et al. 2004) versus expansive supercolonies with low levels of genetic diversity in California (Suarez et al. 1999; Tsutsui et al. 2000), may be to some extent explained by the dissimilar abiotic and biotic pressures acting on these populations. Unlike populations from California and southern Europe that experience

relatively mild winter conditions and also reduced biotic resistance (Human & Gordon 1996; Heller 2004), *L. humile* in the southeastern U.S.A. are exposed to winter subfreezing temperatures and possibly compete with *S. invicta* (Buczkowski et al. 2004). Thus, unfavourable ecological conditions in the temperate southeastern U.S.A. may restrict colony expansion and subsequent intermixing of individuals resulting in mutually aggressive 'diminutive supercolonies', while extreme unicoloniality in regions with Mediterranean or subtropical climate may result from boundary expansion followed by mixing of colony members, thereby creating a blend of recognition cues across the newly fused larger colony.

Colony traits in multicolonial and unicolonial populations may constitute a continuum of variation in social structure in polygynous ant societies (Bourke & Franks 1995). Although reduction in recognition loci diversity offers an evolutionary explanation for extreme unicoloniality in introduced *L. humile* populations (Tsutsui et al. 2000, 2003; Giraud et al. 2002), little attention has been paid to the mechanistic underpinnings of the transition from diminutive to extreme unicoloniality. The mechanisms leading to the development of secondary polygyny in ants, that is, new gyne acceptance and/or colony fusion (Hölldobler & Wilson 1990; Herbers 1993), could also be involved in the variable expression of unicoloniality among introduced *L. humile* populations. Studies examining interactions between mutually aggressive *L. humile* colonies in the absence of ecological constraints would shed light on whether these mechanisms, queen adoption and colony fusion, play a role in shaping invasive population social organization. Fusion events may depend on the intensity of aggressive interactions between *L. humile* colonies and should vary according to the degree of intercolony genetic similarity that seems to regulate aggression (Tsutsui et al. 2000). Moreover, the use of cuticular hydrocarbons as recognition cues in *L. humile* (Liang & Silverman 2000), and the inverse relationship between cuticular hydrocarbon similarity and intraspecific aggression (Suarez et al. 2002), suggests that cuticular hydrocarbon profile similarity between colonies may also modulate intercolony interactions.

The objectives of this study were, therefore, to determine if aggressive *L. humile* colonies from the southeastern U.S.A. can fuse, and if levels of aggression and genetic and phenotypic similarity between colonies influence this process. We predict that low intercolony aggression and high levels of genetic and cuticular hydrocarbon similarity between colonies promote colony fusion. To test this we conducted laboratory assays where we paired experimental *L. humile* colonies showing various levels of intraspecific aggression and for which we determined levels of overall genetic similarity and worker cuticular hydrocarbon profile similarity. We then selected two colony pairs that fused under laboratory conditions to examine the interactions between colony fragments in the field. Although colony fusion might be only one of a variety of mechanisms shaping *L. humile* social structure, we provide evidence for a proximate mechanism by which unrelated colonies fuse to attain extreme unicoloniality in their introduced range.

METHODS

Ant Colonies and Rearing Conditions

We collected colonies of Argentine ants, *L. humile*, from five locations in the southeastern U.S.A.: Cary (CAR), Chapel Hill (CHH), Research Triangle Park (RTP), and Winston-Salem (FOR) in North Carolina; and Greenville (COC) in South Carolina (Fig. 1). Distances between collection sites ranged from 9.7 km (CAR–RTP) to 402.3 km (CAR–COC). Ants were collected from landscaped residential and business lots and in nesting substrates such as pine needles, hardwood mulch, and leaf litter. Experimental colonies from each location were established within 1–2 months after collection to conduct a colony fusion laboratory assay. We tested all 10 pairwise combinations in three trials with five colony pairs per trial and five replicates per pair. Each experimental colony consisted of five queens, ca. 100 pieces of brood, and 500 workers. For each colony pair, all queens and 50 workers from each colony were marked on the thorax and abdomen, respectively, with either pink (colony 1) or yellow (colony 2) water-based paint (Apple Barrel Colors®, Plaid Enterprises, Inc., Norcross, GA, U.S.A.) using a 10/0 brush to observe individuals mixing and determine fusion events. Colonies were maintained in individual Fluon-coated trays (17 × 25 × 11 cm) and provided either plastic petri dishes (9 cm diameter) filled with moist grooved Castone® dental plaster (colony 1) or foil-covered glass tubes half-filled with water and stopped with cotton as artificial nests (colony 2), alternating type of nest assigned across replicates in each colony pair. Containers were connected through a 12-cm-long vinyl tube with soft earplugs initially inserted at each end to prevent contact between colonies. Each colony was provided 25% sucrose solution, artificial diet (Bhatkar & Whitcomb 1970) ad libidum, and a water source during a 24-h acclimation period. To begin the colony fusion experiment, food provided during acclimation was removed and only experimental colonies in plaster nests were provided 25% sucrose solution, artificial diet

ad libidum, and three freshly killed *Blattella germanica* adults once a week. By providing different types of artificial nests and placing food items only in colonies with a plaster nest, we expected to promote continuous encounters. Controls consisted of five queens, ca. 100 pieces of brood, and 500 workers from each location (five replicates per location) placed in individual Fluon™-coated trays, and that were not paired to any foreign colony (unexposed controls). All colonies were maintained at $25 \pm 1^\circ\text{C}$ and $50 \pm 15\%$ relative humidity, on a 12:12 h light:dark cycle. Source colonies from each of the five locations containing ants not used in the experimental colonies were also maintained as described above.

For a field intercolony interaction and fusion assay we used freshly collected Argentine ants from CAR, CHH, and RTP. Field colony fragments (18) consisting of 2 g of workers, 0.2 g of brood, and 30 queens nesting in their original substrate (500 cc) were placed in individual Fluon™-coated plastic containers (23 × 23 × 9 cm). All queens and a fraction of the workers (ca. 1 out of 10) were marked as previously described, while the brood was marked by feeding the colonies sucrose solution (25%) containing 8 mM erioglucine (blue). Within 1 week after collection, colonies provided with a water source and 25% sucrose solution were transferred to the RTP field site in their plastic containers covered with lids to proceed with the field introduction assay described below. Research Triangle Park (RTP) ants were used as controls.

Behavioural Assays

Levels of worker aggression

We assessed the level of worker–worker aggression between all pairwise source colony combinations (CAR–CHH, CAR–COC, CAR–FOR, CAR–RTP, CHH–COC, CHH–FOR, CHH–RTP, COC–FOR, COC–RTP, FOR–RTP) following Roulston et al. (2003). Briefly, individual intruder workers were collected on a toothpick and introduced into trays containing a resident colony. We allowed

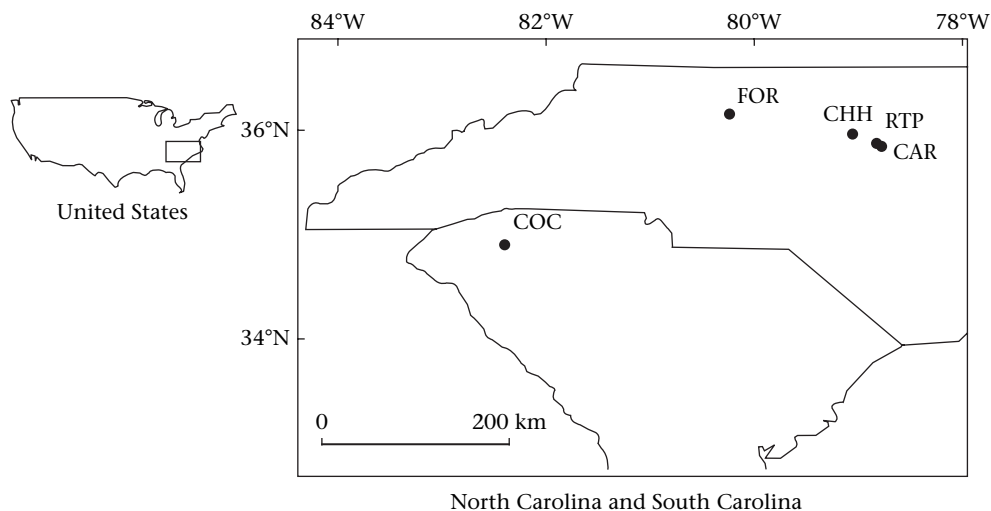


Figure 1. Collection sites of *Linepithema humile* colonies used in the laboratory colony fusion assay. Sampling sites included Cary (CAR), Chapel Hill (CHH), Winston-Salem (FOR) and Research Triangle Park (RTP) in North Carolina and Greenville (COC) in South Carolina.

the intruder up to 25 encounters with resident ants and scored behavioural interactions using a 1–4 scale similar to Tsutsui et al. (2000) in which 1 = touch (prolonged antennation), 2 = avoid (ants retreating in opposite directions), 3 = aggression (lunging, biting, pulling appendages), and 4 = fight (prolonged aggression, gaster flexion). The intruder was discarded after each trial. Twelve trials per colony pair were performed; six trials with colony 1 as the resident and six trials with colony 1 as the intruder, and responses recorded were generally symmetrical within pairs. The observer who recorded the aggression scores did not know the identity of the interacting colonies and was unfamiliar with the hypothesis being tested. To determine if colony fusion could lead to a reduction in intraspecific aggression, we measured aggression levels between fused colonies and their respective controls (unexposed colonies) 6 months after the start of the colony fusion assay. Levels of aggression in replicates that did not fuse were also measured. Data were analysed as the maximum score out of 25 encounters per trial recorded between source colony pairs, and as the difference between maximum aggression scores recorded after 6 months and initial maximum aggression scores.

Laboratory colony fusion assay

We conducted a colony fusion experiment to determine if levels of aggression would predict the outcome of intraspecific interactions. After a 24-h acclimation period, the vinyl tube connecting the previously described colony pairs was unblocked to allow interactions between ants. Total number of workers fighting and total number of dead workers (marked and unmarked) in each container and length of tubing were recorded each hour for 6 h and then at 24 h. Colony pairs were inspected for fusion and dead queens daily, from day 2 to day 30, and monthly, from month 2 to month 6. Fusion was defined as the presence of queens from both colonies and all brood in the same nest. Colonies that did not fuse were characterized by the lack of mixing of individuals and elimination of all queens from one of the colonies forming a pair. Data were analysed as the total number of workers fighting and dead workers within the first 24 h, and percentage of replicates from the same colony pair that fused 24 h and 6 months after the experiment started. Three trials were conducted, the first one from August 2003 through February 2004, the second one from May through November 2004, and the third one from March through September 2005. The first two trials included the following colony pairs: CAR–CHH, CAR–COC, CAR–RTP, CHH–FOR, and CHH–RTP (50 pair and 50 control experimental units); while the third trial included: CAR–FOR, CHH–COC, COC–FOR, COC–RTP, and FOR–RTP (25 pair and 25 control experimental units).

Intercolony interactions under controlled field conditions

Two colony pairs that readily fused in the laboratory assay, CAR–RTP and CHH–RTP, were used to test whether freshly collected colony fragments would fuse with fragments of an established colony following aggressive encounters in the field. Cary (CAR) and CHH

colonies were set up as previously described, and placed individually at the base of six red maples, *Acer rubrum*, along a 200 m transect at the RTP collection site. Research Triangle Park (RTP) ants collected at the base of three individual trees along the same transect served as controls. Treatments (foreign colonies) and controls were randomly assigned as RTP ant densities (workers trailing/minute across a fixed point on the trunk of trees) measured before the experiment started did not differ between trees ($F_{2,2} = 2.35$, $P = 0.2983$). Containers with the introduced colonies were fastened to the tree trunk with Nalgene® premium tubing (130-cm length \times 5-mm diameter) to allow interaction with RTP ants. A section of the trunk (80-cm length) was banded with Tree Tanglefoot® to facilitate the observation of behavioural interactions (i.e. fighting) and prevent foreign ants from escaping. We counted the total number of ants fighting on the connecting tube and on a tree trunk section measuring 5 \times 5 cm around the point of attachment of the tube at 30 min and every hour for 6 h to quantify aggressive interactions and compared them with those recorded in the first 6 h of the laboratory assay. After 6 h, we removed the tubing, collected RTP ants nesting at the base of each tree (approx. 500 cc ants and nesting substrate), placed them in plastic containers (23 \times 23 \times 9 cm), and transferred these and the introduced colony fragments to the laboratory to monitor fusion under controlled conditions. In the laboratory, we first standardized queen number to 30 in each of the freshly collected RTP colony fragments by removing supernumerary queens, and provided all colonies with a water source and 25% sucrose solution dyed with either 8 mM erioglaucine (introduced ants and RTP controls) or 8 mM amaranth (red; RTP colony fragments). After 14 h, we replaced the coloured sucrose with undyed sucrose solution and connected colony fragments that interacted in the field using paper strips measuring 2.5 \times 45 cm as bridges between containers. We inspected for mixed brood and queens present in the same nest (our indicator of colony fusion) and counted the total number of queens alive in each nest at 24 h, 6 days and 12 days after colonies were connected. Colonies that did not fuse were defined as those where all queens from one of the colonies were killed. Two trials were conducted, the first one on 11 August 2005 and the second one on 2 September 2005.

Genetic Similarity between Colonies

We assessed genetic similarity between colonies used in the fusion assay (CAR, CHH, COC, FOR, RTP) using microsatellite markers. Genomic DNA was extracted from 15 workers from each of the source colonies using the DNeasy Tissue Kit (Qiagen, Valencia, CA, U.S.A.) and analysed at eight microsatellite loci: Lhum-11, Lhum-13, Lhum-19, Lhum-28, Lhum-35, Lhum-39 (Krieger & Keller 1999), Lihu-M1 and Lihu-T1 (Tsutsui et al. 2000). PCR reactions were performed as described by Buczkowski et al. (2004). Products were separated on 6.5% KB^{plus} polyacrylamide sequencing gels using a 4300 LI-COR DNA analyser. Microsatellite alleles were scored using GeneImagIR software (Scanalytics, Inc., Billerica, MA, U.S.A.). Genetic

differentiation (F_{ST}) between Argentine ants from different locations was estimated with the program FSTAT v.2.9.3.2 (Goudet 1995). Levels of genetic similarity between colonies were estimated based on the percentage of alleles shared between these groups (Tsutsui et al. 2000).

Extraction, Isolation and Chemical Analysis of Cuticular Hydrocarbons

Worker cuticular hydrocarbon profiles were examined to determine cuticular hydrocarbon similarities between colony pairs and to relate these similarities to worker aggression, colony fusion and genetic similarity. We collected workers from each source colony used in the laboratory fusion assay in groups of 10 and placed them in glass vials (nine vials per colony). Cuticular lipids were extracted by immersion in 1 ml of hexane for 10 min, followed by a brief second rinse in 100 μ l of hexane. Samples were lightly shaken for the first and last 15–20 s of the immersion period. The solvent was removed under a gentle stream of N_2 , the vial rinsed with two 100 μ l of hexane and the concentrated extract (200 μ l) applied to a hexane-pretreated Pasteur pipette minicolumn filled with 500 mg of silica gel (100–200 mesh). The hydrocarbon fraction was eluted with 6 ml of hexane, and the solvent was subsequently removed. Capillary gas chromatography (GC) was carried out using a HP5890 gas chromatograph equipped with a DB-XLB column (film thickness of 30 m \times 0.25 mm \times 0.25 μ m). Extracts were introduced into a split–splitless injector operated at 300°C in splitless mode (2 min purge) and helium was the carrier gas at an average linear velocity of 30 cm/s. Oven temperature was held at 80°C for 2 min, increased to 270°C at a rate of 20°C/min, then to 310°C at 3°C/min and held at 310°C for 20 min. The flame-ionization detector was operated at 310°C with nitrogen make-up gas at 30 ml/min. Extracts were resuspended in 10 μ l of hexane and 2 μ l were injected (two worker equivalents). Quantitative data were obtained by integrating the area under each peak and calculating its percentage of the total cuticular hydrocarbons; only peaks with a mean percentage area of 1% or higher were used for data analysis. All selected peak areas were standardized to 100%.

Statistical Analyses

All analyses were carried out using SAS 8.2 statistical software (SAS Institute 2000). Changes in levels of aggression were determined using PROC MIXED with colony pair as a fixed factor in the model, trials nested within pair as a random variable, and the difference between maximum aggression scores recorded 6 months after and at the start of the laboratory colony fusion assay as the dependent variable. Differences in changes in aggression between colonies that fused and those that did not were tested using PROC MIXED, with fusion after 6 months and pair nested within fusion as fixed factors in the model and trials nested within pair by fusion as a random variable. Mean separation was carried out by least squares means (LSMeans). Comparisons between means of two samples were analysed with Student's *t* tests.

Differences in numbers of workers fighting, worker mortality at 24 h, and the proportion of marked surviving workers (higher over lower survival) in the laboratory fusion assay were determined using PROC MIXED with colony pair as a fixed factor in the model, run nested within pair as a random variable, total workers fighting, workers killed and proportion of surviving workers (after square-root transformation) as dependent variables. Differences in number of workers fighting in the field assay were determined with an ANOVA using PROC GLM, and means were separated with a least significance difference test.

To determine if aggressive interactions could explain the results of the laboratory colony fusion assay, we performed binary logistic regression with the number of workers fighting and the number of dead workers as independent variables and colony fusion within 24 h as the dependent variable. A similar analysis was performed with maximum intercolony aggression as the independent variable and colony fusion, within 24 h and after 6 months, as the dependent variable.

We performed a stepwise discriminant analysis (stepwise DA) on transformed quantitative cuticular hydrocarbon data using PROC STEPDISC to identify variables (GC peaks) that differed significantly between groups of workers. Peak areas were transformed using the formula: $Z_{ij} = \ln[Y_{ij}/g(Y_j)]$, where Z_{ij} is the standardized peak area *i* for individual *j*, Y_{ij} is the peak area *i* for individual *j*, and $g(Y_j)$ is the geometric mean of all peaks for individual *j* (Reyment 1989). Only transformed variables that met the assumptions of homogeneity of variance (Brown and Forsythe's test) were used in stepwise DA. We then performed a canonical DA on the selected peaks using PROC DISCRIM. Pairwise generalized square distances between group means (centroids) were used as an estimate of the degree of cuticular hydrocarbon differentiation between colonies.

Spearman rank correlation coefficients were used to determine relationships between aggression levels and percentage of colony fusion (24 h and 6 months) versus genetic similarity between colonies (pairwise F_{ST} and % alleles shared); and between intraspecific aggression, colony fusion and genetic similarity versus cuticular hydrocarbon similarity. The significance of the regression coefficient was tested by Mantel's (1967) test in GENPOP using 10 000 permutations. All means reported are followed by standard errors.

RESULTS

Intercolony Worker–Worker Aggression

Levels of intercolony aggression at the end of the laboratory fusion assay differed from those recorded at the beginning of the assay as indicated by aggression levels measured between pairs and their respective control colonies 6 months after the start of the experiment, with changes in aggression (increase or decrease) differing between colony pairs ($F_{9,5,5} = 6.46$, $P = 0.0213$; Fig. 2). Aggression levels decreased considerably from initial values in colonies that fused versus those that did not fuse (paired *t* test: $t_{6,5} = 6.16$, $P = 0.0006$). After 6 months, aggression in colony pairs in which all replicates (100%)

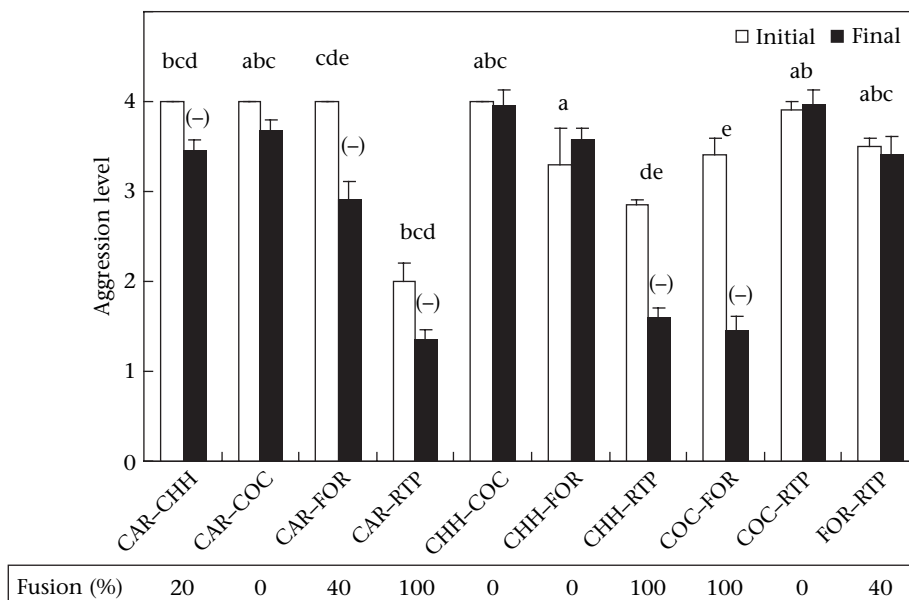


Figure 2. Mean (± 1 SE) worker–worker level of intercolony aggression before (initial) and 6 months after (final) the laboratory colony fusion assay started. Set of bars with different letters indicate significant differences in aggression changes across colony pairs ($P < 0.05$, least significance difference test). Aggression levels decreased significantly (–) in five colony pairs throughout the experiment. See Table 1 for colony abbreviations.

fused (CAR–RTP, CHH–RTP, COC–FOR) was significantly lower than that recorded before fusion, while it remained high between colony pairs that did not fuse (Fig. 2). Aggression averaged across all replicates also decreased in colony pairs with lower (20–40%) fusion rates (CAR–CHH, CAR–FOR, FOR–RTP; Fig. 2), and even greater changes were recorded only in the fused replicates: CAR–CHH, from 4 to 2.6 ($t_{10,8} = -4.69$, $P = 0.0007$); CAR–FOR, from 4 to 2.7 ($t_{6,8} = -3.41$, $P = 0.0118$); and FOR–RTP, from 3.5 to 2.7 ($t_{6,8} = -2.04$, $P = 0.0827$).

Laboratory Colony Fusion Assay

Argentine ant colony fusion varied across colony pairs, with rates of fusion increasing over time in some highly aggressive pairs (Table 1). All CAR–RTP and COC–FOR replicates fused within 24 h (100% fusion). Fusion rates increased from 50% at 24 h to 100% after a month in CHH–RTP. Fewer FOR–RTP and CAR–FOR replicates fused (40%), while all queens were killed in at least one of the paired colonies in the other replicates. Similarly, queens from one of the paired colonies were eliminated in CAR–CHH replicates that did not fuse, with only 20% of the replicates fusing. CAR–COC, COC–RTP, CHH–COC and CHH–FOR did not fuse, and one of the colonies was eliminated in all pairs except CHH–FOR, where workers were mixed in all replicates, while all queens from either one of the colonies were killed. Rates of fusion recorded at 24 h and at 6 months were highly correlated (Pearson correlation: $r_8 = 0.8926$, $N = 10$, $P = 0.0005$).

The total number of workers fighting during the first 24 h was a strong predictor of colony fusion within 24 h ($\chi^2_{75} = 16.45$, $P < 0.0001$) with fusion decreasing as the

number of workers fighting increased (Fig. 3a). Fusion generally occurred when worker mortality was low ($\chi^2_{75} = 11.69$, $P = 0.0006$; Fig. 3b), and an even stronger relationship ($\chi^2_{75} = 13.32$, $P = 0.0003$) was found when CHH–FOR, the colony pair in which only workers but not queens mixed and worker percent mortality was low ($22.27 \pm 5.43\%$), was excluded from the analysis. Colony pairs with lower levels of aggression, CAR–RTP and CHH–RTP, both fused consistently and had low worker percentage mortality at 24 h, $5.60 \pm 0.39\%$ and $30.32 \pm 3.95\%$, respectively, while a pair with higher

Table 1. Initial levels of worker–worker aggression (mean ± 1 SE) and time of fusion for 10 colony pairs

Colony pair	Aggression scores (N=12)	Number of fused replicates*				
		24 h	144 h	1 month	2 months	6 months
CAR–CHH	4.00 \pm 0.00	0	0	0	2	2
CAR–COC	4.00 \pm 0.00	0	0	0	0	0
CAR–FOR	4.00 \pm 0.00	1	2	2	2	2
CHH–COC	4.00 \pm 0.00	0	0	0	0	0
COC–RTP	3.90 \pm 0.10	0	0	0	0	0
FOR–RTP	3.50 \pm 0.10	0	2	2	2	2
COC–FOR	3.40 \pm 0.20	5	5	5	5	5
CHH–FOR	3.30 \pm 0.40	0	0	0	0	0
CHH–RTP	2.85 \pm 0.05	5	9	10	10	10
CAR–RTP	2.00 \pm 0.20	10	10	10	10	10

CAR, Cary; CHH, Chapel Hill; COC, Greenville; FOR, Winston-Salem; RTP, Research Triangle Park. N = number of replicates.

*Ten replicates for CAR–CHH, CAR–COC, CHH–FOR, CHH–RTP and CAR–RTP; and five replicates for CAR–FOR, CHH–COC, COC–RTP, FOR–RTP and COC–FOR.

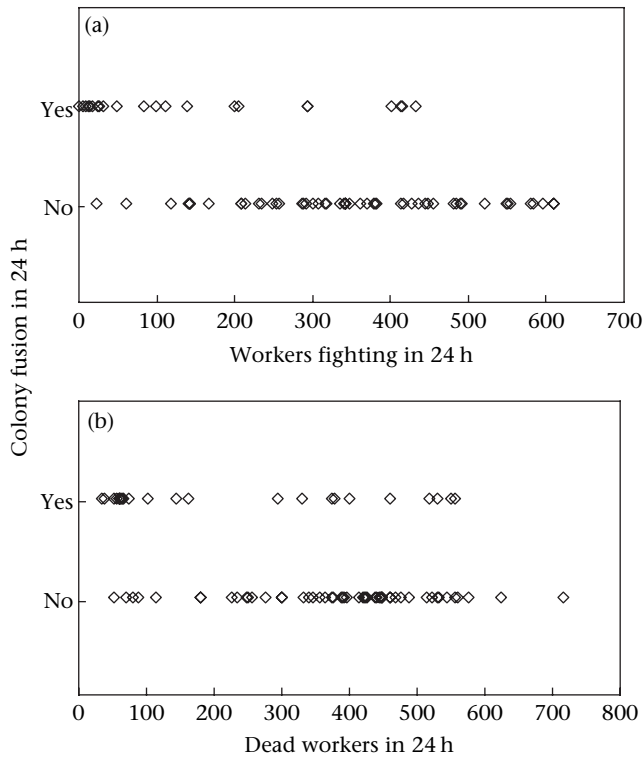


Figure 3. Relationship between number of ants (a) fighting and (b) killed in the first 24 h of contact and colony fusion within 24 h.

aggression in which all replicates fused, COC–FOR, had higher worker mortality, $40.00 \pm 4.10\%$, than either of these two pairs ($t_{13} = 12.13$, $P < 0.0001$, and $t_{13} = 1.60$, $P = 0.0667$, respectively). Worker mortality at 24 h ranged from $34.24 \pm 2.50\%$ (CAR–CHH) to $56.62 \pm 1.44\%$ (CAR–FOR) in colony pairs in which some replicates fused, however, mortality of fused and nonfused replicates was similar ($t_{18} = 0.6124$, $P = 0.2739$). Worker mortality ranged from $38.03 \pm 4.27\%$ (CAR–COC) to $53.46 \pm 4.83\%$ (CHH–COC) in pairs where no replicates fused. Aggression between source colonies measured before the assay started was also a robust predictor of fusion at 24 h ($\chi^2_{75} = 20.02$, $P < 0.0001$) and after 6 months ($\chi^2_{75} = 20.58$, $P = 0.0004$).

Field Experiments

The number of workers fighting in the field assay differed between colony pairs ($F_{2,2} = 159.70$, $P = 0.0062$), with the highest numbers recorded for CHH–RTP (226.50 ± 7.86), while fewer and no workers fought in the CAR–RTP pair (83.67 ± 4.58) and RTP control, respectively. Similarly, more CHH–RTP workers (293.00 ± 42.82) than CAR–RTP (22.60 ± 7.50) workers fought in the laboratory assay where worker fights and mortality were strongly correlated (Pearson correlation: $r_3 = 0.7211$, $N = 5$, $P < 0.0001$).

Cary (CAR) and CHH ants fused with RTP ants with rates of fusion ranging from $50.00 \pm 16.67\%$ (CHH–RTP) to $83.33 \pm 16.67\%$ (CAR–RTP) at 6 days. These rates were similar to those recorded in the laboratory at 24 h, $50.00 \pm 10.00\%$ for CHH–RTP and $100 \pm 0.00\%$ for CAR–RTP ($t_2 = 0.001$, $P = 0.4998$ and $t_2 = 1.00$, $P = 0.2113$,

respectively). However, only CAR–RTP had fusion rates comparable with those in the laboratory at 6 days ($t_2 = 1.00$, $P = 0.2113$) while CHH–RTP fusion rates at 6 days ($90.00 \pm 10.00\%$) were higher in the laboratory ($t_2 = 4.00$, $P = 0.0286$). In addition to the lack of increase in fusion rates between field CHH and RTP ants, queen survival was considerably lower with $62.83 \pm 2.72\%$ of queens alive at 12 days in nests from this assay while $84 \pm 0.00\%$ of queens survived after a month in laboratory colonies ($t_2 = 7.78$, $P = 0.0080$).

Levels of Genetic Similarity between Colonies

Colonies from different locations were genetically differentiated ($F_{ST} = 0.27 \pm 0.062$). We found a relationship between pairwise F_{ST} and colony fusion at 24 h (Spearman correlation: $r_s = -0.7451$, $N = 10$, $P = 0.0109$), and at 6 months ($r_s = -0.8799$, $N = 10$, $P = 0.0090$; Fig. 4a). Percentage alleles shared varied across colony pairs, ranging from 30.3% (CHH–COC) to 63.3% (CAR–RTP). A positive association was found between % alleles shared and rates of colony fusion at 24 h ($r_s = 0.7331$, $N = 10$, $P = 0.0101$), and at 6 months ($r_s = 0.6229$, $N = 10$, $P = 0.0487$; Fig. 4b). Also, a negative relationship was found between maximum aggression score and genetic similarity between colonies ($r_s = -0.8004$, $N = 10$, $P = 0.0167$).

Cuticular Hydrocarbon Analysis

The stepwise DA selected 18 variables that grouped all workers according to their colony of origin (Wilk's

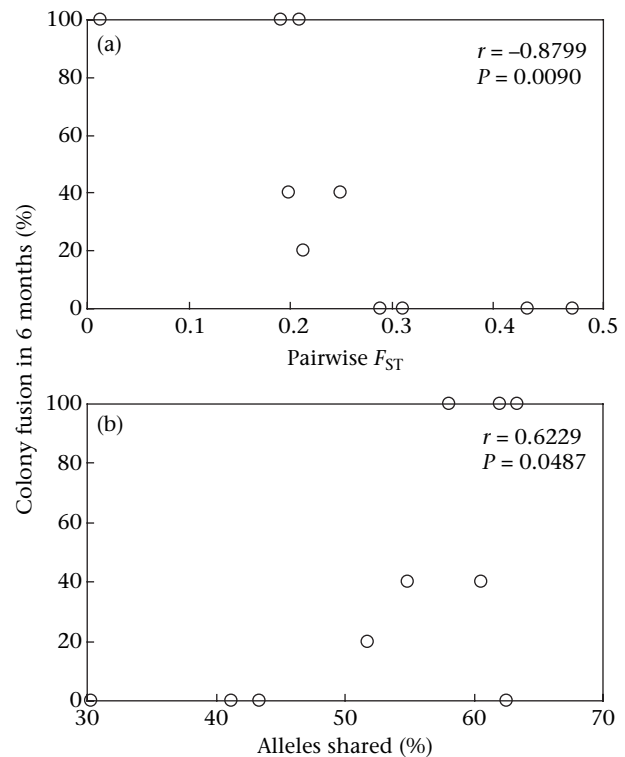


Figure 4. Relationship of (a) pairwise F_{ST} between colonies and (b) genetic similarity (% alleles shared) versus colony fusion at 6 months.

$\lambda < 0.01$, $F_{72,92.8} = 151.23$, $P < 0.0001$) with functions 1 and 2 explaining 68.7% and 26.2% of the total variation, with generalized square distances between colony centroids (cuticular hydrocarbon similarity) ranging from 184 (CAR–RTP) to 4521 (COC–RTP). We found a relationship between worker cuticular hydrocarbon similarities between colonies and levels of worker–worker aggression ($r_S = 0.5378$, $N = 10$, $P = 0.0500$) with aggression increasing with greater worker cuticular hydrocarbon profile dissimilarities between colonies. Also, we found an association between cuticular hydrocarbon similarities and colony fusion at 24 h ($r_S = -0.5125$, $N = 10$, $P = 0.0331$) but not at 6 months ($r_S = -0.4513$, $N = 10$, $P = 0.0896$). A strong relationship between similarities of worker cuticular hydrocarbons and both the percentage of alleles shared ($r_S = -0.7576$, $N = 10$, $P = 0.0260$; Fig. 5a) and pairwise F_{ST} ($r_S = 0.6991$, $N = 10$, $P = 0.0400$; Fig. 5b) was found.

DISCUSSION

We provide evidence that introduced *L. humile* colonies fuse in the absence of barriers preventing their encounters, and that colony fusion is regulated by levels of intraspecific aggression and both genetic and phenotypic similarity between interacting colonies. We suggest that fusion of unrelated but phenotypically similar colonies may be a route by which introduced *L. humile* populations can achieve extreme unicoloniality after strong initial

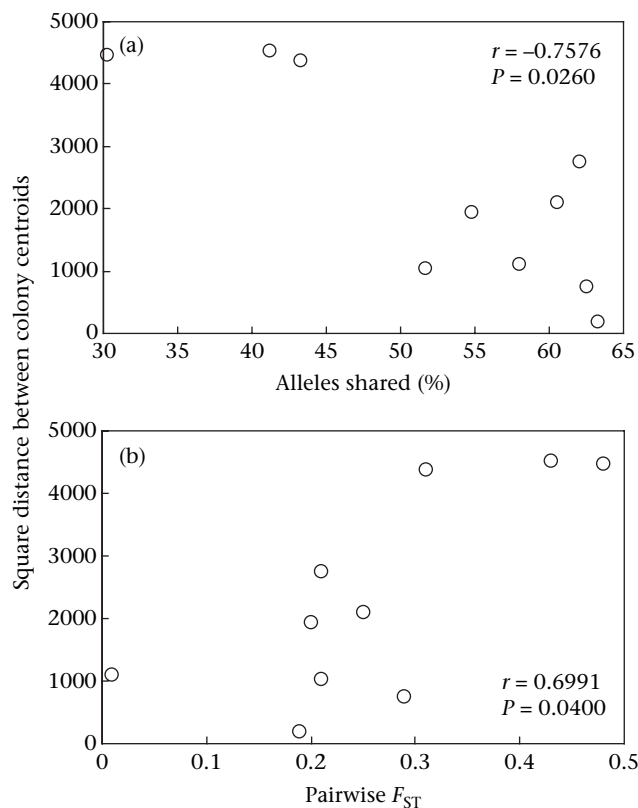


Figure 5. Relationship of (a) genetic similarity (% alleles shared) and (b) pairwise F_{ST} between colonies versus cuticular hydrocarbon similarity (generalized square distance between colony centroids).

levels of intraspecific aggression subside, thereby influencing distribution patterns and the ability to dominate new habitats. Current explanations for the formation of invasive *L. humile* supercolonies invoke a reduction in intraspecific aggression because of loss in recognition cue diversity arising from lack of overall genetic differentiation among separated nests following a genetic bottleneck (Tsutsui et al. 2000), or increased encounter rates between colonies under relaxed ecological constraints leading to emergence of supercolonies composed of individuals sharing the most common recognition alleles (Giraud et al. 2002). We investigated interactions between southeastern *L. humile* colonies, a population that possibly experienced a less severe bottleneck than the California population (Buczowski et al. 2004), and found a range of outcomes, from complete colony elimination to fusion. These outcomes can be explained by the level of intraspecific aggression and genetic similarity between colony pairs, with colony fusion occurring despite distances between collection sites as great as 290 km. Therefore, ecological constraints limiting behavioural interactions and the relatively high phenotypic diversity of *L. humile* populations in the southeastern U.S.A. may be preventing expansive supercolony formation.

The outcome of our colony interactions, fusion or complete elimination of one of the groups, was largely determined by levels of aggression and worker mortality, supporting Roulston et al. (2003), where lower worker mortality resulted in merging, and high aggression decreased chances for fusion; however, we also observed fusion between some colony pairs where extensive worker mortality occurred. In social insects, colony size, presence of surrounding nestmates, habituation and proportion of aggressive individuals can affect aggressive interactions between colonies (Binder 1988; Hölldobler & Wilson 1990; Langen et al. 2000). For example, in an arboreal nesting termite, high aggression leads to elimination of one of a pair of colonies, while either continuous avoidance or merging results after the elimination of the most aggressive individuals in lower aggression pairs (Leponce et al. 1996). Similarly, elimination of the most aggressive *L. humile* phenotypes and/or less genetically similar individuals may have increased the levels of similarity between groups and favoured merging. The different outcomes across replicates within the same colony pair may be partially explained by variation in colony composition (phenotypic heterogeneity, perceptive ability).

Our laboratory experiments used relatively small colonies in restricted spaces with limited food accessibility, which, in contrast to field populations, may have forced interactions. Our laboratory conditions could have either hindered fusion because of the lack of gradual acceptance with time or increased competition because of restricted food availability, or increased fusion by using small colonies that stopped fighting to prevent complete elimination. Even after the apparent elimination of the most aggressive individuals, possibly older workers (Hölldobler & Wilson 1990), thereby perhaps facilitating fusion between the least aggressive individuals (e.g. callows, brood and queens), not all colonies fused, indicating that intrinsic colony factors may also govern fusion. Among these

factors, queen number may play an important role in intercolony interactions, evidenced by increased tolerance to foreign ant conspecifics due to queenlessness (Boulay et al. 2003). Although numbers of workers, queens and brood in our experimental colonies do not reflect those of established field colonies, our results may be most relevant to incipient field colonies or peripheral colony satellites that contain fewer queens than central nests (Ingram 2002b). Similarly, queen number may, in part, explain the lower fusion rates observed between field colony fragments that had six times more queens than colonies used in the laboratory fusion assay.

Thomas et al. (2006) reported that high levels of aggression and genetic differentiation presumably prevented the exchange of individuals between adjacent *L. humile* field colonies, suggesting that only nonaggressive colonies fuse into larger and more productive supercolonies (Holway et al. 1998). Yet, we recorded fusion between genetically distinct colonies. These disparities may reflect a broader range of aggression and genetic similarity levels among the colonies we studied, which may represent a transitional state towards expansive supercolony development. Estimates of genetic differentiation (F_{ST}) between colonies that did not fuse were higher than those from colonies that fused, and comparable with estimates between aggressive supercolonies in which individuals did not intermix (Jaquierey et al. 2005; Thomas et al. 2006). Prior experience seems to increase aggression between *L. humile* colonies (Thomas et al. 2005) yet, we did not detect an increase in aggression following prolonged interactions between distant colony pairs. Our work and that of Tsutsui et al. (2000, 2003) support a genetic basis for aggressive behaviour and for modulating the outcome of intercolony interactions in introduced *L. humile* populations, which may be similar to the mechanism underlying fusion in some colonial marine invertebrates (Scofield et al. 1982; Grosberg & Hart 2000).

A model for nestmate recognition postulates that individuals match the phenotypic recognition cues of the encountered conspecific with the individual's neural template, which is an internal representation of the colony's recognition cues or labels derived from the environment, the individual's own phenotype, or all colony members (Breed & Bennett 1987). Hence, colony odour similarity may modulate fusion (Astruc et al. 2001) and merged colonies may form a more homogenous colony Gestalt if individuals in the mixed group bear only common labels (e.g. Boulay et al. 2003). Alternatively, merging of genetically heterogeneous groups can increase recognition cue diversity and produce an expanded neural template, thereby reducing aggression because of acceptance of more labels (Vander Meer & Morel 1998). Reduced intraspecific aggression towards unpaired control (source) colonies in our study suggests that fused colony pairs possess the most common recognition cues of both sources and/or a broader neural template than either source colony. However, whether this was achieved by cue transfer and chemical profile homogenization, or elimination of individuals bearing labels that made them more easily recognized as foreign is unclear. Overall, our findings suggest that fusion between small groups can gradually lead to

colony odour homogenization on a larger scale, and this recognition system plasticity may improve group success through increased colony size and superior competitive ability.

Following Reeve's (1989) acceptance threshold model, colony members will accept conspecifics when levels of cue-template dissimilarities are below the acceptance threshold, and fusion between colonies should vary accordingly. Phenotypic similarity between groups may also affect the balance between the costs of fighting versus merging, with the costs of fighting being higher when levels of similarity, and, therefore, reduced ability to distinguish non-nestmates from nestmates, are considerable. By examining worker cuticular hydrocarbon profiles we found that quantitative variation in cuticular hydrocarbons not only reflects colony identity but is also correlated with worker-worker aggression, fusion and overall genetic similarity between colony pairs. The association between overall genetic similarity and cuticular hydrocarbon similarity also suggests a possible association between variability at loci coding for recognition cues and variability in microsatellite loci.

Aggression attenuation through sharing of a common diet may have also promoted fusion between our moderately aggressive laboratory colony pairs (Buczowski et al. 2005). While this is possible, we also recorded fusion between freshly collected field colonies that did not have prior exposure to the same laboratory diet. Furthermore, in the field, it is likely that neighbouring colonies access the same food and nesting materials, which may facilitate fusion by masking key intrinsic recognition cue distinctions. Lower rates of fusion between colonies interacting in the field may reflect an influence of colony composition and queen reproductive status, position relative to colony boundaries, and environmental and seasonal effects.

Territoriality and well-established foraging ranges in termites and ants may limit opportunities for mixing of workers from different colonies (Adams 2003; Vargo 2003). However, highly saturated habitats may promote fusion between initially aggressive ant colonies under natural conditions (e.g. Foitzik & Heinze 1998). Additionally, reproduction by budding, constant internest exchange of individuals, extreme vagility and ability to cope with frequent colony fragmentation in polydomous ants (Hölldobler & Wilson 1977) may promote opportunities for colony fusion at natural contact zones. Although our colonies were collected from sites up to 402 km (CAR-COC) apart, human-mediated dispersal and the ability of incipient *L. humile* colonies to successfully establish in new habitats (Aron 2001; Suarez et al. 2001) may increase the chances for colonies of distinct origin to meet and fuse.

Colony fusion has been suggested as a route towards polygyny and unicoloniality in ants (Herbers 1993; Bourke & Franks 1995; Crozier & Pamilo 1996) and as a mechanism leading to complex family structure in some termites, particularly *Reticulitermes* species (Clément 1986; Matsuura & Nishida 2001; Deheer & Vargo 2004). Changes in colony structure may be due to powerful colony-level selection, in the context of unusual ecological conditions that seem to have overridden the selfish

interests of nest members (Sudd & Franks 1987). Thus, fusion may represent an adaptive tactic if the cost of fusion is lower than that of an intercolony battle, and if it benefits the colony by increasing its labour force and/or expanding its foraging range, thereby increasing colony survival and productivity (Su & Scheffrahn 1988; Matsuura & Nishida 2001). Fusion of nonaggressive *L. humile* experimental colony pairs increased rates of resource retrieval, brood and worker production (Holway et al. 1998), interference competition and exploitative ability (Holway & Case 2001). Similarly, we expect that fusion of aggressive colonies would result in larger and more productive colonies if the benefits from increased colony size supersede the costs of initial high mortality.

Recent evidence indicates that gene flow between relatively close or contiguous aggressive supercolonies is absent (Jaquiere et al. 2005; Thomas et al. 2006). However, this does not preclude that these and other *L. humile* supercolonies were once distinct colonies that subsequently fused. Our results may reflect the varying degree of interactions occurring between colonies from different origins in their early stages of establishment (recently invaded areas), or between foreign colony fragments encountering newly budded nests from established colonies where mixing of individuals is more likely than between nests of well-established colonies (Ingram & Gordon 2003).

Our findings on colony fusion in the Argentine ant raise two major questions: (1) does fusion produce a homogenization of recognition cues, that is, cuticular hydrocarbons, and (2) does fusion of unrelated colonies increase colony productivity and provide clear colony fitness benefits, that is, numerical advantage and greater chances to monopolize resources? A better knowledge of the conditions and factors affecting the expression of uniclonality in ants would shed light on the selection pressures shaping this social organization, the levels at which selection may be acting upon, and its benefits and costs, and would greatly contribute our understanding of invasive ant species and the complexity of their ecological success.

Acknowledgments

We thank C. Schal, E. Vargo, W. Watson and two anonymous referees for helpful comments on the manuscript. We also thank C. Brownie for statistical advice, and A. Carper, S. Hutchens, J. Leonard, and P. Labadie for technical assistance. This study was supported by the Blanton J. Whitmire Endowment at North Carolina State University and the David R. Nimocks Jr. Fellowship (G.V.)

References

- Adams, E. S. 2003. Experimental analysis of territory size in a population of the fire ant *Solenopsis invicta*. *Behavioral Ecology*, **14**, 48–53.
- Aron, S. 2001. Reproductive strategy: an essential component in the success of incipient colonies of the invasive Argentine ant. *Insectes Sociaux*, **48**, 25–27.
- Astruc, C., Malosse, C. & Errard, C. 2001. Lack of intraspecific aggression in the ant *Tetramorium bicarinatum*: a chemical hypothesis. *Journal of Chemical Ecology*, **27**, 1229–1248.
- Bhatkar, A. D. & Whitcomb, W. H. 1970. Artificial diet for rearing various species of ants. *Florida Entomologist*, **53**, 229–232.
- Binder, B. F. 1988. Intercolonial aggression in the subterranean termite *Heterotermes aureus* (Isoptera: Rhinotermitidae). *Psyche*, **95**, 123–137.
- Boulay, R., Katzav-Gozansky, T., Vander Meer, R. K. & Hefetz, A. 2003. Colony insularity through queen control on worker social motivation in ants. *Proceedings of the Royal Society of London, Series B*, **270**, 971–977.
- Bourke, A. F. G. & Franks, N. R. 1995. *Social Evolution in Ants*. Princeton, New Jersey: Princeton University Press.
- Breed, M. D. & Bennett, B. 1987. Kin recognition in highly eusocial insects. In: *Kin Recognition in Animals* (Ed. by D. J. C. Fletcher & C. D. Michener), pp. 243–285. New York: J. Wiley.
- Buczowski, G., Vargo, E. L. & Silverman, J. 2004. The diminutive supercolony: the Argentine ants of the southeastern United States. *Molecular Ecology*, **13**, 2235–2242.
- Buczowski, G., Kumar, R., Suib, S. L. & Silverman, J. 2005. Diet-related modification of cuticular hydrocarbon profiles of the Argentine ant, *Linepithema humile*, diminishes intercolony aggression. *Journal of Chemical Ecology*, **31**, 829–843.
- Chapman, R. E. & Bourke, A. F. G. 2001. The influence of sociality on the conservation biology of social insect. *Ecology Letters*, **4**, 650–662.
- Clément, J.-L. 1986. Open and closed societies in *Reticulitermes* termites (Isoptera, Rhinotermitidae), geographic and seasonal variations. *Sociobiology*, **11**, 311–323.
- Crozier, R. H. & Pamilo, P. 1996. *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. New York: Oxford University Press.
- Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. *Biological Journal of the Linnaean Society*, **61**, 153–181.
- Deheer, C. J. & Vargo, E. L. 2004. Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. *Molecular Ecology*, **13**, 431–441.
- Foitzik, S. & Heinze, J. 1998. Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. *Behavioral Ecology*, **4**, 367–375.
- Gamboa, G. J., Reeve, H. K., Ferguson, I. D. & Wacker, T. L. 1986. Nestmate recognition in social wasps: the origin and acquisition of recognition odours. *Animal Behaviour*, **34**, 685–695.
- Giraud, T., Pedersen, J. S. & Keller, L. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences, U.S.A.*, **99**, 6075–6079.
- Goudet, J. 1995. FSTAT (v.1.2): a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486. <http://www2.unil.ch/popgen/softwares/fstat.htm>.
- Grosberg, R. K. & Hart, M. W. 2000. Mate selection and the evolution of highly polymorphic self/nonself recognition genes. *Science*, **289**, 2111–2114.
- Heller, N. E. 2004. Colony structure in introduced and native populations of the invasive Argentine ant, *Linepithema humile*. *Insectes Sociaux*, **51**, 378–386.
- Herbers, J. M. 1993. Ecological determinants of queen number in ants. In: *Queen Number and Sociality in Insects* (Ed. by L. Keller), pp. 262–293. Oxford: Oxford University Press.
- Hölldobler, B. & Wilson, E. O. 1977. The number of queens: an important trait in an evolution. *Naturwissenschaften*, **64**, 8–15.
- Hölldobler, B. & Wilson, E. O. 1990. *The Ants*. Cambridge, Massachusetts: Harvard University Press.
- Holway, D. A. & Case, T. J. 2001. Effects of colony-level variation on competitive ability in the invasive Argentine ant. *Animal Behaviour*, **61**, 1181–1192.

- Holway, D. A. & Suarez, A. V. 2004. Colony structure variation and interspecific competitive ability in the invasive Argentine ant. *Oecologia*, **138**, 216–222.
- Holway, D. A., Suarez, A. V. & Case, T. J. 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science*, **282**, 949–952.
- Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D. & Case, T. J. 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics*, **33**, 181–233.
- Human, K. G. & Gordon, D. M. 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia*, **105**, 405–412.
- Ingram, K. K. 2002a. Flexibility in nest density and social structure in invasive populations of the Argentine ant, *Linepithema humile*. *Oecologia*, **133**, 492–500.
- Ingram, K. K. 2002b. Plasticity in queen number and social structure in the invasive Argentine ant (*Linepithema humile*). *Evolution*, **56**, 2008–2016.
- Ingram, K. K. & Gordon, D. M. 2003. Genetic dispersal dynamics in an invading population of Argentine ants. *Ecology*, **84**, 2832–2842.
- Jaquiéry, J., Vogel, V. & Keller, L. 2005. Multilevel genetic analyses of two European supercolonies of the Argentine ant, *Linepithema humile*. *Molecular Ecology*, **14**, 589–598.
- Keller, L. & Ross, K. G. 1998. Selfish genes: a green beard in the red fire ant. *Nature*, **394**, 573–575.
- Krieger, M. J. B. & Keller, L. 1999. Low polymorphism at 19 microsatellite loci in a French population of Argentine ants (*Linepithema humile*). *Molecular Ecology*, **8**, 1075–1092.
- Lacy, R. C. & Sherman, P. W. 1983. Kin recognition by phenotype matching. *American Naturalist*, **121**, 489–512.
- Langen, T. A., Tripet, F. & Nonacs, P. 2000. The red and the black: habituation and the dear-enemy phenomenon in two desert *Pheidole* ants. *Behavioral Ecology and Sociobiology*, **48**, 285–292.
- Leponce, M., Roisin, Y. & Pasteels, J. M. 1996. Intraspecific interactions in a community of arboreal nesting termites (Isoptera: Termitidae). *Journal of Insect Behavior*, **9**, 799–817.
- Liang, D. & Silverman, J. 2000. 'You are what you eat': diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften*, **87**, 412–416.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- Matsuura, K. & Nishida, T. 2001. Colony fusion in a termite: what makes the society 'open'? *Insectes Sociaux*, **48**, 378–383.
- Moller, H. 1996. Lessons for invasion theory from social insects. *Biological Conservation*, **78**, 125–142.
- Passera, L. 1994. Characteristics of tramp species. In: *Exotic Ants: Biology, Impact and Control of Introduced Species* (Ed. by D. F. Williams), pp. 23–43. Boulder, Colorado: Westview Press.
- Reeve, H. K. 1989. The evolution of conspecific acceptance thresholds. *American Naturalist*, **133**, 407–435.
- Reyment, R. A. 1989. Compositional data analysis. *Terra Nova*, **1**, 29–34.
- Ross, K. G. & Keller, L. 1995. Ecology and evolution of social organization: insights from fire ants and other social insects. *Annual Review of Ecology and Systematics*, **26**, 631–656.
- Roulston, T. H., Buczkowski, G. & Silverman, J. 2003. Nestmate discrimination in ants: effect of bioassay on aggressive behaviour. *Insectes Sociaux*, **50**, 151–159.
- SAS Institute. 2000. *SAS Online Document, Version 8.2*. Cary, North Carolina: SAS Institute.
- Scofield, V. L., Schlumberger, J. M., West, L. A. & Weissman, I. L. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. *Nature*, **295**, 499–502.
- Sherman, P. W. & Holmes, W. G. 1985. Kin recognition: issues and evidence. In: *Experimental Behavioral Ecology and Sociobiology* (Ed. by B. Hölldobler & M. Lindauer), pp. 437–460. Massachusetts: Sinauer.
- Su, N.-Y. & Scheffrahn, R. H. 1988. Intra- and interspecific competition of the Formosan and the eastern subterranean termite, evidence from field observations (Isoptera: Rhinotermitidae). *Sociobiology*, **14**, 157–164.
- Suarez, A. V., Tsutsui, N. D., Holway, D. A. & Case, T. J. 1999. Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biological Invasions*, **1**, 43–53.
- Suarez, A. V., Holway, D. A. & Case, T. J. 2001. Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. *Proceedings of the National Academy of Sciences, U.S.A.*, **98**, 1095–1100.
- Suarez, A. V., Holway, D. A., Liang, D. S., Tsutsui, N. D. & Case, T. J. 2002. Spatiotemporal patterns of intraspecific aggression in the invasive Argentine ant. *Animal Behaviour*, **64**, 697–708.
- Sudd, J. H. & Franks, N. R. 1987. *The Behavioural Ecology of Ants*. New York: Chapman & Hall.
- Thomas, M. L., Tsutsui, N. D. & Holway, D. A. 2005. Intraspecific competition influences the symmetry and intensity of aggression in the Argentine ant. *Behavioral Ecology*, **16**, 472–481.
- Thomas, M. L., Payne, C., Suarez, A. V., Tsutsui, N. D. & Holway, D. A. 2006. When supercolonies collide: territorial aggression in an invasive and unicolonial social insect. *Molecular Ecology*, **15**, 4303–4315.
- Tsutsui, N. D. & Case, T. J. 2001. Population genetics and colony structure of the Argentine ant (*Linepithema humile*) in its native and introduced range. *Evolution*, **55**, 976–985.
- Tsutsui, N. D., Suarez, A. V., Holway, D. A. & Case, T. J. 2000. Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences, U.S.A.*, **97**, 5948–5953.
- Tsutsui, N. D., Suarez, A. V. & Grosberg, R. K. 2003. Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *Proceedings of the National Academy of Sciences, U.S.A.*, **100**, 1078–1083.
- Vander Meer, R. K. & Morel, L. 1998. Nestmate recognition in ants. In: *Pheromone Communication in Social Insects* (Ed. by R. K. Vander Meer, M. Breed, M. Winston & K. E. Espelie), pp. 79–103. Boulder, Colorado: Westview Press.
- Vargo, E. L. 2003. Genetic structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) colonies in an urban habitat and tracking of colonies following treatment with hexaflumuron bait. *Environmental Entomology*, **32**, 1271–1282.
- Walters, A. C. & Mackay, D. A. 2003. An experimental study of the relative humidity preference and survival of the Argentine ant, *Linepithema humile* (Hymenoptera, Formicidae): comparisons with a native *Iridomyrmex* species in South Australia. *Insectes Sociaux*, **50**, 355–360.