The diminutive supercolony: the Argentine ants of the southeastern United States

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Abstract

Native to Argentina and Brazil, the Argentine ant (*Linepithema humile*) is an invasive species that has become established on six continents and many oceanic islands. In several parts of its introduced range, including the western United States, southern Europe and Chile, the Argentine ant is unicolonial, forming extensive supercolonies. We examined population genetic structure and intercolony aggression in two regions of the introduced range of this species in the United States: California and the southeastern United States. Our results show that the southeastern *L. humile* population has high genotypic variability and strong intercolony aggression relative to the California population. In the California population, intercolony aggression was absent and 23 alleles were found across seven polymorphic microsatellite loci. However, in the Southeast, aggression between colonies was high and 47 alleles were present across the same seven loci in an equal number of colonies. We suggest that distinctly different colonization patterns for California and the Southeast may be responsible for the striking disparity in the genetic diversity of introduced populations. Southeastern colonies may have descended from multiple, independent introductions from the native range, undergoing a bottleneck at each introduction. In contrast, the California supercolony may have originated from one or more colonies inhabiting the southeastern United States, thus experiencing a double bottleneck. The differences in present-day distribution patterns between California and the Southeast may be due to the combined effect of two factors: lower winter temperatures in the Southeast and/or competition with another successful and widely distributed ant invader, the fire ant *Solenopsis invicta*.

Keywords: Argentine ant, introduced species, invasive ants, microsatellites, nestmate recognition, unicoloniality

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Introduction

Populations of living organisms can undergo dramatic evolutionary changes due to natural disasters and/or human-mediated changes in the environment. When a population is severely reduced in size, the genetic makeup of the surviving population is unlikely to be representative of the original population and the surviving population may suffer from the bottleneck effect (Wright 1931; Nei *et al*. 1975). Genetic bottlenecks cause a loss of genetic variation, and produce genetically atypical populations (Nei *et al*. 1975; Chakraborty & Nei 1977). Certain alleles are lost among the survivors, affecting their ability to survive

changes in the environment (Allendorf & Leary 1986; Newman & Pilson 1997). Despite the negative consequences of genetic bottlenecks, such as inbreeding depression and limited opportunities for evolution, many bottlenecked invasive species thrive in their introduced environments. In several social insects, changes in genetic composition associated with colonization events have been accompanied by dramatic changes in social organization and behaviour of the founding population (e.g. Ross *et al*. 1993, 1996; Ross & Keller 1995; Tsutsui *et al*. 2000).

The invasive Argentine ant (*Linepithema humile*) is one of the best-studied examples of an introduced ant species. Native to South America, the Argentine ant has become established in many parts of the world (Suarez *et al*. 2001), where it is a serious ecological, agricultural and urban pest

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(Newell & Barber 1913; Human & Gordon 1996; Holway 1998). The success of the Argentine ant can be attributed to changes in its social behaviour and colony structure (Tsutsui *et al*. 2000, 2003; Giraud *et al*. 2002). Recent evidence from behavioural and population genetic studies indicates that in its native range the Argentine ant is multicolonial, whereby territorial boundaries between colonies are well defined, nests are aggressively defended against conspecifics and colonies are genetically differentiated (Suarez *et al*. 1999; Tsutsui *et al*. 2000). In its introduced range, including California, southern Europe and Chile, however, the Argentine ant is unicolonial, forming large supercolonies with poorly defined boundaries and multiple interconnected nests (Suarez *et al*. 1999; Tsutsui *et al*. 2000; Tsutsui & Case 2001; Giraud *et al*. 2002). Both workers and queens move freely between nests (Newell & Barber 1913; Markin 1970). Such unicolonial behaviour promotes efficient food discovery, defence and retrieval (Human & Gordon 1996; Holway *et al*. 1998; Holway 1999), characteristics which may give the Argentine ant an advantage over native ant species (Holway & Case 2001). In California and southern Europe, the majority of the introduced populations exist as a single behaviourally defined supercolony, with intraspecific aggression being very rare (Tsutsui *et al*. 2000; Giraud *et al*. 2002).

Recently, two hypotheses have been proposed to explain the evolution of unicoloniality in *L. humile* (Tsutsui *et al*. 2000; Giraud *et al*. 2002). Tsutsui *et al*. (2000) proposed that the formation of the California supercolony was a direct consequence of a genetic bottleneck that reduced the genetic diversity of introduced populations. Low levels of genetic variability produced insufficient phenotypic variability in the chemical cues underlying nestmate recognition. Nestmate recognition in the Argentine ant is influenced by genetic (Tsutsui *et al*. 2000; Suarez *et al*. 2001) and environmental (Chen & Nonacs 2000; Liang & Silverman 2000) inputs and when cues are genetically based, the precision of recognition depends ultimately on the degree of polymorphism and allelic frequencies at loci conferring recognition cues. As a result, low levels of genetic diversity may have led to the formation of a large, ecologically dominant supercolony. Furthermore, Tsutsui *et al*. (2003) demonstrated that the establishment and maintenance of the large California supercolony is due to strong directional selection against genetically diverse colonies. By contrast, Giraud *et al*. (2002) hypothesized that the genetic bottleneck experienced by colonies introduced into southern Europe was weak, relative to colonies introduced into California, playing only a minor role in the formation of the European supercolony. Instead, these authors proposed that introduction of colonies into a new habitat combined with relaxed ecological constraints resulted in accelerated colony growth and expansion, increasing competition and encounter rates between colonies. Giraud *et al*. (2002) suggested that during aggressive encounters, colonies possessing rare recognition alleles were eliminated, while colonies harbouring the most common recognition alleles gained a selective advantage. Consequently, Giraud *et al*. (2002) proposed that colonies possessing the most common recognition labels enjoyed a selective advantage due to reduced intercolony aggression. These colonies survived and dominated the landscape.

In contrast to *L. humile* populations reported from California, southern Europe and Chile, where supercolonies extend over thousands of square kilometres (Suarez *et al*. 1999; Tsutsui *et al*. 2000; Tsutsui & Case 2001; Giraud *et al*. 2002), our surveys in North Carolina revealed that Argentine ant colonies from the southeastern United States were small and patchily distributed. These colonies were almost always restricted to relatively small patches within urban landscapes (< 2500 m²). In preliminary trials, we recorded high intraspecific aggression between southeastern colonies over relatively small spatial scales (tens of kilometres), which suggested that the expansive supercolonies reported earlier for introduced populations (Tsutsui *et al*. 2000; Giraud *et al*. 2002) are not universal and may depend on genetic factors and/or regional environmental characteristics. To investigate more closely the apparent regional variation in Argentine ant population structure, we compared aggressive behaviour and genetic diversity among colonies from sites across similar spatial scales in California and portions of the southeastern United States.

Materials and methods

Study sites and rearing procedures

We collected ants along two 700-km transects. One transect was through an area of a supercolony in California (37.8 N–33.0 N and 122.2 W–117.0 W) described previously by Tsutsui *et al*. (2000); the other spanned three southeastern states: North Carolina, South Carolina and Georgia (33.0 N–36.1 N and 72.0 W–84.4 W). We sampled 16 sites in California: Berkeley, Cambria, Corona, Escondido, Guadalupe, King City, Lompoc, Monterey, Ojai, Pleasanton, Point Piedras Blancas, Refugio State Park, Riverside, San Luis Obispo, San Mateo and Santa Barbara. All sites sampled in California were different from those in Tsutsui *et al*. (2000). We did not know a priori whether ants collected in California belonged to the main supercolony or some of the smaller supercolonies, which have been discovered occasionally in the introduced range (Tsutsui *et al*. 2000; Tsutsui & Case 2001). We therefore refer to each of the collection sites as colonies rather than nests, although some of the collection sites may have belonged to the same supercolony. In the southeastern United States we sampled 16 sites: North Carolina (nine); Chapel Hill, Emerald Isle, Greenville, Holden Beach (two sites),

Jacksonville, Research Triangle Park, Shallotte and Winston-Salem; South Carolina (three): Greenville and Greer (two sites); and Georgia (four): Barnesville, Fayetteville, Gainesville and Griffin. At each collection site (except Chapel Hill and Winston-Salem) ants were sampled from a single nest. In Chapel Hill and Winston-Salem, Argentine ants used in behavioural assays and genetic analyses were collected from two to four neighbouring nests (5–20 m apart; often connected by visible trails). Our analysis of internest aggression and colony genetic structure at these two locations indicated that workers from adjacent nests were not aggressive [average aggression score 0.0 ± 0 on an 0–4 scale of Suarez *et al*. (1999)]. Moreover, genetic analysis on 10 workers from four nests indicated no significant difference in the genotypic distribution across nests in both Chapel Hill (χ^2 = 15.3, P = 0.75) and Winston-Salem (χ^2 = 16.1, P = 0.88) (GENEPOP; version 3.3, updated version of Raymond & Rousset 1995).

For each location, we established a single laboratory colony consisting of 10 000–15 000 workers, a few hundred queens and numerous brood collected from a single field nest, with the exception of Chapel Hill and Winston-Salem, as described above. Colonies were maintained in soil-free, Fluon™-coated trays. Nests were plastic dishes filled with moist grooved plaster. Colonies were provided with 25% sucrose solution *ad libitum*, hard-boiled egg once a week, and artificial diet (Bhatkar & Whitcomb 1970). For microsatellite genotyping, ants were placed in 95% ethanol at the time of collection and stored at –20 °C until DNA extraction.

Aggression tests (nestmate recognition bioassays)

We assessed intraspecific aggression with a colony introduction assay that measured the level of aggression in single worker introductions into a foreign colony (Roulston *et al*. 2003). Randomly selected 'intruder' workers were introduced into rearing trays containing 'resident' ants. Both intruder and resident workers were from recently collected field colonies and all tests were conducted within a week of collection. For each test, we allowed the intruder up to 25 encounters with resident ants. Each instance of direct physical contact between the intruder and any of the residents was regarded as an encounter. Aggressive behaviours were scored on a 0–4 scale described by Suarez *et al*. (1999) [0 = ignore, 1 = touch, 2 = avoid, 3 = aggression (lunging, brief bouts of biting and/or pulling), 4 = fighting (prolonged aggression, also abdomen-curling to deposit defensive compounds)]. Ten replicates per colony pair were performed: each colony served as residents five times, and five workers from that colony served as intruders. For each of the two geographical regions, we tested all 16 colonies in all possible pairwise colony combinations (120 intercolony pairings per region). Resident colonies were allowed to recover between trials to avoid any possible effects of sensitization or habituation to intruder workers. The maximum score per trial was used in the analysis.

Molecular techniques

Genomic DNA was extracted from 15 workers from each of 16 sites (240 individual genotypes per region) using the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) and analysed at seven polymorphic microsatellite loci: *Lhum-11*, *Lhum-13*, *Lhum-19*, *Lhum-28*, *Lhum-35*, *Lhum-39* (Krieger & Keller 1999) and *Lihu-T1* (Tsutsui *et al*. 2000). The polymerase chain reaction (PCR) products were labelled with an IRD dye using the method of Oetting *et al*. (1995), in which the forward primer in each primer pair had the first 19 base pairs (bp) of the M13 forward sequence attached to the 5′-end, and IRD-labelled M13 forward primer was included in the PCR. PCR reactions were multiplexed (*Lhum-35* with *Lhum-39*, *Lhum-11* with *Lhum-13*, and *Lhum-19* with *Lhum-28*) and performed with a Peltier PTC-200 thermocycler (MJ Research, Reno, NV, USA) with the following cycle parameters: initial denaturation at 94 °C (2 min), followed by 32 cycles at 94 °C (50 s), 56 °C (50 s) and 72 °C (1 min), and one final elongation step at 72 °C (5 min). PCR reactions were carried out in a 10 μL volume: 4 μL of genomic DNA (~20 ng), 1 μL 10× NH$_4$ reaction buffer, 0.2 μg bovine serum albumin (BSA), 2 mM MgCl$_2$, 0.2 mM of each dNTP, 0.32 μM M13F-29 IRD standard labelled primer, 0.4 U *Taq* polymerase and 0.1 μM of each primer. Amplification products were separated on 6.5% KB^{Plus} (Li-Cor) polyacrylamide sequencing gels using a 4000 L Li-Cor DNA sequencer and microsatellite alleles were scored using GeneImagIR software (Scanalytics Inc., Billerica, MA, USA).

Data analysis

Genetic diversity measures, including the number of observed alleles per locus (A_O) and the expected heterozygosity (H_E, unbiased for sample size), were calculated using the program Genetic Data Analysis (GDA; Lewis & Zaykin 2001). Private alleles, defined as those occurring in only one population, were identified in pairwise comparisons of colonies.

Genetic structure in each of the two regions was estimated using Wright's (1951, 1965) *F*-statistics as implemented in the program ARLEQUIN version 2.000 (Schneider *et al*. 2000), and their significance was tested using a nonparametric permutation procedure (Excoffier *et al*. 1992) with 10 000 permutations.

Pairwise F_{ST} values calculated between colonies were plotted against geographical distance to test for genetic isolation by distance. The significance of the regression coefficients was tested by Mantel's (1967) test in GENEPOP using 10 000 permutations.

Allele frequency data were tested for evidence of a recent genetic bottleneck by testing for heterozygosity excess (Cornuet & Luikart 1996; Luikart *et al.* 1998) using the program BOTTLENECK version 1.2.02 (Piry *et al.* 1999). Calculations were performed using both the infinite allele model (IAM) and the stepwise mutation model (SMM) of mutation. Two statistical tests, the sign test and the Wilcoxon signed-rank test, were conducted to determine whether there was significant heterozygosity excess. The tests were performed on each of the 20 replicate data sets for each of the two regions. In total, 80 tests were performed for each population (20 resampled data sets × two tests × two models for mutation drift equilibrium). The overall significance of the bottleneck tests across the re-sampled data sets was examined using Fisher's combined probability test (Sokal & Rolf 1995).

Estimates of *F*-statistics and tests for the genetic bottleneck were conducted using a reduced set of loci for each region. In California, we excluded *Lhum-28* and *Lihu-T1* from the analysis because they were monomorphic. In the Southeast, we excluded *Lhum-39* and *Lihu-T1* because they showed large differences between expected and observed heterozygosities, possibly due to the presence of a null allele or other scoring anomalies.

Results

Geographic variation in genetic diversity

Relative to Argentine ants from California, ants from the Southeast are genetically more diverse both in terms of allele numbers and heterozygosity (Table 1) ($P < 0.03$; two-tailed paired *t*-test, both values). Across the seven loci examined, the southeastern population had more than twice the number of alleles detected in the California population. All 23 alleles found in California were a subset
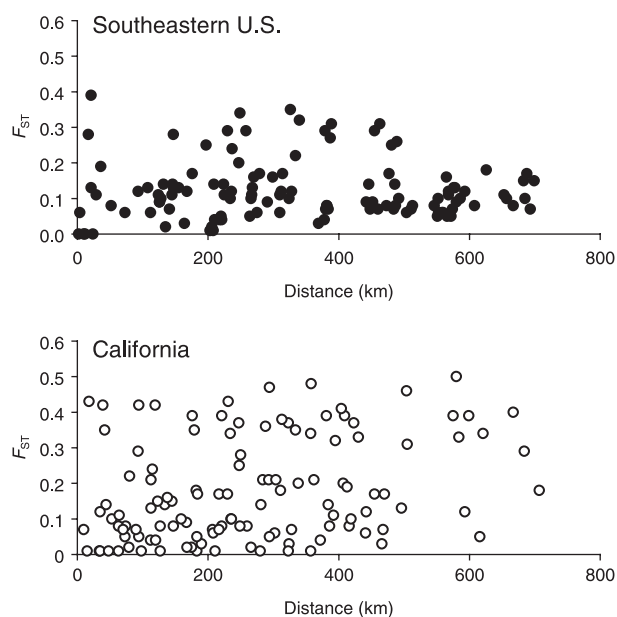

Fig. 1 Relationship between pairwise F_{ST} and geographical distance between colonies in the Southeast (●) and in California (○).

of those found in the Southeast. Similarly, the average expected multilocus heterozygosity in the Southeast was 33% higher than California. On average, colony pairs in California shared 75% of their alleles (range 50–95%, median 76%). In contrast, colony pairs in the Southeast shared only 57% of their alleles (range 30–77%, median 58%, *t*-test, $P < 0.0001$). A total of five private alleles were observed in the Southeast (mean frequency = 0.11), whereas no private alleles were observed in California.

Population genetic differentiation

A comparison of the genetic structure in the two ranges revealed that the degree of genetic differentiation between colonies was not significantly different between the ranges. The fixation index values (F_{ST}) were 0.126 ($P < 0.0001$) for the Southeast and 0.194 ($P < 0.0001$) for California, suggesting moderate to strong differences among sites within each range. The relationship between pairwise F_{ST} and geographical distance was not significant either in California ($r^2 = 0.099$, $P = 0.974$) or in the Southeast ($r^2 = 0.004$, $P = 0.732$) (Fig. 1A,B). We also tested for isolation by distance by using Slatkin's (1993) M, an estimator of *Nm*. The relationship between M and the geographical distance was not significant either in California ($r^2 = 0.063$, $P = 0.889$) or in the Southeast ($r^2 = 0.091$, $P = 0.065$).

Tests for genetic bottleneck

The results of the tests, which examined the allelic data for possible deviations from mutation drift equilibrium, were
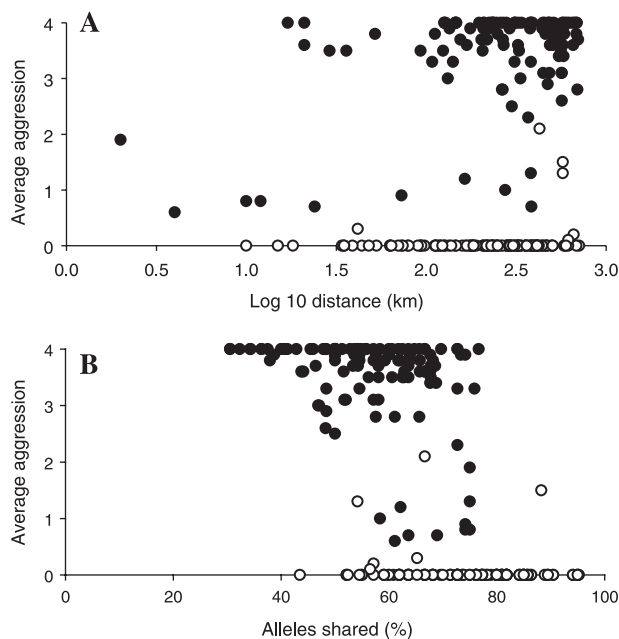
Table 1 The observed number of alleles (A_O) and the expected heterozygosity (H_E) for southeastern (North Carolina, South Carolina, Georgia) and Californian populations of *Linepithema humile*

Locus	Southeast (*n* = 240)		California (*n* = 240)	
	A_O	H_E	A_O	H_E
Lhum-11	8	0.806	4	0.641
Lhum-13	6	0.729	4	0.727
Lhum-19	7	0.736	4	0.660
Lhum-28	2	0.407	1	0.000
Lhum35	13	0.815	6	0.696
Lhum-39	6	0.554	3	0.326
Lihu-T1	5	0.497	1	0.000
Total	47		23	
Mean (SE)		0.649 (0.061)		0.436 (0.123)

Fig. 2 Relationships among intraspecific aggression, geographical distance, and genetic similarity in the Southeast (●) and in California (○). (A) Relationship between intraspecific aggression and distance between colonies. (B) Relationship between intraspecific aggression and genetic similarity between colony pairs (% alleles shared).

dependent on the mutation model, as well as the statistical test. The Wilcoxon signed-rank test, which is considered more robust than the sign test (Piry *et al.* 1999), revealed significant heterozygosity excess ($P < 0.001$) in California and in the southeastern United States under the infinite-alleles model. The sign test performed with the infinite-alleles model revealed significant heterozygosity excess in the Southeast ($P < 0.001$) but not in California ($P > 0.1$). The stepwise mutation model revealed no significant heterozygosity excess in either population using either of the two statistical tests. Our results suggest that both populations experienced a genetic bottleneck; however, the bottleneck appears to be more severe in the California population, where the allelic diversity is only half that in the Southeast.

Geographic variation in intraspecific aggression

The results of behavioural assays revealed striking disparities in intraspecific aggression among colonies in the two regions. In contrast to California, where intraspecific aggression was absent, Argentine ants in the Southeast showed extremely high levels of aggression even at relatively small geographical scales (Fig. 2A). The average (± SE) level of aggression across all population pairs was 3.49 ± 0.08 in the Southeast and 0.05 ± 0.02 in California (*t*-test, $P < 0.0001$). In the Southeast, 85.8% of all intercolony

encounters resulted in fighting (aggression level 3 or higher). In California, none of the encounters resulted in fighting and level 2 was the highest level of aggression reached between any of the colony pairs. Aggression between colonies across regions was always high (average = 3.99 ± 0.01). In the Southeast, high aggression was present even between colonies that shared relatively high proportions of alleles (Fig. 2B), whereas in California aggression was absent irrespective of the proportion of alleles shared.

Discussion

Our results indicate profound genetic and behavioural differences between introduced North American populations of the Argentine ant, with the southeastern US population revealing higher levels of genetic diversity and intercolony aggression than the Californian population. That all 23 alleles found in California were a subset of those in the Southeast suggests that the California population was derived probably from the southeastern population. In the United States, the Argentine ant was first detected in 1891 in New Orleans and by 1907 it was present in California (Newell & Barber 1913). The relatively high genetic diversity of Southeastern *L. humile* populations may be due to multiple introductions from the native range during the decades of shipping trade between South and North America. One or possibly a few introductions may have reached California from the Southeast, with a single successful introduction spreading inside California by human mediated 'commercial jumps' (Suarez *et al.* 2001) without significant additional introductions of ants from the Southeast. The lower genetic diversity (both in terms of allele numbers and heterozygosity) of the California supercolony may be a result of a double bottleneck; a minor one upon entry into the United States, and a major one during transport from the Southeast to California. Our results suggest that both populations underwent bottlenecks; however, the bottleneck appears to be more severe in the California population based on the reduced number of alleles present. In studies of different indices of genetic diversity, allelic diversity, in contrast to heterozygosity excess, has proved to be the most powerful measure for detecting demographic bottlenecks (Luikart *et al.* 1998; Spencer *et al.* 2000), provided data are available for an ancestral, bottleneck-free population. Our study provides clear evidence for a unidirectional chain of invasion accompanied by successive genetic bottlenecks (see also Stone & Sunnucks 1993).

Native populations of the Argentine ant display a pattern of genetic isolation by distance, whereas this pattern is absent in introduced populations in California (Tsutsui & Case 2001) and the southeastern US population, despite large genotypic variation and high intraspecific aggression in the latter population. This may reflect differences in the

mode of dispersal of Argentine ants in the native vs. introduced ranges. In the native range, Argentine ants are rarely associated with urban habitats and gene flow, mediated almost exclusively by male flight and short-range colony dispersal (budding), is relatively limited (Suarez *et al.* 2001). In introduced ranges, gene flow occurs not only on a local scale but also through long-distance jumps, which are human-mediated: the rate of spread by long-range dispersal is three orders of magnitude higher than that due to budding (Suarez *et al.* 2001). Long-range dispersal most probably acts to disrupt the pattern of isolation by distance in the introduced populations by increasing genetic homogeneity among spatially isolated colonies. Alternatively, the time since introduction may have not been long enough to allow for genetic equilibrium between migration and drift to occur (Hutchinson & Templeton 2001).

While Tsutsui *et al.* (2000) reported that *L. humile* workers from nests sharing 60% or fewer alleles displayed moderate to high intraspecific aggression and those from nests sharing > 75% alleles were nonaggressive, we found no clear relationship between genetic similarity and intraspecific aggression in either introduced region. In California, colony pairs were always nonaggressive, even when they shared a relatively low (~50%) proportion of alleles, while southeastern US colony pairs were almost always aggressive despite high allele sharing (70%). Therefore, it was difficult identifying an aggression threshold using our neutral microsatellite markers as these may not represent alleles coding for recognition behaviour.

Two factors could contribute to the slow spread and the patchy distribution of Argentine ants in the areas of the southeastern United States that we sampled. One of these factors is the relatively low winter temperature in the Southeast. Low winter temperatures may not only delay the spread of existing colonies, but more importantly may severely limit the survival of newly transplanted colony fragments. We compared low winter temperatures for the month of January by averaging temperatures across 22 cities from this study. In California the average low was 4.0 °C, while in the Southeast it was −1 °C ($t = −8.75$, d.f. = 10, $P = 0.0001$). Furthermore, the average low winter (January) temperature calculated for the cities in southern Europe harbouring the large supercolony described by Giraud *et al.* (2002) was 3.8 °C, which is not different from the study sites in California ($t = 1.81$, d.f. = 10, $P = 0.41$). We suspect that ambient subfreezing conditions, experienced by our southeastern population, restrict winter nests to human-modified refugia, thereby limiting colony expansion. In addition, the difference of approximately 5 °C between the southeastern United States and the geographical regions where supercolonies form may affect the foraging activity of Argentine ants. In California, Argentine ants still forage at 5 °C (Markin 1970). In the Southeast, lack of foraging at subfreezing temperatures may have negative consequences on colony growth and survival. An additional factor that may limit range expansion of *L. humile* in the Southeast is competition with another introduced species, the red imported fire ant, *Solenopsis invicta*. The red imported fire ant is now distributed widely in the Southeast (Vinson 1997), and it may compete with the Argentine ant for nesting sites and access to nutritional sources. According to Wilson (1951), the red imported fire ant has displaced Argentine ants previously from parts of the Southeast. Recent studies have addressed the effect of abiotic (temperature and humidity) and biotic (presence of competitiors) factors on Argentine ant survival and colony expansion rates (Holway *et al.* 2002; Walters & Mackay 2003; Holway & Suarez 2004).

Unlike prior reports of extreme unicoloniality within the Argentine ant's introduced range, we have identified a geographical region where colonies occupy relatively small territories and display high levels of intraspecific aggression and genetic variability. Southeastern US *L. humile* may represent a transitional stage between native populations having high genetic diversity and high intraspecific aggression and the introduced California population characterized by low genetic diversity and limited intraspecific aggression. Consequently, this population may be useful in identifying mechanisms involved in the transition to unicoloniality in invasive ants. In particular, the southeastern population may provide insights into the relative effects of a genetic bottleneck (Tsutsui *et al.* 2000) vs. ecological interactions (Giraud *et al.* 2002) as mechanisms underlying supercolony formation. Tsutsui *et al.* (2000) proposed that the formation of the California supercolony was due to a genetic bottleneck. In the Southeast the genetic diversity is relatively high, intercolonial aggression is strong and no expansive supercolony, on the order of those reported from California or Europe, exists in this region. Therefore, our results support Tsutsui's hypothesis — low genetic diversity leads to the formation of supercolonies. At the present time, however, we cannot rule out the hypothesis proposed by Giraud *et al.* (2002) who suggested that ecological factors promoted the evolution of the supercolony in southern Europe which, according to these authors, experienced only a relatively minor genetic bottleneck. Giraud *et al.* (2002) proposed that supercolony formation in southern Europe involved release from ecological constraints (native parasites and competitors) and increase in colony density. It remains unknown whether colonies in the southeastern United States are free from ecological pressures to a degree that would allow systematic colony expansion. While *L. humile* in the Southeast are most probably not affected by native competitors, they might be affected adversely by abiotic (low winter temperatures) and biotic (competition with *S. invicta*) factors. Currently, Argentine ant colonies in the southeastern United States are relatively rare and their distribution is patchy (Newell & Barber 1913;

Suarez *et al.* 2001). Such characteristics may limit intraspecific interactions, which may maintain relatively high genetic diversity at the loci important in nestmate recognition by preventing selection against rare recognition alleles. Possible future colony range expansion might promote inter-colonial fighting and selection for the most common recognition alleles. Alternatively, laboratory competition studies between mutually aggressive colony fragments from the Southeast might provide quicker answers about the role of ecological factors in supercolony formation in this region.

Given that Argentine ants were introduced into the Southeast before they were introduced into California, it is evident that certain factors are either slowing down or possibly even preventing colony expansion in the Southeast. Additional studies are needed to follow the spread of Argentine ants in the Southeast to examine how intrinsic (high genotypic variability) and extrinsic (temperature, competing species, and interaction with humans) factors interact to shape the evolution of *L. humile* social organization. The investigation of population structure of Argentine ants in and around New Orleans might offer unique insights into the role of these factors and possible interaction between them. On the one hand, mild winter temperatures might promote the spread of colonies. Such spread might lead to polarized aggression between adjoining colonies, selection against genetically diverse colonies and ultimately formation of large supercolonies (Tsutsui *et al.* 2003). On the other hand, competition with *S. invicta* might severely impede such spread. Careful studies of the possible roles of temperature and competition between *L. humile* and *S. invicta* in the New Orleans area might help reveal the events that shaped the present-day distribution patterns of both invaders and may predict future distribution patterns.

Acknowledgements

References

Allendorf FW, Leary RF (1986) Heterozygosity and fitness in natural populations of animals. In: *Conservation Biology: the Science of Scarcity and Diversity* (ed. Soule ME), pp. 57–76. Sinauer Associates Inc., Sunderland, MA.

Bhatkar AP, Whitcomb WH (1970) Artificial diet for rearing various species of ants. *Florida Entomologist*, **53**, 229–232.

Chakraborty AB, Nei M (1977) Bottleneck effect on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution*, **31**, 34–356.

Chen JSC, Nonacs P (2000) Nestmate recognition and intraspecific aggression based on environmental cues in Argentine ants (Hymenoptera: Formicidae). *Annual Entomological Society of America*, **93**, 1333–1337.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Giraud T, Pedersen JS, Keller J (2002) Evolution of supercolonies: the Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences*, **99**, 6075–6079.

Holway DA (1998) Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. *Oecologia*, **116**, 252–258.

Holway DA (1999) Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology*, **80**, 238–251.

Holway DA, Case TJ (2001) Effects of colony-level variation on competitive ability in the invasive Argentine ant. *Animal Behaviour*, **61**, 1181–1192.

Holway DA, Suarez AV (2004) Colony-structure variation and interspecific competitive ability in the invasive Argentine ant. *Oecologia*, **138**, 216–222.

Holway DA, Suarez AV, Case TJ (1998) Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science*, **282**, 949–952.

Holway DA, Suarez AV, Case TJ (2002) Role of abiotic factors in governing susceptibility to invasion: a test with Argentine ants. *Ecology*, **83**, 1610–1619.

Human KG, Gordon DM (1996) Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia*, **105**, 405–412.

Hutchinson DW, Templeton AR (2001) Correlation of pair-wise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.

Krieger MJB, Keller L (1999) Low polymorphism at 19 microsatellite loci in a French population of Argentine ants (*Linepithema humile*). *Molecular Ecology*, **8**, 1075–1092.

Lewis PO, Zaykin D (2001) GENETIC DATA ANALYSIS: *Computer Program for the Analysis of Allelic Data*, version 1.0 (d16c). Free program distributed by the authors over the internet from: *http://lewis.eeb.uconn.edu/lewishome/software.html.*

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.

Luikart G, Sherwin WB, Steele BM *et al.* (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology*, **7**, 963–974.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Markin GP (1970) The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae) in southern California. *Annals of the Entomological Society of America*, **63**, 1238–1242.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Newell W, Barber TC (1913) The Argentine ant. *USDA Bureau of Entomology Bulletin*, **122**, 1–98.

Newman D, Pilson D (1997) Increased probability of extinction due to decreased effective population size: experimental populations of *Clarkia pulchella. Evolution*, **51**, 354–362.

Oetting WS, Lee H, Flanders D, Wiesner G, Sellers T, King RA (1995) Linkage analysis with multiplex short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics*, **30**, 450–458.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution*, **49**, 1280–1283.

Ross KG, Keller L (1995) Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. *Annual Review of Ecology and Systematics*, **26**, 631–656.

Ross KG, Vargo EL, Keller L (1996) Social evolution in a new environment: the case of introduced fire ants. *Proceedings of the National Academy of Sciences*, **93**, 3021–3025.

Ross KG, Vargo EL, Keller L *et al.* (1993) Effect of a founder event on variation in the genetic sex-determining system of the fire ant *Solenopsis invicta*. *Genetics*, **135**, 843–854.

Roulston TH, Buczkowski G, Silverman J (2003) Nestmate discrimination in ants: effect of bioassay on aggressive behavior. *Insectes Sociaux*, **50**, 151–159.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, *version 2.000: a Software for Population Genetic Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Sokal RR, Rolf FJ (1995) *Biometry: the Principles and Practice of Statistics in Biological Research*. W.H. Freeman, New York.

Spencer CC, Neigel JE, Leberg PL (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology*, **9**, 1517–1528.

Stone GN, Sunnucks P (1993) Genetic consequences of an invasion through a patchy environment — the cynipid gallwasp *Andricus quercuscalicis* (Hymenoptera: Cynipidae). *Molecular Ecology*, **2**, 251–268.

Suarez AV, Holway DA, Case TJ (2001) Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. *Proceedings of the National Academy of Sciences*, **98**, 1095–1100.

Suarez AV, Tsutsui ND, Holway DA *et al.* (1999) Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biological Invasions*, **1**, 43–53.

Tsutsui ND, Case TJ (2001) Population genetics and colony structure of the Argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution*, **55**, 976–985.

Tsutsui ND, Suarez AV, Grosberg RK (2003) Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *Proceedings of the National Academy of Sciences*, **100**, 1078–1083.

Tsutsui ND, Suarez AV, Holway DA *et al.* (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences*, **97**, 5948–5953.

Vinson SB (1997) Invasion of the red imported fire ant (Hymenoptera: Formicidae): spread, biology, and impact. *American Entomologist*, **43**, 23–39.

Walters AC, Mackay DA (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.

Wilson EO (1951) Variation and adaptation in the imported fire ant. *Evolution*, **5**, 68–79.

Wright S (1931) Evolution in Mendellian populations. *Genetics*, **16**, 97–159.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 395–420.

Wright S (1965) The interpretation of population structure by *F*-statistics with special regards to systems of mating. *Evolution*, **19**, 395–420.