AUSTRALIAN JOURNAL of ENTOMOLOGY

Australian Journal of Entomology (2009) **48**, 79–83

Genetic and behavioural evidence for a city-wide supercolony of the invasive Argentine ant *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) in southeastern Australia

Elissa L Suhr,[1,2]* Stephen W McKechnie[1] and Dennis J O'Dowd[2]

[1]*Centre of Environmental and Stress Adaptation Research, School of Biological Sciences, Monash University, Vic. 3800, Australia.*
[2]*Australian Centre for Biodiversity, School of Biological Sciences, Monash University, Vic. 3800, Australia.*

Abstract The success of invasive ants is frequently attributed to genetic and behavioural shifts in colony structure during or after introduction. The Argentine ant (*Linepithema humile*), a global invader, differs in colony genetic structure and behaviour between native populations in South America and introduced populations in Europe, Japan, New Zealand and North America. However, little is known about its colony structure in Australia. We investigated the genetic structure and behaviour of *L. humile* across Melbourne, Victoria by quantifying variation at four microsatellite loci and assaying intraspecific aggression at neighbourhood (30–200 m), fine (1–3.3 km) and regional (5–82 km) spatial scales. Hierarchical analyses across these scales revealed that most genetic variation occurred among workers within nests (~98%). However, although low genetic differentiation occurred among workers between nests at the fine and regional scales (~2%), negligible differentiation was detected among workers from neighbouring nests. Spatial genetic autocorrelation analysis confirmed that neighbouring nests were genetically more similar to each other. Lack of aggression within and across these scales supported the view that *L. humile* is unicolonial and forms a large supercolony across Melbourne. Comparisons of genetic structure of *L. humile* among single nests sampled from Adelaide, Brisbane, Hobart and Perth with Melbourne showed no greater levels of genetic differentiation or dissimilar spatial structure, suggesting an Australia-wide supercolony.

Key words biological invasions, intraspecific aggression, invasive ants, microsatellite markers, supercoloniality.

INTRODUCTION

Many invasive ants share a remarkable social feature – unicoloniality – that may contribute to their ecological success in introduced ranges (Suarez & Tsutsui 2007). Founder effects and selection against genetic diversity during or after introduction may lead to loss of intraspecific aggression and supercolony formation because of the invader's inability to differentiate between nestmates and non-nestmates (Suarez *et al.* 1999; Giraud *et al.* 2002). By reducing costs associated with territoriality, unicoloniality may lead to high worker densities promoting ecological dominance and impact (Holway *et al.* 1998; Holway & Suarez 2004). In many instances, unicoloniality is associated with the formation of supercolonies, large aggregations without colony boundaries where mixing of individuals between socially interconnected but spatially separate nests can occur (Suarez & Tsutsui 2007).

The Argentine ant *Linepithema humile* (Mayr), native to South America, has been introduced throughout the world via human commerce and is now established on six continents and many oceanic islands (Suarez *et al.* 2001). Colony structure differs markedly between its area of origin and introduction. In the native range, *L. humile* colony structure ranges from single nests to very small supercolonies across 10–100 s of metres that are mutually antagonistic towards each other (Tsutsui *et al.* 2000). In contrast, introduced populations in Europe, Japan, New Zealand and North America are unicolonial and form diminutive to expansive supercolonies spanning <10 to 100–1000 s of kilometres (Suarez *et al.* 1999; Tsutsui *et al.* 2000; Giraud *et al.* 2002; Buczkowski *et al.* 2004; Corin *et al.* 2007a; Sunamura *et al.* 2007; Hirata *et al.* 2008).

In Australia, *L. humile* was first recorded in Melbourne in 1939 and detected thereafter across the continent (Western Australia [1941], New South Wales [1950], Tasmania [1951], South Australia [1979] and Queensland [2002]) (Pasfield 1968; Madge 1979; M Elson-Harris pers. comm. 2007). Its invasion across Australia has been associated with reductions in or loss of native ant species in both urban and natural environments (Walters 2006; Rowles & O'Dowd 2007). Despite its notoriety as an invader and pest, almost no information is available on the colony structure of *L. humile* in Australia. Here we use microsatellite loci and behavioural assays to quantify genetic structure and intraspecific

*elissa.suhr@sci.monash.edu.au

doi:10.1111/j.1440-6055.2008.00688.x

aggression of *L. humile* across Melbourne, Victoria, and compare genetic structure across the continent.

MATERIALS AND METHODS

Linepithema humile workers were collected in 2003 and 2004 from nests at increasing spatial scales: 30–200 m, 1–3.3 km, 5–82 km and 549–3606 km (Table 1). At the finest scale we sampled nine nests – three neighbouring nests between 30 and 200 m apart at each of three adjacent sites between 1 and 3.3 km apart – along a 3.3-km urban-bushland interface at Mornington Peninsula National Park (MPNP; Rowles & O'Dowd 2009). At the regional scale, nine nests were sampled across Melbourne that comprised one nest from MPNP and two nests between 5 and 40 km apart in each of the northern, eastern, southern and western quadrants of urban Melbourne. At the continental scale, we sampled a single nest from each of Adelaide, Brisbane, Hobart and Perth. Voucher specimens were deposited in the Australian National Insect Collection, Canberra.

To determine genetic structure, we analysed 15 workers from each of 21 nests ($n = 315$) at five microsatellite loci: Lhum-11, Lhum-13, Lhum-19, Lhum-28 and Lhum-33 (Krieger & Keller 1999). Workers were placed in 100% EtOH and stored at −80°C until DNA was extracted individually using a modified standard phenol chloroform procedure (Sambrook *et al.* 1989). DNA pellets were resuspended in 60 µL ddH_20 and stored at −20°C. Polymerase chain reaction (PCR) amplifications were performed in 25 µL reaction volumes with 2.5 µL reaction buffer (1×), 2.5 µL $MgCl_2$ (2 mM), 2 µL dNTPs (0.2 mM), 0.05 µL labelled IR-dye forward primer (1 pmol), 0.45 µL unlabelled forward primer (10 pmol), 0.5 µL unlabelled reverse primer (10 pmol), 14.5 µL ddH_20, 0.5 µL *Taq* DNA polymerase (1 µg/µL) and 2 µL DNA template in the Applied Biosystems PCR Gene Systems 2700. PCR cycle parameters were 95°C (2 min), 36 cycles of denaturation of 95°C (30 s), annealing (Lhum-11: 55°C, Lhum-13 and Lhum-19: 53°C and Lhum-28 and Lhum-33: 60°C) (1 min), 72°C (3 min) and a final extension step 72°C (2 min). PCR products were diluted between 1:1 and 1:3 with ddH_20, run on a LICOR® 4200 Global Edition IR^2 system and analysed using SAGA 2.1 software.

Table 1 Information for sampling locations of *Linepithema humile*

Location	No. nests	Latitude (S)	Longitude (E)
Mornington Peninsula National Park			
Diamond Bay	3	38.361	144.752
Ronald Avenue	3	38.347	144.737
Sorrento Cemetery	3	38.342	144.728
Melbourne			
Bundoora	1	37.718	145.051
Greenvale	1	37.638	144.898
Glen Waverley	1	37.868	145.162
Blackburn	1	37.828	145.139
Brighton East	1	37.916	145.011
Mount Martha	1	38.272	145.008
Altona	1	37.864	144.837
Taylors Lakes	1	37.703	144.785
Australia			
Adelaide	1	34.980	138.550
Brisbane	1	27.370	153.030
Hobart	1	42.900	147.320
Perth	1	32.020	115.900

The number of alleles (A) and expected (H_e) and observed (H_o) heterozygosities, fit to Hardy–Weinberg equilibrium and linkage disequilibrium, were assessed using GENEPOP 3.4 (Raymond & Rousset 1995). Private alleles were identified in pairwise nest comparisons. Lhum-33 was monomorphic and excluded from further analyses. Genetic structure was assessed with analysis of molecular variance (AMOVA) with 10 000 permutations using Arlequin 3.01 (Excoffier *et al.* 2005). At the finest scale, we used a three-level hierarchy and for the regional and continental scales we used a two-level hierarchy. AMOVA was calculated locus-by-locus using F_{ST} (Weir & Cockerham 1984). Spatial genetic autocorrelation was assessed with GenALEx 6 (Peakall & Smouse 2006). Pairwise geographical and pairwise squared genetic distance (F_{ST}) matrices were used to calculate a genetic correlation coefficient (r) for distance classes across each scale (neighbourhood and fine (MPNP), regional (across Melbourne) and continental (across Australia)). Spatial autocorrelation within variable distance classes was assessed relative to 10 000 permutated r-values with 9999 permutations for tests of significance and 999 permutations for estimating 95% confidence intervals. Spatial autocorrelation is positive or negative where significant r-values indicate the level of genetic similarity or dissimilarity between pairs of nests that fall within the specified distance class. Random spatial structure is inferred when no statistically significant spatial autocorrelation exists.

We examined intraspecific aggression with a standard live 1–1 assay (Holway *et al.* 1998). One worker from each of two nests was placed into an 8-mL (1.5 cm × 5 cm) glass vial internally coated with Fluon™. The maximum aggression score was recorded over 5 min (0 = ignore, 1 = touch (antennation), 2 = avoid (ants antennate and one or both retreat in opposite direction), 3 = aggression (raising of gaster) and 4 = fighting (prolonged biting, pulling and chemical spraying)). Behavioural scores between 0 and 2 are considered non-aggressive whereas 3–4 are aggressive. Five assays were conducted in the field for each pairwise combination of nests at MPNP ($n = 225$) and between one MPNP nest and the eight other nests in Melbourne ($n = 220$). Each worker was only used once. Aggression scores for each pairwise comparison were averaged and plotted against distance between nests.

RESULTS

Overall, 25 alleles were detected at four microsatellite loci across all genotyped *L. humile* from 21 nests. The number of alleles per locus varied from four to 10 (Table 2). Expected

heterozygosities (H_e) ranged from 0.133 to 0.655 (overall H_e 0.457). Five private alleles were found in four nests at frequencies of 0.036–0.136 (two in Hobart and one in Adelaide, Brisbane and Diamond Bay-2). None of the four loci departed significantly from Hardy–Weinberg equilibrium and linkage disequilibrium was not detected.

At the finest scale (MPNP), three-level hierarchical AMOVA revealed that most of the genetic variation occurred among workers within nests (98.69%, Table 3). The little remaining genetic differentiation was explained by variation among sites 1–3.3 km apart. No significant component of the variation occurred among neighbouring nests <200 m apart. Two-level AMOVA found similar patterns across both Melbourne and Australia, only approximately 2% of the variation occurring among nests. Genetic differentiation was always highest among workers within nests (97.98% and 97.25%, respectively).

Spatial autocorrelation analysis revealed a significantly positive genetic correlation within the <200 m distance class ($r = 0.024$, $P < 0.000$) indicating that these nests were genetically more similar than expected under the hypothesis of no association between genetic similarity and spatial distance apart (Fig. 1). No significant genetic correlation was detected at other distance classes.

Intraspecific aggression did not occur between *L. humile* workers at the neighbourhood, fine or regional scales we examined ($n = 445$ trials, Fig. 2). The highest aggression score between any pairwise combination of nests was 1 (non-aggressive). Aggression scores showed no relationship with distance between nests, even at large distances.

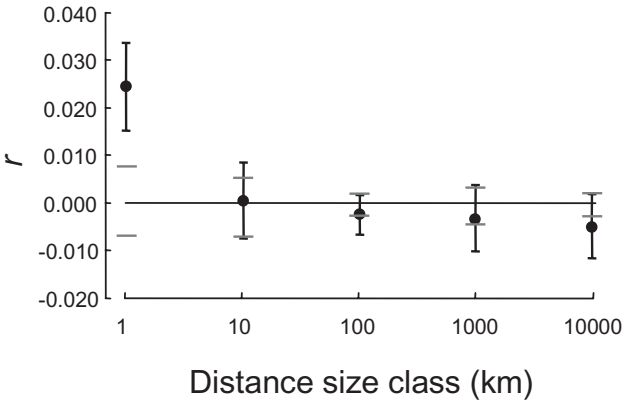

Fig. 1. Spatial autocorrelogram for *Linepithema humile* across distance classes 1–10 000 km that encompass neighbourhood (30–210 m), fine (1–3.3 km), regional (5–82 km) and continental (549–683 km and 1004–3606 km) scales, respectively. Solid circles, genetic (F_{ST}) autocorrelation coefficients (r) ± 95% confidence intervals; grey lines, upper and lower 95% confidence intervals about the null hypothesis of no spatial structure.

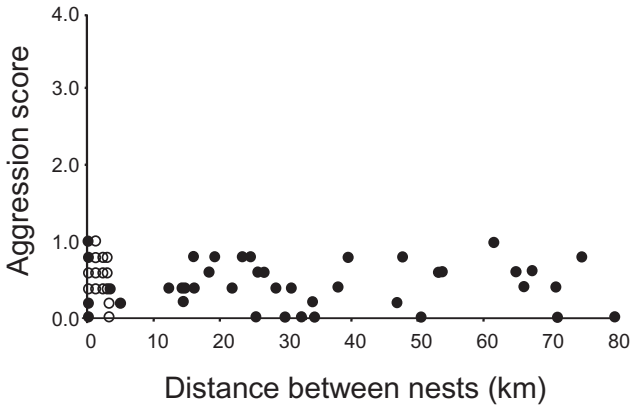

Fig. 2. Mean aggression scores for pairwise nest combinations ($n = 5$ assays for each pairwise comparison) at the neighbourhood to fine (30 m–3.3 km, Mornington Peninsula) [○] and regional (5–82 km, Melbourne) [●] scales.

Table 2 Number of alleles (A) and expected (H_e) and observed (H_o) heterozygosities for the entire sample of *Linepithema humile* ($n = 315$)

	A	H_e	H_o
Lhum-11	4	0.394	0.360
Lhum-13	10	0.655	0.591
Lhum-19	7	0.646	0.643
Lhum-28	4	0.133	0.133
All	25		
Mean		0.457	0.432

Table 3 Hierarchical analyses of molecular variance across three spatial scales (neighbourhood to fine across Mornington Peninsula National Park (MPNP), 30 m–3.3 km; regional across Melbourne, 5–82 km; and continental across Australia, 549–3606 km). The percentage of genetic variance explained by each hierarchical level is given for each and over all loci

	MPNP			Melbourne		Australia	
	Within nests	Among nests within sites	Among sites	Within nests	Among nests	Within nests	Among nests
Lhum-11	100.62	–1.97	1.35	100.94	–0.94	98.34	1.66
Lhum-19	98.45	1.08	0.47	97.02*	2.98*	97.54*	2.46*
Lhum-28	97.77	–1.07	3.30	97.56*	2.44*	96.79**	3.21**
Lhum-33	98.99	–0.98	1.99	96.78*	3.22*	94.96***	5.04***
All	98.69	–0.46	1.76*	97.98*	2.02*	97.25***	2.75***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

DISCUSSION

Genetically and behaviourally, Argentine ants are unicolonial and form a large supercolony across Melbourne. The Argentine ant showed little genetic structuring across the neighbourhood, fine, and regional scales we examined in Mornington Peninsula National Park (MPNP) and across Melbourne. The great majority of variation (~98%) was among ants within nests with only ~2% of the variation occurring among nests at the fine and regional scales. Notable was that no significant component of the variation could be attributed to nests within 200 m of each other. Analysis of spatial structure across Melbourne revealed significant positive autocorrelation only between nests up to 200 m apart. These data are consistent with the idea of population viscosity where neighbouring ants are very closely related, as with the supercolonial native ant *Polyrhachis robsoni* (van Zweden *et al.* 2007). In our study these close nest comparisons all occurred at MPNP. The simplest explanation for this pattern is local dispersal by budding at neighbourhood scales (nests <200 m apart), similar to that reported in California at the early stage of colony expansion (Ingram & Gordon 2003), and human-mediated jump dispersal (Suarez *et al.* 2001) at larger scales to account for the low level of overall nest differentiation. Moreover, a complete lack of intraspecific aggression between workers from nests across Melbourne indicates an absence of colony boundaries for distances up to 82 km. Pedersen *et al.* (2006) sampled workers across 10 nests in Melbourne (Bundoora, <600 m from one of our nests) using 11 microsatellite loci and reported no evidence for a significant inbreeding coefficient or increased relatedness among nestmate workers, consistent with the idea of a genetically homogeneous supercolony occurring in and around Melbourne.

Additional genetic analyses across Australia showed that genetic differentiation remained low from nests as close as 30 m to as far as 3606 km apart. Most variation was among workers within nests (~98%). Although here we only sampled single nests for the widespread cities, our data suggest that the colony structure of the Argentine ant across Australia mirrors the expansive supercolonies spanning 1000 s of kilometres in California and Europe (Tsutsui *et al.* 2000; Giraud *et al.* 2002). Confirmation of an Australia-wide supercolony and identification of potential introduction pathways awaits more extensive sampling of genotypes and haplotypes (using both an expanded number of nuclear markers and mitochondrial markers) in combination with more intraspecific aggression assays across the continent. We note that the Argentine ant mtDNA cytochrome *b* haplotypes from a single nest in Perth (Tsutsui *et al.* 2001) and in Melbourne (Bundoora; Chiotis *et al.* 2000) are different (Corin *et al.* 2007b). This suggests that at least one other colony in Perth might be from a different source.

The Melbourne supercolony may have originated from one or more founder events from Argentina followed by human-mediated redistribution across Australia. The Bundoora haplotype is identical to that from a nest at Ocampo, Argentina (Corin *et al.* 2007b). Although nearly all port interception records for the Argentine ant in Australia are from other areas of introduction, a single record from Argentina indicates that a direct pathway is possible from the area of origin (EL Suhr unpubl. results 2008). Furthermore, the Bundoora haplotype is the only one known in New Zealand suggesting that southeastern Australia served as the source for the New Zealand supercolony (Corin *et al.* 2007b). Asymmetries between southeastern Australia and New Zealand in dates of introduction and in introduction pressure support this view. The Melbourne population established >50 years before that in New Zealand and introduction pressure of Argentine ants from Australia into New Zealand, as measured by port interception records, has been higher than from any other source region (37% of records, 14/38 – Corin *et al.* (2007b)). In contrast, introduction pressure from New Zealand into Australia is relatively low, accounting for 7% (13/175) of port interceptions since 1988 (EL Suhr unpubl. results 2008). Thus, preliminary data suggest that the Argentine ant forms a supercolony (*sensu* Suarez & Tsutsui 2007) across Australasia.

ACKNOWLEDGEMENTS

We thank A Walters, J Holley, J Meggs and M Widmer for collecting Argentine ants, D Mackay for statistical help and A Rowles for field and laboratory assistance. R Crozier, B Schlick-Steiner and F Steiner commented on a previous version of the manuscript. The Australian Quarantine and Inspection Service and Market Access and Biosecurity, Department of Agriculture, Fisheries, and Forestry provided the port interception records for Australia. We acknowledge support of the Department of Primary Industries, Victoria and the Australian Research Council through their Special Research Centre Program. This is publication No. 133 from the Australian Centre for Biodiversity at Monash University.

REFERENCES

Buczkowski G, Vargo EL & Silverman J. 2004. The diminutive supercolony: the Argentine ants of the southeastern United States. *Molecular Ecology* **13**, 2235–2242.

Chiotis M, Jermiin LS & Crozier RH. 2000. A molecular framework for the phylogeny of the ant subfamily Dolichoderinae. *Molecular Phylogenetics and Evolution* **17**, 108–116.

Corin SE, Abbott KL, Ritchie PA & Lester PJ. 2007a. Large-scale unicoloniality: the population and colony structure of the invasive Argentine ant (*Linepithema humile*) in New Zealand. *Insectes Sociaux* **54**, 275–282.

Corin SE, Lester PJ, Abbott KL & Ritchie PA. 2007b. Inferring historical introduction pathways with mitochondrial DNA: the case of introduced Argentine ants (*Linepithema humile*) into New Zealand. *Diversity and Distributions* **13**, 510–518.

Excoffier L, Laval G & Schneider S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47–50.

Giraud T, Pedersen JS & Keller L. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 6075–6079.

Hirata M, Hasegawa O, Toita T & Higashi S. 2008. Genetic relationships among populations of the Argentine ant *Linepithema humile* introduced into Japan. *Ecological Research* **23**, 883–888.

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.

Ingram KK & Gordon DM. 2003. Genetic analysis of dispersal dynamics in an invading population of Argentine ants. *Ecology* **84**, 2832–2842.

Krieger MJB & Keller L. 1999. Low polymorphism at 19 microsatellite loci in a French population of Argentine ants (*Linepithema humile*). *Molecular Ecology* **8**, 1078–1080.

Madge PE. 1979. *Argentine Ant*. South Australia Department of Agriculture, Adelaide, South Australia, Australia. Fact Sheet AGDEX 612.

Pasfield G. 1968. Argentine ants. *Australian Natural History* **16**, 12–15.

Peakall R & Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288–295.

Pedersen JS, Krieger MJB, Vogel V, Giraud T & Keller L. 2006. Native supercolonies of unrelated individuals in the invasive Argentine ant. *Evolution* **60**, 782–791.

Raymond M & Rousset F. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.

Rowles AD & O'Dowd DJ. 2007. Interference competition by Argentine ants displaces native ants: implications for biotic resistance to invasion. *Biological Invasions* **9**, 73–85.

Rowles AD & O'Dowd DJ. 2009. Impacts of the invasive Argentine ant on native ants and other invertebrates in coastal scrub in southeastern Australia. *Austral Ecology* **34**, in press. doi:10.111/j.1442-9993.2008.01922.x

Sambrook S, Fritsch EF & Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Suarez AV & Tsutsui ND. 2007. The evolutionary consequences of biological invasions. *Molecular Ecology* **17**, 351–360.

Suarez AV, Tsutsui ND, Holway DA & Case TJ. 1999. Behavioural and genetic differentiation between native and introduced populations of the Argentine ant. *Biological Invasions* **1**, 43–53.

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 of the United States of America* **98**, 1095–1100.

Sunamura E, Nishisue K, Terayama M & Tatsuki S. 2007. Invasion of four Argentine ant supercolonies into Kobe Port, Japan: their distributions and effects on indigenous ants (Hymenoptera: Formicidae). *Sociobiology* **50**, 659–674.

Tsutsui ND, Suarez AV, Holway DA & Case TJ. 2000. Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 5948–5953.

Tsutsui ND, Suarez AV, Holway DA & Case TJ. 2001. Relationships among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source of introduced populations. *Molecular Ecology* **10**, 2151–2161.

Walters AC. 2006. Invasion of Argentine ants (Hymenoptera: Formicidae) in South Australia: impacts on community composition and abundance of invertebrates in urban parklands. *Austral Ecology* **31**, 567–576.

Weir BS & Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

van Zweden JS, Carew ME, Henshaw MT, Robson SKA & Crozier RH. 2007. Social and genetic structure of a supercolonial weaver ant, *Polyrhachis robsoni*, with dimorphic queens. *Insectes Sociaux* **54**, 34–41.

Accepted for publication 13 June 2008.