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Relationships among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source of introduced populations

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Abstract

The Argentine ant (*Linepithema humile*) is a damaging invasive species that has become established in many Mediterranean-type ecosystems worldwide. To identify likely sources of introduced populations we examined the relationships among native *Linepithema* populations from Argentina and Brazil and introduced populations of *L. humile* using mitochondrial cytochrome *b* sequence data and nuclear microsatellite allele frequencies. The mitochondrial phylogeny revealed that the populations in Brazil were only distantly related to both the introduced populations and the native populations in Argentina, and confirmed that populations in Brazil, previously identified as *L. humile*, are likely a different species. The microsatellite-based analysis provided resolution among native and introduced populations of *L. humile* that could not be resolved using the mitochondrial sequences. In the native range, colonies that were geographically close to one another tended to be genetically similar, whereas more distant colonies were genetically different. Most samples from the introduced range were genetically similar, although some exceptions were noted. Most introduced populations were similar to native populations from the southern Rio Parana and were particularly similar to a population from Rosario, Argentina. These findings implicate populations from the southern Rio Parana as the most likely source of introduced populations. Moreover, these data suggest that current efforts to identify natural enemies of the Argentine ant for biological control should focus on native populations in the southern Rio Parana watershed.

Keywords: Argentine ants, biological control, biological invasions, cytochrome *b*, introduced species, *Linepithema humile*, microsatellites

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Introduction

Identifying the geographical source of invasive species is a critical component of invasion biology. Such knowledge can facilitate the reconstruction of invasion history (Ross & Trager 1990; Williams 1994; Stiller & Denton 1995; Haymer *et al*. 1997; Villablanca *et al*. 1998; Davies *et al*. 1999a,b; Amsellem *et al*. 2000), allow transport vectors to be more easily identified (Fonseca *et al*. 2000), direct efforts for the discovery of appropriate biological control agents (Porter *et al*. 1995), and illuminate important changes in the biology of the invader during or shortly after introduction (Novak & Mack 1993; Ross *et al*. 1996; Anttila *et al*. 2000; Tsutsui *et al*. 2000; Tsutsui & Case 2001). Because historical human records are often incomplete, inaccurate or simply nonexistent, molecular genetics offers a powerful tool for the identification of putative source populations.

Many factors, however, make this seemingly straightforward task difficult. Population bottlenecks during introduction and genetic drift in small founding populations can dramatically reduce levels of genetic diversity, necessitating the use of highly polymorphic markers to retrieve meaningful population genetic information (Villablanca *et al*. 1998; Davies *et al*. 1999a,b). In contrast, within the native range of an organism high levels of genetic diversity

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within populations, genetic differentiation among populations, and the presence of closely related or cryptic species may necessitate the use of more highly conserved markers to resolve the relationships among populations.

Native to South America, the Argentine ant is an invasive species that has become established in at least 15 countries on six continents and many oceanic islands (Suarez *et al*. 2001). In the United States, Argentine ants were first detected in 1891 in New Orleans (Louisiana), where they were thought to have arrived by ship from either Argentina or Brazil (Newell & Barber 1913). In its introduced range, the Argentine ant is an economic concern, primarily as an agricultural and urban pest (Newell & Barber 1913; Smith 1936; Knight & Rust 1990). Argentine ants are also ecologically destructive as they displace ecologically important native ant species (Erickson 1971; Ward 1987; Human & Gordon 1996; Suarez *et al*. 1998; Holway 1999) and have been implicated in the disruption of arthropod communities (Human & Gordon 1997; Cole *et al*. 1992; Bolger *et al*. 2000). The loss of native ant species may then have detrimental indirect effects on other taxa (Bond & Slingsby 1984; Suarez *et al*. 2000).

Several factors necessitate the use of both conserved and highly polymorphic markers to determine the source of introduced *Linepithema humile* populations. Conserved markers are necessary because native populations of Argentine ants possess high levels of genetic diversity relative to introduced populations (Suarez *et al*. 1999; Tsutsui *et al*. 2000; Tsutsui & Case 2001), and are often highly genetically differentiated at small spatial scales (< 1 km) (Tsutsui & Case 2001). In contrast, introduced populations are characterized by low levels of genetic diversity relative to native populations (Suarez *et al*. 1999; Tsutsui *et al*. 2000) and lower levels of genetic differentiation across large spatial scales (> 900 km) (Tsutsui *et al*. 2000; Tsutsui & Case 2001). Thus, highly polymorphic loci are required to achieve informative levels of polymorphism. Additionally, Argentine ants in their native range occur within a species complex (P. Ward and A. Wild, personal communication). Different species can be found in sympatry, and the identity of *L. humile* has been a subject of confusion. In this study, we used two types of molecular markers to examine the genetic relationships among native and introduced populations of the invasive Argentine ant. We first used mitochondrial DNA sequences to identify and exclude as potential sources native populations that are highly divergent from introduced populations. We then used nuclear microsatellite loci to resolve relationships at a finer scale, among genetically similar native and introduced populations.

Materials and methods

Samples were collected from 42 sites in the native and introduced ranges of the Argentine ant (Table 1). At each site, we collected ants from one or more nests. We define a nest as an aggregation of queens, workers and brood. In introduced populations of Argentine ants intraspecific aggression is typically absent among nests, a form of colony structure known as unicoloniality (Newell & Barber 1913; Markin 1968). When multiple nests were collected within the same location (except in South Africa and Australia), we identified colony boundaries using a behavioural assay (described in Suarez *et al*. 1999; Tsutsui *et al*. 2000). A colony was then defined as the collection of all nests among which intraspecific aggression was absent. This grouping is appropriate because behaviourally defined colonies are genetically distinct from one another and may represent independent introductions, whereas spatially separate nests within these colonies are genetically similar (Tsutsui & Case 2001). Because behavioural data were not collected for the sites in South Africa or Australia, it is possible that these nests may actually belong to the same colony. Furthermore, since behavioural data were not collected among some distant locations (e.g. among continents), we cannot determine if some of these geographically distant populations belong to the same behaviourally defined colony.

In its native range, *Linepithema humile* was collected from 11 sites in Argentina and one site in Brazil. In addition, related *Linepithema* species were collected from three sites in Brazil, including one site where *L. humile* also occurred (Passo do Lontra). In Argentina, ants were collected from two sites on the Rio Uruguay, seven sites along the Rio Parana, and three sites in and around Buenos Aires (including the Reserva Ecologia Costanera Sur located near downtown Buenos Aires) (Fig. 1). All *Linepithema* sampled in Argentina closely resembled the introduced form morphologically (A. Wild, personal communication). In Brazil, *L. humile* was sampled from Passo do Lontra (PL1; Fig. 1). In addition, other *Linepithema* species were sampled from Monte Verde (MV), Serra do Japi (SJ1, SJ2) and Passo do Lontra (PL2; Table 1; Fig. 1). Additional information about these Brazilian sites is provided in Orr & Seike (1998). At Passo do Lontra, where *L. humile* co-occurs with a second *Linepithema* species, the latter is attacked by phorid flies (Orr & Seike 1998) and is morphologically distinct from *L. humile* (P. Ward & A. Wild, personal communication). These two *Linepithema* species can be distinguished by colour, scape length, and pronotal hair number (Orr *et al*. 2001). We included these other *Linepithema* in our analyses because they have previously been misidentified as *L. humile* (Orr & Seike 1998) and subsequently discussed as such in the literature (Holway & Suarez 1998; Chapin *et al*. 2000; Feener 2000).

In its introduced range, we collected *L. humile* from 17 sites (35 nests) in California (Table 1). Twenty-two nests, representing seven colonies, were collected in San Diego County (La Jolla, Encinitas, Solana Beach, Mission Trails,

Table 1 Sampling locations in the native and introduced ranges and the genetic markers used. Letters in parentheses indicate the abbreviations for each location. Numbers in parentheses indicate the number of individuals examined

Site	No. nests	No. colonies	Cytochrome *b*	Microsatellites
***Linepithema* spp.**				
Monte Verde, Brazil (MV)	2	2	yes (1)	no
Serra do Japi, Brazil (SJ1)	1	1	yes (1)	no
Serra do Japi, Brazil (SJ2)	1	1	yes (1)	no
Passo do Lontra, Brazil (PL2)	1	1	yes (1)	no
***Linepithema humile* — Native range**				
Passo do Lontra, Brazil (PLI)	1	1	yes (1)	yes (5)
Ita lbate, Argentina (IT)	3	1	yes (1)	yes (30)
Isla de Cerritas, Argentina (IC)	1	1	no	yes (10)
Ocampo, Argentina (OC)	1	1	yes (3)	yes (10)
Colon, Argentina (CO)	1	1	yes (1)	yes (10)
Alvear, Argentina (AL)	1	1	yes (1)	yes (10)
Pre-delta, Argentina (PD)	1	1	yes (1)	yes (10)
Rosario, Argentina (RO)	1	1	no	yes (10)
Ibicuy, Argentina (IB)	1	1	yes (1)	yes (10)
Costanera Sur, Argentina (CS)	9	4	yes (1)	yes (95)
Otamendi, Argentina (OT)	6	4	no	yes (80)
Buenos Aires, Argentina (BA)	3	2	no	yes (30)
***Linepithema humile* — Introduced range**				
California (overall)	35	8		
La Jolla	8	1*	yes (1)	yes (105)
Encinitas (EN)	9	3*	no	yes (130)
Solana Beach	1	1*	no	yes (15)
Temecula (TH)	2	2*	no	yes (30)
Lake Skinner (LS)	2	1	yes (1)	yes (30)
Mission Trails (MT)	1	1	no	yes (15)
Lake Hodges (LH)	1	1	no	yes (15)
Sweetwater (SW)	2	2	yes (2)	yes (30)
Los Angeles	1	1*	yes (1)	yes (10)
Santa Barbara	1	1*	no	yes (10)
Morro Bay	1	1*	no	yes (10)
Santa Maria	1	1*	no	yes (10)
King City	1	1*	no	yes (10)
Salinas	1	1*	no	yes (10)
San Jose	1	1*	no	yes (10)
Sausalito	1	1*	no	yes (10)
Ukiah	1	1*	no	yes (10)
Kilauea, Hawaii (HI)	4	2	yes (2)	yes (40)
Haleakala, Maui	1	1	no	yes (10)
New Orleans, Louisiana	1	1	yes (1)	no
Bermuda	2	1	yes (1)	no
Perth, Australia	1	1	yes (1)	yes (10)
Melbourne, Australia	1	1	yes (1)	no
South Africa (SA)	3	n.a.	yes (1)	yes (15)
Vina del Mar, Chile	1	1	yes (1)	no
Rome, Italy	1	1	no	yes (10)

*Sites that contained at least one nest belonging to the large supercolony in California.

Lake Hodges, and Sweetwater Reservoir). Argentine ants were also collected from four nests in Temecula, California, two of which belonged to a eighth colony (LS). The remaining nine nests were collected along U.S. Route 101, and together with 15 of the San Diego nests and two of the Temecula sites, comprise a single large supercolony (noted with asterisks in Table 1). In Hawaii, Argentine ants were collected from four sites (two colonies) at Kilauea Volcano in Volcanoes National Park, and from one site at Haleakala Volcano on Maui. In South Africa, Argentine ants were collected from Betty's Bay (SA1), Cape Point (SA2) and Caledon (SA3). Other introduced populations were sampled in Bermuda (two sites in one colony), and at single sites in New Orleans (USA), Viña del Mar (central

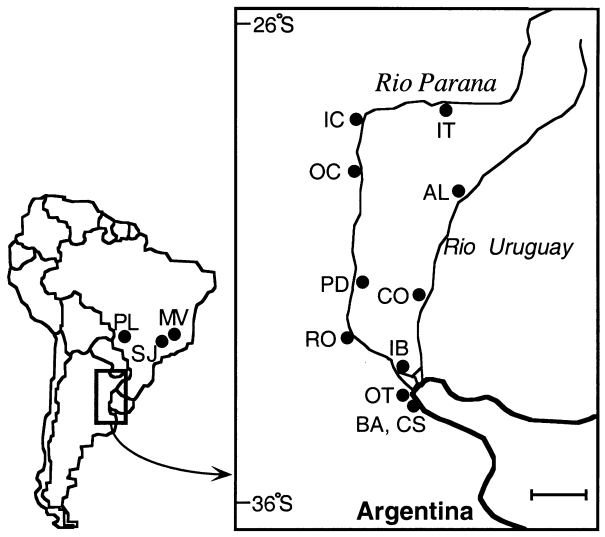

Fig. 1 Sampling locations in the native range of the Argentine ant. Full names of the sites are listed in Table 1. Scale bar represents 100 km.

Chile), Perth (Australia), Melbourne (Australia), and Rome (Italy) (Table 1).

To resolve the relationships among the more distantly related populations, we amplified and sequenced 405 bp of the mitochondrial cytochrome *b* gene from 31 individual workers (15 from native populations and 16 from introduced populations; Table 1). As an outgroup we used the published cytochrome *b* sequence for *Forelius chalybaeus*, a species from the sister genus to *Linepithema* (Chiotis *et al*. 2000; GenBank accession no. AF146718). The *L. humile* sequence from this study (from Bundoora, Australia; GenBank accession no. AF146720) was also included in our analysis. These sequences were then used to reconstruct phylogenetic trees by maximum likelihood, using the program TREE-PUZZLE 5.0 (Strimmer & von Haeseler 1996), and by maximum parsimony in PAUP 4.0b4a (Swofford 1998). TREE-PUZZLE uses quartet puzzling to reconstruct all possible quartet maximum likelihood trees, then repeatedly combines these quartets into an overall tree. A majority rule consensus is calculated and support is assigned to internal branches based on the number of times a particular group is reconstructed (Strimmer & von Haeseler 1996; Strimmer *et al*. 1997).

Many of the native populations from Argentina and all introduced populations could not be distinguished using cytochrome *b*. To resolve the relationships among these populations, we genotyped 5–30 individual workers from each nest at seven polymorphic microsatellite loci (Suarez *et al*. 1999; Krieger & Keller 2000; Tsutsui *et al*. 2000) using standard protocols. We then performed three types of analysis to identify potential source populations in the native range.

First, we calculated pairwise genetic distances (Nei 1972) between colonies in the program PHYLIP 3.5c (Felsenstein 1993) and visualized the relationships among populations using two dimensional nonmetric multidimensional scaling (NMDS) in the program Syn-Tax 5.02 (Podani 1995). NMDS is a type of ordination analysis in which metric information or the distance between objects (colonies) is iteratively arranged into a nonhierarchical solution that best preserves the rank order of the original data set (Lessa 1990). Thus, NMDS is useful for visualizing the reticulate relationships that can arise when multiple populations are founded from a single source. The fit of the NMDS rendering to the original data is measured by a stress coefficient between the two, with stress approaching zero when the fit is perfect. In this case, 30 iterations were performed using a resemblance matrix from a principle coordinates analysis as the input, and stress was always < 0.2.

Second, we used an assignment test to compare the multilocus microsatellite genotype of each individual from the introduced range to each of the native populations. This analysis was performed using the program ARLEQUIN 2.000 (Schneider *et al*. 2000), which implements the methods described by Paetkau *et al*. (1995, 1997) and Waser & Strobeck (1998) to calculate the log-likelihood of each individual's genotype in each potential source population.

Finally, because founder events and genetic drift may produce extreme changes in allele frequency in introduced populations, we compared the allelic identity of colonies in the native and introduced ranges at all microsatellite loci. Specifically, we determined how many of the alleles in each introduced colony could not be accounted for by alleles present in each putative (native) source. This provided a measure of allelic nestedness for each introduced colony within each native colony. A high value for a particular comparison indicates that the introduced population possesses many alleles that are absent in the corresponding native population. Thus, the native populations in high-scoring pairs are unlikely to be the source of the corresponding introduced population. Low values indicate that few of the alleles in the introduced population are absent in the corresponding native population, and that the native population in the pair, or one genetically similar to it, may be a potential source of the introduced population. Because the number of alleles sampled increases with the number of individuals genotyped, introduced colonies with larger sample sizes (i.e. CA*) will tend to have more alleles that do not match alleles sampled in native populations. Although this bias should not reduce the ability to identify likely sources of a particular introduced population, comparison of the number of unexplained alleles (found in introduced colonies, but not in native colonies) across colonies from different introduced populations may not be appropriate.

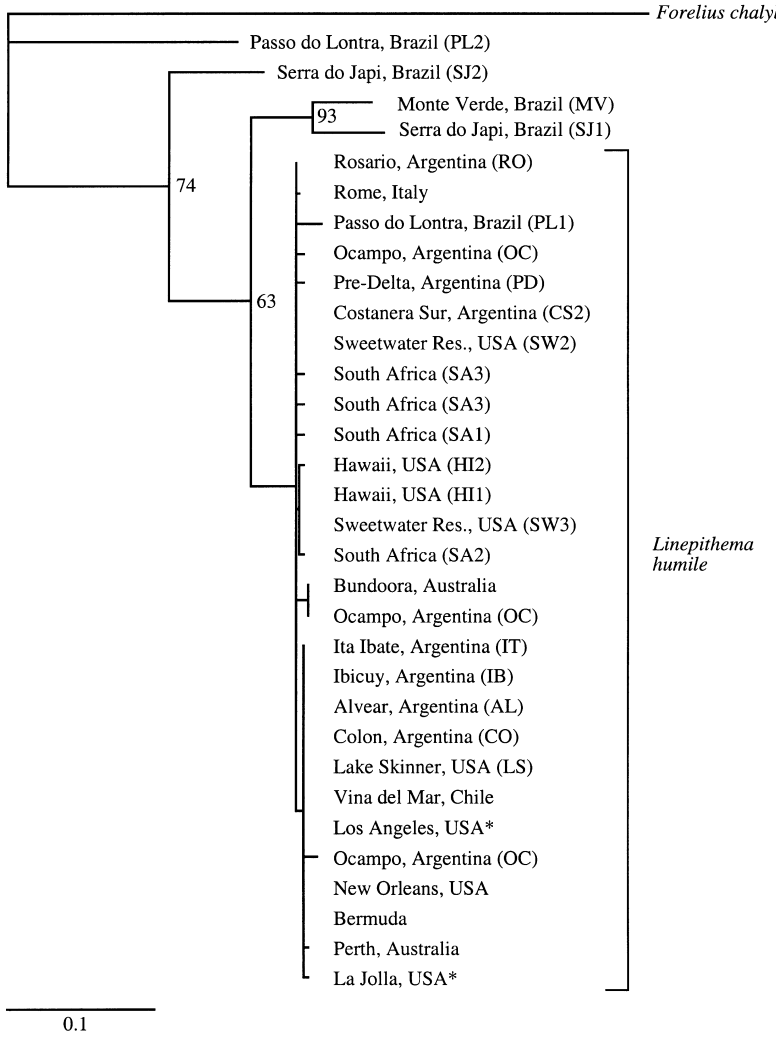

Fig. 2 Maximum likelihood phylogeny of native and introduced Argentine ant populations using 405 bp of the mitochondrial cytochrome *b* gene. Numbers indicate the reliability of each node as determined by the percentage of time each corresponding cluster was formed during 10 000 quartet puzzling steps (Strimmer & von Haeseler 1996). Sites that belong to the large California supercolony are designated with an asterisk (*).

Results

We first used mitochondrial cytochrome *b* sequences to reconstruct the relationships among native and introduced populations. All of the introduced populations, all native populations in Argentina and one population in Brazil (PL1) possessed similar mitochondrial haplotypes and formed a single group in the maximum likelihood tree (Fig. 2). The other four Brazilian samples possessed different and unique haplotypes. One group included a light-coloured species from Serra do Japi (SJ1) and a species from Monte Verde (MV; Fig. 2). A dark-coloured species from Serra do Japi (SJ2) and a population from Passo do Lontra (PL2; a site where *Linepithema humile* is also present) were more basally located, and were distinctly different from all *L. humile* populations from both the native and introduced ranges (Fig. 2). These Brazilian *Linepithema* were also placed outside the *L. humile* clade when maximum parsimony was used (not shown).

To resolve the relationships among the more similar native and introduced populations, we first calculated genetic distances among colonies using data from seven microsatellite loci, and visualized the relationships using NMDS. Several biogeographic patterns were evident in the native range. First, the Brazilian population (PL1) that was similar to ants from Argentina and the introduced range at cytochrome *b* was genetically different from these populations when microsatellites were used for the analysis (PL1; Fig. 3). Second, Argentine populations from the southern Rio Parana (IB, BA, PD, OT and RO) clustered together, separated along axis 2 from populations on the Rio Uruguay (AL and CO) and from the northern Rio Parana (IT, OC and IC). Third, genetic similarity within sampling locations was evident in the native range. On the southern Rio Parana, nests from the Buenos Aires area (BA and CS, Fig. 3) clustered together, as did nests collected in Reserva Otamendi (OT, Fig. 3).

In general, populations from the introduced range of the Argentine ant grouped together (Fig. 3). Within this group were both colonies from geographically close sites (i.e. sites in California) as well as some geographically distant sites.

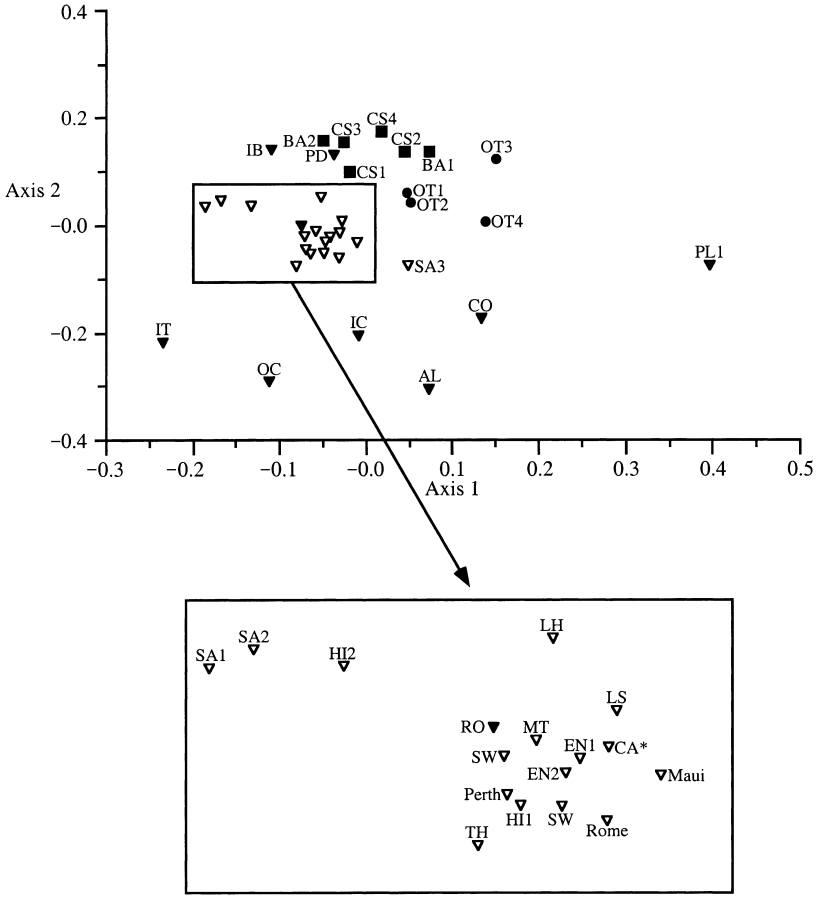

Fig. 3 Results from ordination analysis using non-metric multidimensional scaling (NMDS). The analysis was conducted using genetic distances among populations estimated from microsatellite allele frequencies. Open triangles represent introduced populations. Closed symbols represent native populations. Colonies in Buenos Aires (BA-urban sites; CS-sites in Reserva Costanera Sur) are represented by closed squares. Colonies from Reserva Otamendi (OT) are represented by closed circles. All other native populations are represented by closed triangles. The large California supercolony is labelled CA*. The full names of all sites are listed in Table 1. When multiple colonies were collected within a site, abbreviations include a number corresponding to the same colony in Tables 2 and 3.

In other cases, however, geographically distant sites were genetically similar. Colonies from Perth (Australia) and one site from Hawaii (HI), for example, were more similar to each other than to any other sites (Fig. 3). Similarly, ants from Rome, Italy, were similar to colonies in California (Fig. 3; SW and EN). Only one native population, from Rosario, Argentina (RO), was located within the cluster of introduced populations (Fig. 3), suggesting that many introduced populations may have originated from a similar native colony.

The introduced colonies that fell outside this grouping were from either Hawaii (HI2) or South Africa (SA1, SA2 and SA3). One of the colonies from South Africa (SA3), which was different from other parts of the introduced range and genetically quite distinct when compared to the other colonies in South Africa (Fig. 3). Interestingly, this population was also one of the two introduced populations that were most different from one another (SA1 and SA3; Fig. 3), and these two were geographically separated by a linear distance of less than 50 km. A similar pattern of genetic differentiation between spatially close sites was also evident in other parts of the introduced range. The two colonies from Volcanoes National Park, Hawaii (HI), were collected from nearby sites (< 2.0 km apart) on Kilauea Volcano and were genetically distinct from each other (Fig. 3). Similarly, two colonies in California (TH and LH) were genetically different from each other (Fig. 3) but were separated by only 50 km.

The similarity between RO and introduced populations was also observed when assignment tests were performed on individuals from the introduced range (Table 2). In 13 of the 16 introduced colonies examined, more individuals were assigned to RO than to any other introduced population. In nine of these cases, 80% or more of the individuals were most similar to RO, and all individuals from three sites in the introduced range (MT, EN2 and Maui) were assigned to RO. Other than RO, only five colonies from the native range (IC, PD, OT1, OT2 and BA2) were assigned more than 10% of individuals from an introduced colony. All five of these sites are in Argentina, three of them (OT1, OT2 and BA2) are located within 60 km of Buenos Aires, and all but IC are on the southern Rio Parana (Fig. 1).

The three introduced colonies in which the majority of individuals were assigned to a site other than RO (Rome, SA1 and SA3) appeared to be most similar to sites in and around Buenos Aires (Table 2). Six of the 10 individuals from Rome were assigned to a colony from Otamendi

Table 2 Results of the assignment test comparing individuals in the introduced range (top) to colonies in the native range (left). Values within the matrix indicate the per cent of individuals in the introduced population assigned to the corresponding native population. Bold numbers indicate the native population to which most individuals in each introduced population were assigned. Sample sizes (number of individuals genotyped) are shown in parentheses

	Introduced range															
	California, USA								Hawaii, USA				Cape Town, South Africa			
Native range	CA* (300)	MT (15)	EN1 (40)	EN2 (15)	LH (30)	SW2 (15)	SW3 (15)	LS (30)	HI1 (20)	HI2 (20)	Maui (10)	Perth (10)	SA1 (5)	SA2 (5)	SA3 (5)	Rome (10)
PL1 (5)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AL (10)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CO (10)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IC (10)	0.3	—	—	—	13.3	—	20.0	—	15.0	—	—	—	—	—	—	—
IT (30)	—	—	—	—	3.3	—	—	—	—	—	—	—	—	—	—	—
OC (10)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PD (10)	0.3	—	—	—	20.0	6.7	—	—	—	—	—	—	—	—	—	—
RO (10)	**88.9**	**100.0**	**82.5**	**100.0**	**43.3**	**93.3**	**53.3**	**63.3**	**85.0**	**95.0**	**100.0**	**90.0**	—	**80.0**	20.0	40.0
IB (10)	—	—	—	—	—	—	—	3.3	—	—	—	—	—	—	—	—
OT1 (10)	6.2	—	12.5	—	—	—	—	13.3	—	—	—	10.0	40.0	—	**60.0**	**60.0**
OT2 (15)	1.3	—	—	—	3.3	—	26.7	13.3	—	—	—	—	—	—	—	—
OT3 (45)	0.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
OT4 (10)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BA1 (20)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BA2 (10)	1.3	—	—	—	6.7	—	—	—	—	—	—	—	**60.0**	20.0	20.0	—
CS1 (10)	0.7	—	2.5	—	—	—	—	6.7	—	5.0	—	—	—	—	—	—
CS2 (55)	—	—	—	—	10.0	—	—	—	—	—	—	—	—	—	—	—
CS3 (20)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CS4 (10)	0.7	—	2.5	—	—	—	—	—	—	—	—	—	—	—	—	—

*Denotes nests from throughout California that belong to a single, large supercolony.

(OT1), whereas four were assigned to RO. The other two sites assigned to native colonies other than RO were both from South Africa (SA1 and SA3). Three of the five individuals sampled from SA1 were assigned to a colony in Buenos Aires (BA2), and two were assigned to a colony from Otamendi (OT1). In SA3, one individual was assigned to BA2, three to OT1 and one to RO. Although the individuals sampled from these locations in South Africa appear to be quite different than individuals from other parts of the Argentine ant's introduced range, more extensive sampling may be necessary to identify likely sources with confidence.

The analysis of allelic nestedness also indicated that native populations from the southern Rio Parana are the most likely sources of introduced populations (Table 3). Overall, alleles that were present in native populations matched alleles in colonies from Rosario (RO), Otamendi (OT1 and OT2), Costanera Sur (CS1 and CS3), Buenos Aires (BA2) and Ibicuy (IB). None of the colonies from the introduced range possessed a suite of alleles that were best matched by native populations from Brazil, from the Rio Uruguay (Argentina) or from north of Rosario on the Rio Parana (Argentina). The native colonies with the largest sample sizes (IT, OT3 and CS2) were not the best matches for any introduced colonies, suggesting that sampling differences among native populations did not contribute to the observed matches between introduced and native colonies.

Discussion

All of the *Linepithema humile* populations examined possessed identical, or extremely similar, mitochondrial haplotypes. In contrast, the other *Linepithema* species from Brazil were genetically distinct from both introduced and native populations of Argentine ants (Fig. 1). These data are consistent with unpublished morphological patterns (A. Wild & P. Ward, personal communication; Orr *et al*. 2001). Despite the similarity at cytochrome *b* of *L. humile* from Passo do Lontra (PL1) and introduced populations of Argentine ants, the analyses using microsatellites indicate that the *L. humile* population from Brazil (PL1) is genetically different from introduced populations, and is an unlikely source (Tables 2 and 3; Fig. 3). The microsatellite allele frequency data also revealed that native populations from the southern Rio Parana are, in general, more similar

Table 3 Allelic nestedness of introduced colonies within each native colony. Values indicate the number of alleles (at seven microsatellite loci) present in the introduced colony (top) but absent in the corresponding native colony (left). Bold numbers indicate the native colonies that best explain the alleles present in the corresponding introduced colony. A value of zero indicates that all of the alleles found in the introduced colony were also found in the putative source colony. Sample sizes are shown in Tables 1 and 2

	Introduced range															
	California, USA								Hawaii, USA				Cape Town, South Africa			
Native range	CA*	MT	EN1	EN2	LH	SW2	SW3	LS	HI1	HI2	Maui	Perth	SA1	SA2	SA3	Rome
PLl	17	10	11	10	15	14	14	16	9	10	9	8	9	9	10	8
AL	10	6	6	5	10	8	8	11	5	6	5	4	5	5	5	4
CO	11	5	6	4	9	9	9	11	4	5	4	3	6	6	5	3
IC	10	4	5	4	7	7	8	11	4	4	3	3	7	6	4	3
IT	12	5	6	5	9	8	7	10	4	5	3	4	6	6	5	4
OC	10	4	4	3	8	5	5	8	3	5	3	2	4	4	4	2
PD	7	3	3	3	6	6	7	8	3	5	2	4	4	4	3	4
RO	6	**1**	2	**1**	4	**4**	5	6	**1**	**1**	**0**	2	**3**	3	**1**	2
IB	7	1	2	2	9	7	7	11	3	6	2	3	5	4	3	3
OT1	**5**	2	2	2	6	5	**4**	**4**	2	4	1	**1**	4	4	2	**1**
OT2	**5**	2	**1**	2	5	**4**	**4**	**4**	2	2	1	2	**3**	3	2	2
OT3	9	3	4	3	8	6	6	7	3	6	2	4	5	5	4	4
OT4	12	5	7	6	11	9	10	9	5	7	4	5	7	7	6	5
BA1	6	3	3	4	6	6	6	7	3	4	2	4	4	4	2	4
BA2	7	5	5	5	8	9	9	9	6	4	4	4	**3**	**2**	3	4
CS1	7	2	2	3	**3**	5	6	8	3	2	2	2	4	3	3	2
CS2	7	4	4	4	6	5	6	9	4	5	3	4	6	5	3	4
CS3	5	3	3	3	6	7	7	6	3	3	2	2	**3**	3	2	2
CS4	9	5	5	4	8	8	7	9	4	5	3	4	5	5	4	4

*Denotes nests from throughout California that belong to a single, large supercolony.

to introduced populations than are native populations from farther north on the Rio Parana (IT, OC and IC) or populations from the Rio Uruguay (AL and CO). Moreover, the microsatellite data indicate that the *L. humile* population from Rosario, Argentina (RO) is most similar to introduced populations, and warrants consideration as a potential source. Although populations in Rosario and Buenos Aires (BA and CS) occur in urban areas, and may have been introduced by humans from elsewhere in the native range, the genetic similarity of these populations to Argentine ants in nearby undeveloped areas (OT and IB) suggests that any such transport has occurred locally (Fig. 3; Tsutsui & Case 2001). Interestingly, the *L. humile* population at Passo do Lontra, Brazil also occurs in and near sites of human habitation, whereas the other *Linepithema* species (PL2) from this site, which hosts phorid fly parasitoids, is genetically distant from all populations of *L. humile* and occurs in natural seasonal wetland habitat (Orr & Seike 1998). Therefore, we cannot exclude the possibility that Argentine ants may have been introduced to this site during the establishment of human developments.

Within the introduced range, the observation that some spatially close colonies are genetically differentiated from each other suggests that multiple introductions from different sources may have occurred. Consistent with this possibility, the most divergent South African populations (SA1/SA2 vs. SA3) each possess alleles that are absent from the other population, and the two groups appear to be fixed for different alleles at one of the seven microsatellite loci sampled. It is important to note, however, that the individuals collected from sites SA1 and SA2 were collected in 1999, whereas those from SA3 were collected in 1997 (C. Christian, personal communication). Thus, the genetic differences observed in South Africa may represent temporal genetic changes that occurred between 1997 and 1999 (e.g. new introductions). Closer examination of historical collections as well as additional sampling from more locations may reveal if secondary introductions occurred during this time period, or if other processes have produced genetic changes over time in South African populations.

The two adjacent colonies from Volcanoes N.P. (Hawaii) also have unique alleles relative to each other, suggesting that these two colonies may have independent origins. Interestingly, the Argentine ant population on the neighbouring island of Maui contained a nested subset of the alleles present in one of the colonies from Volcanoes N.P.,

suggesting that the Maui colony may have been introduced from this colony on Hawaii. Because the population on Maui is also one of the most genetically depauperate (fixed for a single allele at six of the seven loci), it is possible that the founders of this population passed through a series of intermediate introductions, and concomitant population bottlenecks, before arriving on Maui.

Overall, it appears that several parts of the introduced range are occupied by colonies from multiple introductions. One possibility is that different independent introductions from the native range may have occurred, each from a slightly different source. An alternative possibility is that genetic differences among different introduced populations arose as the lineages followed different routes of introduction before becoming established in their current locales. Because this process would involve multiple population bottlenecks and periods of small population size, different lineages from the same source could rapidly become genetically different through a series of founder effects and genetic drift. Although one expectation is that this process would produce a series of genetically nested introduced populations, closer examination of the alleles present within the locations studied here failed to reveal any such pattern (N. Tsutsui, unpublished data).

Molecular genetic tools have been successfully applied to the study of other invasive species. Another particularly well studied case is the agriculturally damaging Mediterranean fruit fly, *Ceratitis capitata*. A broad range of molecular markers, including mitochondrial restriction fragment length polymorphisms (Gasparich *et al*. 1997), allozymes (Baruffi *et al*. 1995), random amplified polymorphic DNAs (Baruffi *et al*. 1995; Haymer *et al*. 1997), nuclear intron sequences (Villablanca *et al*. 1998; Davies *et al*. 1999b), and microsatellites (Bonizzoni *et al*. 2000) have documented the loss of genetic diversity associated with population bottlenecks and clarified the genetic relationships among native and introduced populations. Additionally, the use of intron sequences and new statistical techniques has established that individuals captured in the introduced range in different years represent separate introduction events rather than captures from an infestation that has persisted at low levels (Davies *et al*. 1999b). In this species, highly polymorphic nuclear loci have been particularly useful because lekking behaviour (Hoelzer 1997) and the haploid, maternally inherited nature of mitochondria reduces the effective population size of mitochondrial genomes, allowing a single haplotype to reach high frequencies or become fixed in many introduced populations (e.g. Gasparich *et al*. 1997).

The use of molecular tools to reconstruct the history of invasions and to identify the source of introduced populations may also facilitate the identification of effective biological control agents for introduced species. Current efforts to identify biological controls for *L. humile* (and invasive ants more generally) have focused on phorid fly parasitoids (Orr *et al*. 1995; Porter *et al*. 1995; Orr & Seike 1998). Phorid flies alter the behaviour of ants and can change the outcome of competitive interactions between ant species (Feener & Brown 1997; Orr *et al*. 1995; Feener 2000). Because many phorids are host-specific (Disney 1994; Porter *et al*. 1995; Feener & Brown 1997; Gilbert & Morrison 1997), it is important to identify the appropriate host population for biological control of introduced populations. However, recent studies have confirmed that the phorid flies that attack Brazilian *Linepithema* populations do not attack Argentine ants from either the native or introduced ranges (Orr *et al*. 2001). These behavioural data, coupled with the morphological (P. Ward & A. Wild, personal communication) and genetic (Fig. 2) dissimilarity between Brazilian *Linepithema* and *L. humile*, suggest that the use of phorid flies for the biological control of Argentine ants may not be effective. In theory, parasitoids of Argentine ant populations located on the southern Rio Parana would be more appropriate candidates for the biological control of introduced populations. At present, however, no phorid flies are known to parasitize these populations (Orr *et al*. 2001), and we have not discovered phorid parasitoids during our studies of native populations.

The success of Argentine ants in their introduced range has also been attributed to their escape from phorid fly parasitoids (Chapin *et al*. 2000; Feener 2000), as has the success of the red imported fire ant (*Solenopsis invicta*) in the United States (Orr *et al*. 1995). Although escape from other natural enemies and co-evolved competitors probably plays a role in the successful establishment and spread of the Argentine ant in its introduced range, the apparent absence of phorid fly parasitoids of Argentine ants in both the native and introduced ranges suggests that escape from phorid flies has not led to the Argentine ant's success in the introduced range.

The ability of Argentine ants to become a damaging invader may arise, in part, from behavioural and genetic changes that have occurred during the introduction and establishment of this species. In introduced populations, Argentine ants are unicolonial (Newell & Barber 1913; Markin 1968; Way *et al*. 1997; Suarez *et al*. 1999; Krieger & Keller 2000; Tsutsui *et al*. 2000). This colony structure is characterized by the formation of expansive supercolonies that contain numerous interconnected and cooperative nests (Newell & Barber 1913; Markin 1968). A lack of intraspecific aggression within supercolonies is thought to allow Argentine ant populations to reach high densities (Holway 1998; Holway *et al*. 1998) allowing them to achieve numerical superiority in contests with native ant species (Suarez *et al*. 1998; Holway 1999). In contrast, native populations of Argentine ants are typically not unicolonial (Suarez *et al*. 1999; Tsutsui *et al*. 2000; Tsutsui & Case 2001), and Argentine ants in their native range coexist with other ants in species-rich communities (Suarez *et al*. 1999). These

differences in colony structure between the two ranges are associated with the loss of genetic diversity during the introduction of the Argentine ant (Tsutsui *et al*. 2000; Tsutsui & Case 2001). Because many other invasive ant species are also unicolonial in their introduced ranges (Passera 1994), this mechanism may be a common route to invasive success for introduced ants generally. Studies investigating the source of introduced populations for other invasive ants will reveal if changes in social structure, such as those seen in Argentine ants (Tsutsui *et al*. 2000; Tsutsui & Case 2001) and red imported fire ants (*Solenopsis invicta*; Ross *et al*. 1996), have occurred and will clarify the role of unicoloniality (if any) in their success as biological invaders.

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