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The worldwide expansion of the Argentine ant

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ABSTRACT

Aim The aim of this study was to determine the number of successful establishments of the invasive Argentine ant outside native range and to see whether introduced supercolonies have resulted from single or multiple introductions. We also compared the genetic diversity of native versus introduced supercolonies to assess the size of the propagules (i.e. the number of founding individuals) at the origin of the introduced supercolonies.

Location Global.

Methods We used mitochondrial DNA (mtDNA) markers and microsatellite loci to study 39 supercolonies of the Argentine ant *Linepithema humile* covering both the native (n = 25) and introduced range (n = 14).

Results Data from three mitochondrial genes and 13 nuclear microsatellites suggest that the introduced supercolonies studied originated from at least seven founding events out of the native area in Argentina (primary introductions). The distribution of mtDNA haplotypes also suggests that supercolonies in the introduced range each derive from a single source supercolony and that one of these source supercolonies has been particularly successful, being the basis of many introduced populations spread across the world. Comparison of the genetic diversity of supercolonies based on the five most diverse loci also revealed that native and introduced supercolonies have greatly overlapping ranges of diversity, although the genetic diversity is on average less in introduced than in native supercolonies.

Main conclusions Both primary introductions (from the native range) and secondary introductions (from sites with established invasive supercolonies) were important in the global expansion of the Argentine ant. In combination with the similar social organization of colonies in the native and introduced range, this indicates that invasiveness did not evolve recently as a unique and historically contingent event (e.g. reduction of genetic diversity) in this species. Rather, native *L. humile* supercolonies have characteristics that make them pre-adapted to invade new – and in particular disturbed – habitats when given the opportunity. These results have important implications with regard to possible strategies to be used to control invasive ants.

Keywords

Biological invasions, genetic bottlenecks, invasion history, *Linepithema humile*, social insects, supercolonies.

INTRODUCTION

Biological invasions by non-indigenous species are widely recognized as a significant component of human-caused global environmental change, often resulting in a significant loss in the economic value, biological diversity and function of invaded ecosystems (Elton, 1958; D'Antonio & Vitousek, 1992; Lodge, 1993; Vitousek *et al.*, 1996, 1997). Studies of the phylogeographic structure (Stone & Sunnucks, 1993; Bastrop *et al.*, 1998; Blum *et al.*, 2007; Taylor & Keller, 2007), genetic

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diversity and the potential for the rapid evolution of these species may provide novel insights into the colonization dynamics and spread of invasive taxa. Colonization events may result in population bottlenecks because the number of initial colonists is often small. Genetic drift during colonization may also bring about reduced genetic variation in the newly established population (Nei *et al.*, 1975). This effect will be especially strong when all colonists are drawn from the same source population. Thus, a newly established population is likely to be much less genetically diverse than the population from which it is derived. Reduced genetic diversity can have two consequences. First, inbreeding depression may limit population growth and lower the probability that the population will persist (Ellstrand & Elam, 1993; Newman & Pilson, 1997; Nieminen *et al.*, 2001). The effects of reduced genetic diversity will be especially strong if the population remains small for a number of generations. Second, reduced genetic diversity will limit the ability of the population to evolve adaptively (Allendorf & Lundquist, 2003). Thus, we face a paradox: if population bottlenecks are harmful, then why are invasive species that have gone through a founding bottleneck so successful?

There have been several proposals to explain how invasive populations overcome the challenge of low genetic diversity (Allendorf & Lundquist, 2003). First, it has become clear that introduced populations can succeed despite reduced diversity at neutral genetic loci such as microsatellites. This might be because variation at most molecular markers underestimates non-neutral genetic diversity and so is of limited importance in assessing ability to respond to new environments (Reed & Frankham, 2001; Lee, 2002; Hufbauer, 2004). Alternatively, demographic bottlenecks might have positive impacts on introduced populations by purging deleterious alleles (Kristensen & Sorensen, 2005; Schmid-Hempel *et al.*, 2007), preserving highly adapted clonal lineages from sexual recombination (Ren *et al.*, 2005; Liu *et al.*, 2006; Mergeay *et al.*, 2006). Finally, successful invasions might be those where a minimum level of genetic diversity was maintained after introduction. Indeed, recent studies suggest that there simply are no dramatic diversity losses in most successful invasions (Roman & Darling, 2007; Dlugosch & Parker, 2008), possibly because of multiple introductions combined with interbreeding between the introduced populations (Johnson & Starks, 2004; Kolbe *et al.*, 2004; Bossdorf *et al.*, 2005; Genton *et al.*, 2005; Wilson *et al.*, 2009). Therefore, a key step in the comprehension of biological invasions lay in the estimation of the frequency with which a species has been introduced into a specific area, the number of individuals introduced (propagule size) and the subsequent pattern of spread across the natural landscape.

Invasion success varies among taxonomic group (Williamson & Fitter, 1996), but ants are some of the most damaging invaders at both ecological and economical levels (Clark *et al.*, 1982; Porter & Savignano, 1990). Many invasive ants share a suite of characteristics that facilitate their introduction, establishment and subsequent range expansion. One feature of particular importance is the ability to form numerically large, ecologically dominant colonies (Helanterä *et al.*, 2009). In his review McGlynn (1999) reported that of the 147 ant species found out of their native range the most widespread share the characteristics of tramp species described by Hölldobler & Wilson (1990) and Passera (1994), these being small body size, monomorphism of the worker caste, reproduction by budding, high interspecific aggression, polygyny (i.e. the presence of multiple reproductive queens in a mature colony) and often a close association with human activities.

The Argentine ant *Linepithema humile* (Mayr) is one of the most studied invasive species (Pyšek *et al.*, 2008). Native to South America, it has been introduced all over the world particularly in Mediterranean climates and occurs on six continents and many oceanic islands (Suarez *et al.*, 2001). Once established, the Argentine ant reduces populations of native ants and other arthropods (Ward, 1987; Cole *et al.*, 1992; Cammell *et al.*, 1996; Human & Gordon, 1996; Holway, 1998; Suarez *et al.*, 1998; Sanders *et al.*, 2001, 2003), and is considered as a significant agricultural and urban pest (Newell & Barber, 1913; Knight & Rust, 1990). The ecological domination of this species in its introduced range is thought to stem from its social organization, whereby individuals mix freely within large supercolonies containing a high number of interconnected nests (Hölldobler & Wilson, 1977; Heller, 2004; Heller *et al.*, 2008). By reducing the costs associated with territoriality, this social structure allows high worker densities and effective habitat monopolization by the competitive exclusion of other ant species (Human & Gordon, 1996; Holway, 1998; Holway & Suarez, 2004; see however Heller *et al.*, 2006).

The first aim of this study was to determine the number of successful establishments of the Argentine ant out of its native area and to see whether introduced supercolonies have resulted from a single or multiple introductions. To this end, we performed a global study of native and introduced supercolonies covering six continents. We used three mitochondrial DNA (mtDNA) markers and microsatellite loci to study five localities from the native range and 40 localities from the introduced range. The second aim was to compare the genetic diversity of native versus introduced supercolonies to assess the size of the propagules (i.e. the number of founding individuals) at the origin of the introduced supercolonies.

METHODS

Sampling

Workers of *L. humile* were sampled from 179 nests in 45 localities [5 from the native range and 40 from the introduced range (Wild, 2004)] spanning all six continents where the species occurs and several oceanic islands (Table 1). The individual genetic data of Pedersen *et al.* (2006) are included and reanalysed here. To serve as an outgroup for the phylogenetic analyses, we also sampled *Linepithema oblongum* workers, the sister species to *L. humile*

Table 1 Samples of *Linepithema humile* from 45 localities of the native (N) or introduced (I) ranges with information on the distance between the most remote nests sampled and the number of nests (*N*) and individuals (*n*) analysed genetically. Name label and number (*S*) of supercolonies are given for each locality (see text for details).

Supercolonies	*S*	Localities	Positions	Range	Distance (km)	Haplotypes		Micro-satellites	
						N	*n*	*N*	*n*
Corrientes S1–S2	2	Argentina, Corrientes, Arroyo Cuay Grande	28°41′31″ S 56°14′16″ W	N	0.6	23	31	23	340
Otamendi S1–S19	18*	Argentina, Buenos Aires, Otamendi	34°11′49″ S 58°52′47″ W	N	6.0	25	190	25	369
Buenos Aires	1*	Argentina, Buenos Aires, Buenos Aires City	34°41′24″ S 58°27′32″ W	N	–	1	10	1	20
Boca S1–S3	3	Argentina, Buenos Aires, Buenos Aires City, Boca	34°38′23″ S 58°21′55″ W	N	1.1	11	30	11	211
Santa Coloma	1	Argentina, Buenos Aires, Santa Coloma	34°3′35″ S 59°31′27″ W	N	0.05	10	10	6	96
Tucumán	1	Argentina, Tucumán, Ticucho	26°30′55″ S 65°14′43″ W	I	–	1	10	1	11
La Rioja	1	Argentina, La Rioja: Chuquis & Aminga (two localities)	28°53′22″ S 66°58′17″ W; 28°50′56″ S 66°56′4″ W	I	6.9	20	10	47	64
Chile	1	Chile: Conceptión, University Campus → Valparaíso, Los Andes (10 localities)	36°49′38″ S 73°2′16″ W; 32°44′S 70°38′W	I	540	10	20	10	100
Ecuador	1	Ecuador, Pichincha, Carapungo	0°5′S 78°27′W	I	–	1	20	1	35
California	1	California, Davis, Putah Creek	38°31′31″ N 121°47′28″ W	I	0.1	10	20	10	100
Hawaii	1	Hawaii, Haleakala	20°44′44″ N 156°14′19″ W	I	5.0	10	20	10	100
Bermuda	1	Bermuda: Main Island, Hog Bay → Saint David's Island (six localities)	32°17′N 64°52′W; 32°22′N 64°39′	I	22	6	30	6	30
Catalonia	1	Spain: Sant Cugat del Vallès → Guardamar del Segura (three localities)	41°28′31″ N 2°4′26″ E; 38°8′50″ N 0°39′4″ W	I	958	3	20	3	60
Europe Main	1	Europe: Italy, Moneglia → Spain, Calpe (10 localities)	44°15′52″ N 9°26′46″ E; 38°43′38″ N 0°6′38″ E	I	2279	10	20	10	100
Australia	1	Australia, Melbourne, La Trobe	37°43′09″ S 145°2′53″ E	I	0.6	10	20	10	100
New Zealand	1	New Zealand, Wellington	41°17′11″ S 174°45′53″ E	I	–	1	20	1	35
South Africa	1	South Africa, Western Cape, Stellenbosch	33°55′55″ S 18°51′50″ E	I	–	1	20	1	100
Japan S1	1	Japan, Kobe, Port Island, Kita-Kouen & Naha-Kouen (two localities)	34°40′27″ N 135°12′29″ E; 34°40′35″ N 135°12′18″ E	I	0.4	2	20	2	39
Japan S2	1	Japan, Hiroshima, Hatsukaichi-shi	34°21′N 132°20′E	I	–	1	20	1	18
Total	39					156	541	179	1928

*One supercolony (genetic group) is represented both in Otamendi and Buenos Aires City.

(Wild, 2007, 2009) from two localities in the province of Tucumán in Argentina (Quebrada de la Mesada, one nest, 26°21′28″ S 65°31′56″ W; Carapunco, five nests, 26°21′28″ S 65°31′56″W). Detailed sample information is available upon request from the authors.

Genetic analyses

DNA was extracted and purified from a total of 1928 worker samples of *L. humile* (Table 1) using a standard phenol–chloroform protocol or by the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). All individuals were analysed at the five microsatellite loci *Lhum*-3, *Lhum*-11, *Lhum*-19, *Lhum*-28 and *Lhum*-35. To have a better resolution of the relationship between introduced populations, we analysed individuals from the introduced range at additional eight loci: *Lhum*-13, *Lhum*-33, *Lhum*-39, *Lhum*-52, *Lhum*-62, *Lihu*-M1, *Lihu*-S3 and *Lihu*-T1 (Krieger & Keller, 1999; Tsutsui *et al.*, 2000). These 13 loci did also amplify for *L. oblongum* supporting the close relationship of the two species. Polymerase chain reaction (PCR) products were separated in polyacrylamide gels and visualized by autoradiography or run on an ABI 377 XL automated sequencer (Applied Biosystems, Foster City, CA, USA) with subsequent scoring of alleles using GeneMapper 4.0 (Applied Biosystems). The mean number of individuals analysed per locality was 43 (range 11–369).

Three different fragments of the mitochondrial DNA were amplified: 524 bp of the cytochrome *b* gene (Pedersen *et al.*, 2006), 803 bp of the cytochrome *c* oxidase subunit I (COI) gene (Vogel *et al.*, 2009) and 748 bp of the cytochrome *c* oxidase subunit II (COII). Specific primers were designed for COII as part of this study (Table 2). Polymerase chain reaction reactions were performed in 50-μL volumes with a final concentration of 50 pM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ for Cyt *b* and 2.5mM for the other primers and 0.5 U of Taq polymerase (Qiagen, Valencia, CA, USA). Amplification for all sequences involved an initial step at 94 °C for 3 min, then 35 cycles of 92 °C for 30 s, 30 s at 60 °C for Cyt *b* and 1 min at 45 or 47 °C for the other markers (Table 2), and finally 72 °C for 30 s. After checking PCR products on a 1.5% agarose gel, they were purified using the QIAquick purification kit (Qiagen) and directly double-stranded sequenced by the automated sequencer using the BigDye terminator ready-reaction kit. Sequence data were edited and compiled using Lasergene 7.1.0 (Dnastar Inc., Madison, WI, USA).

Statistical analyses of microsatellite data

Because the Argentine ant forms supercolonies that are highly genetically differentiated from each other, even when very closely located (Pedersen *et al.*, 2006; Vogel *et al.*, 2009), we used the Bayesian clustering method implemented in BAPS 4.14 (Corander *et al.*, 2003, 2004) to assign nests to distinct genetic groups. Previous studies showed that the clustering of nests on the basis of genetic data is congruent with results of aggressive nests, hence indicating that nests can reliably be assigned to supercolonies on the basis of genetic data (Jaquiéry *et al.*, 2005; Pedersen *et al.*, 2006; Vogel *et al.*, 2009). This method clusters groups of individuals likely to come from the same randomly mating subpopulation without a priori assumptions (Corander *et al.*, 2003; Pedersen *et al.*, 2006). Data from the native and introduced ranges were analysed separately because samples were analysed with a different number of microsatellite loci (5 and 13, respectively). For the analyses, each nest was considered as a group, and the maximum number of genetically divergent groups (*K*) was set to the number of nests included in the data set (54 and 113, respectively). Each analysis was repeated 10 times to ensure consistency of results between different runs. A total of 25 genetic groups were identified in the native range and 14 genetic groups in the introduced range. Tests of aggression between workers of different nests had been conducted in 18 of the 45 localities, in both the native and introduced ranges. In all cases, there was a perfect agreement between genetic and behavioural data with the distinct genetic clusters corresponding to the supercolonies identified by behavioural data (Giraud *et al.*, 2002; Jaquiéry *et al.*, 2005; Vogel *et al.*, 2009; see also Discussion). Because supercolonies form closed breeding units with most or all the matings occurring within supercolonies and consequently gene flow being extremely limited between supercolonies (Jaquiéry *et al.*, 2005; Pedersen *et al.*, 2006; Vogel *et al.*, 2009), we will refer to these distinct genetic groups as supercolonies.

To assess the relationship between the introduced supercolonies identified by the clustering method, we performed a principal component analyses (PCA) on the microsatellites data using the program Pcagen 1.2 (Goudet, 2000; http://

Table 2 Primers used for the amplification of three mitochondrial fragments (cytochrome *b*, cytochrome oxidase sub-units I and II) with annealing temperature (AT) and elongation time (ET) given.

Marker	Primer	Sequence	AT (°C)	ET (min)
Cyt *b*	L-Lh-Cb	5′-GGGCAACAGTTATTACAAACTTAGTG-3′	60	1
	R-Lh-Cb	5′-TAAGGGTATTCAATTGGTTGGG-3′		
COI	L-Lh-COI	5′-TAATATGGCAGATAAGTGCA-3′	45	1.5
	R-Lh-COI	5′-TCATATCTTCAATATCATTG-3′		
COII	L-Lh-COII	5′-TAATATGGCAGATAAGTGCA-3′	47	1.5
	R-Lh-COII	5′-TCATATCTTCAATATCATTG-3′		

www2.unil.ch/popgen/softwares/pcagen.htm). The percent inertia of each principal component axis and its respective *P* value were estimated by performing 10,000 randomizations of multilocus genotypes.

To further investigate the invasive history of introduced supercolonies, we also used the Bayesian clustering method implemented in Structure 2.1 (Pritchard *et al.*, 2000; http://pritch.bsd.uchicago.edu/). Similarly to BAPS, this software infers the number of different clusters (*K*) that best suit a dataset and assigns individuals to the inferred clusters. All simulations performed in Structure featured 500,000 runs following a burnin period consisting of 100,000 runs. Ten separate runs were performed for each value of *K* tested. We ensured accurate estimates of the simulation values by checking that model parameters equilibrated before the end of the burnin phase and that posterior probabilities were consistent across all ten runs. Then, we determined the appropriate value of *K* for each dataset using the ΔK method of Evanno *et al.* (2005).

To estimate the number of independent introductions, we ran Structure on the dataset composed of only introduced supercolonies. We tested all values of *K* from 1 to 20. According to the ΔK method developed by Evanno *et al.* (2005), the most likely number of genetic groups in our dataset was $K = 6$. Unfortunately, we could not interpret the results because the assignment of supercolonies into genetic groups was not consistent between the different Structure runs. Interestingly, all the individuals that were assigned to supercolonies according to BAPS were always assigned to the same genetic groups with a high posterior probability (> 0.8) but the different supercolonies were not always clustered the same way. For example, depending on the run, the Catalonian supercolony either formed its own genetic group or was clustered with the supercolonies of Tucumán and/or La Rioja. It is currently unclear what may have caused the discrepancy observed across the different runs of Structure. Thus, to infer introduction pathways, we analysed separately each group of supercolonies suspected to have a common history based on the PCA analysis and genetic differentiation. This was the case for two groups of supercolonies (see Results for more details). Accordingly, we ran Structure on the supercolonies sharing the mtDNA haplotype H1 (without South Africa) and on the supercolonies sharing the mtDNA haplotypes H3. For both analyses, we tested values of *K* from 1 to 10.

Estimations of genetic diversity and genetic differentiation were performed with Fstat 2.9.3.2 (Goudet, 1995). Genetic diversity within each supercolony was quantified by two estimators unbiased with regard to sample size: Nei's (1978) measure of expected heterozygosity (H_{exp}) and allelic richness (k'), which corresponds to the number of alleles adjusted to a minimum common sample size using rarefaction statistics (Petit *et al.*, 1998). Statistical significance of differences in expected heterozygosity or allelic richness on average per locus between native and introduced supercolony were assessed in two-sided permutation tests applying 15,000 randomizations.

The genetic differentiation both overall and between all pairs of supercolonies was quantified as F_{ST} with significance testing based on 10,000 randomizations and for the pairwise estimates correcting significance levels for multiple tests (Bonferroni correction). To investigate the relationship between the different introduced supercolonies, we also estimated the allelic nestedness: that is, for each pair of supercolonies we calculated the percentage of alleles found in one supercolony that were also present in the other supercolony and vice versa. This analysis was based on the principle that supercolonies derived from more recent secondary introductions should contain a subset of the alleles present in the supercolony of origin.

Statistical analyses of mtDNA data

To evaluate the variability of haplotypes, the three mtDNA fragments were analysed in at least one individual per genetic group. When applicable, this sample size was increased when several haplotypes were found with the Cyt *b* marker. Then to evaluate within genetic group variability, Cyt *b* (the most variable fragment) was analysed in a minimum of 10 individuals per native supercolony and 20 for each introduced supercolony. In total, 188 individuals were studied at all three fragments with an additional 353 studied at Cyt *b* only, so that this haplotype was scored for an average of 13.5 individuals (range 10–30) per supercolony. For *L. oblongum*, all three fragments were analysed in 10 individuals (five of the first locality and one per nest of the second locality).

The phylogenetic relationship of the supercolony haplotypes was determined using three different approaches: maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference analysis (BI). The parsimony analysis was performed using Paup* 4.0b8. A heuristic search option with 100 random-addition replicates was used with equal weighting of all characters and TBR branch swapping. Phylogenetic analyses under the maximum likelihood criteria (ML) was also performed over the combined data set with Paup* 4.0b8. A heuristic search was conducted using stepwise addition with 100 random replications. Finally, the Bayesian analysis was performed with MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) on the entire data set, but model parameters were estimated for all partitions separately. The number of generations was set to 5×10^6. The average standard deviation of split frequencies of the two simultaneous and independent runs (four chains implemented in each run) performed by MrBayes 3.1 reached stationarity much before 50,000 generations (SD = 0.0055). A tree was sampled every 100 generations. A consensus tree was constructed by MrBayes 3.1 with a burnin period of 500. The appropriate model of DNA substitution was determined for the ML analyses using Modeltest 3.06 (Posada & Crandall, 1998) and for the BI using MrModeltest (Nylander, 2004). These procedures implement hierarchical likelihood ratio tests to determine the model that best fit the data. The evolutionary models selected with the Hierarchical Likelihood Ratio Tests (hLRTs) and Akaike Information Criterion (AIC) by Modeltest and MrModeltest were the same; a HKY model taking a gamma distribution into account and having the following

parameters: base frequencies; A = 0.3626, C = 0.1476, G = 0.1476, T = 0.3772; Ti/Tv ratio = 10.4996; gamma shape parameter, $\alpha = 0.7976$; and proportion of invariable site, I = 0.6556). Support for nodes was assessed by bootstrapping (1000 replicates) (Felsenstein, 1985) for the MP and ML analyses and with the posterior probabilities of reconstructed clades as estimated by MrBayes 3.1 for the BI analysis. In all phylogenetic analyses, *L. oblongum* was used as the outgroup because it is considered to be the sister species of *L. humile* (Wild, 2007). All methods used for reconstructing phylogenies gave virtually identical topologies. In order to provide the results of all methods, bootstrap values of the MP, ML and the posterior probability inferred from the Bayesian analysis are given for each node.

Finally, to obtain an alternative presentation of the relationship among haplotypes found, we used the parsimony-based analysis implemented in TCS 1.21 (Clement *et al.*, 2000) to construct a minimum spanning network of haplotypes. Because the sampling effort was not the same for all supercolonies, we did not take into account the frequencies of haplotypes.

RESULTS

Assignment to genetic groups (supercolonies)

The clustering method implemented in BAPS identified 14 distinct genetic groups in the 40 localities sampled in the introduced range. The clustering of nests were identical in all 10 runs and strongly supported by a posterior probability $P = 1.000$.

In the native range, 25 genetic groups were identified in the five localities sampled. These groups corresponded exactly to the supercolonies identified by Pedersen *et al.* (2006). The locality of Buenos Aires City, which had not been studied previously, clustered with one of the supercolonies (S19) collected in Otamendi. This partition of nests was strongly supported by a posterior probability $P = 0.998$ in each of the 10 runs.

Nucleotide composition and sequence variation

Over the entire 2075 bp region there were 62 variable sites. When the outgroup *L. oblongum* was also considered, the number of sites was 178. A total of 21 haplotypes were identified, with 18 haplotypes being species-specific for *L. humile* and three for *L. oblongum*. Overall, the haplotype divergence was 0.05–1.4% within *L. humile* and 5.6–6.3% between the two *Linepithema* species.

Introduction pathways

Seven haplotypes were discovered in the 14 introduced supercolonies with each of these supercolonies exhibiting a single haplotype except California where one individual out of the 20 analysed had another haplotype (Figs 1 & 2).

The minimum spanning network illustrates that some of the haplotypes are differentiated by more than 20 mutations, while others differ by a single mutation (Fig. 3). Some nodes are not significantly supported and cannot be considered as resolved. However, we considered that the haplotypes found in introduced supercolonies are originally from the native range because the worldwide spread of the species started only about hundred years ago. Thus, the probability of a single mutation occurring and becoming established in a supercolony within that amount of time is very low.

The ordination of introduced supercolonies according to the first two axes of the principal components analysis based on microsatellite genetic data is presented in Fig. 4. The first two axes accounted for almost half of the variance and were significant according to the broken stick model ($P < 0.05$; test in Pcagen) but not in permutation tests (PC1 = 28%, $P = 0.28$; PC2 = 18%, $P = 0.26$). There was a very high and statistically significant genetic differentiation between the introduced supercolonies with an average $F_{ST} = 0.419 \pm 0.043$ SE ($P < 0.0001$). All pairwise F_{ST} values were significant after correction for multiple tests and covered a wide range from 0.11 between Australia and New Zealand to 0.65 between Tucumán and Hawaii (Table 3).

The number of primary introductions was assessed from the worldwide distribution of haplotypes and from the genetic differentiation between supercolonies at microsatellite loci. Four haplotypes (H2, H8, H9 and H17) were each found in a single introduced supercolony (Japan S1, La Rioja, Catalonia and Tucumán, respectively). These four haplotypes were not found in any of the other introduced supercolonies. Thus, the Japan S1, La Rioja, Catalonia and Tucumán supercolonies most likely originate from four independent introductions from the species' native range in Argentina.

Another haplotype (H1) was found in seven introduced supercolonies (Australia, New Zealand, California, Hawaii, Main Europe, South Africa and Japan S2). An analysis of the nuclear markers revealed relatively low genetic differentiation between six of these supercolonies ($0.10 <$ pairwise $F_{ST} < 0.40$, Table 3). By contrast, the seventh supercolony (South Africa) showed a comparatively high differentiation to the other six supercolonies (all pairwise $F_{ST} > 0.45$, Table 3). The genetically divergent nature of the South African supercolony was also revealed by the PCA analysis where the South African supercolony was far from the six other supercolonies that clustered together. Altogether these data suggest that while the supercolonies from Australia, New Zealand, California, Hawaii, Main European and Japan S2 may originate from a single primary introduction event, the South African supercolony most likely represents a separate introduction from a different source in Argentina.

To investigate the dynamics of secondary introductions (i.e. populations founded by propagules from established introduced supercolonies) between Australia, New Zealand, California, Hawaii, Main Europe and Japan S2, we identified pairs of supercolonies characterized by (1) a low genetic differentiation (pairwise F_{ST} among the 5% lowest values) and/

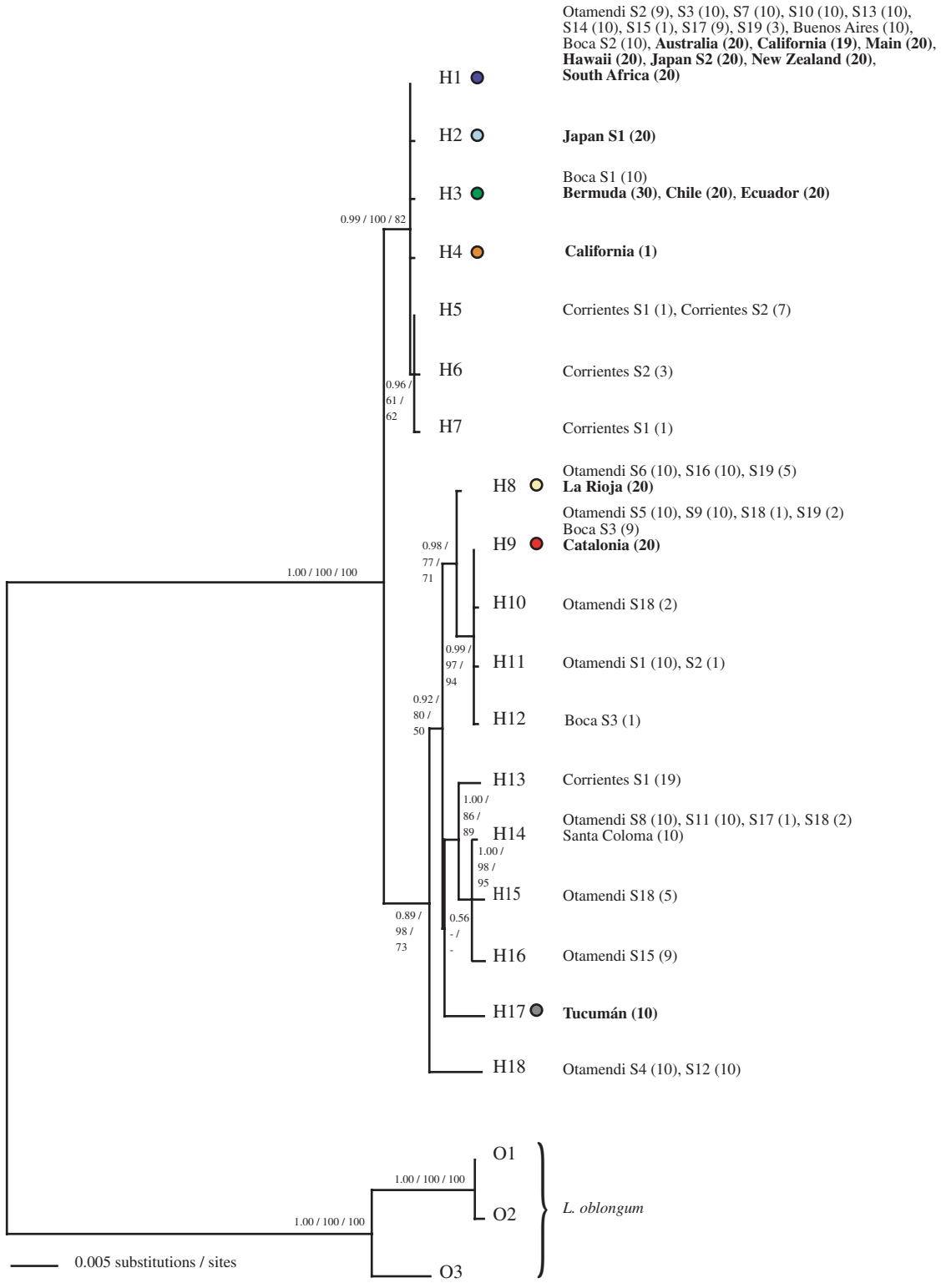

Figure 1 Maximum-likelihood phylogeny based on combined sequences of Cyt *b*, COI and COII from 39 *Linepithema humile* supercolonies. Bootstrap values of maximum parsimony and maximum-likelihood analyses, respectively, and the posterior probability inferred from the Bayesian analysis are given for each node. Nodes that were under the 50% majority rule were collapsed. Names of supercolonies where the relevant haplotype have been found are shown by the tip of branches with the number of individuals in brackets. Introduced populations are in bold and their haplotypes are assigned a colour marker. GenBank accession numbers for H1–H18 are FJ466647–FJ466664 for Cyt *b*, FJ466666–FJ466683 for COI, and FJ535653–FJ535670 for COII. GenBank accession numbers for O1–O3 are FJ496346–FJ496348 for Cyt *b*, FJ496349–FJ496351 for COI, and FJ496352–FJ496354 for COII.

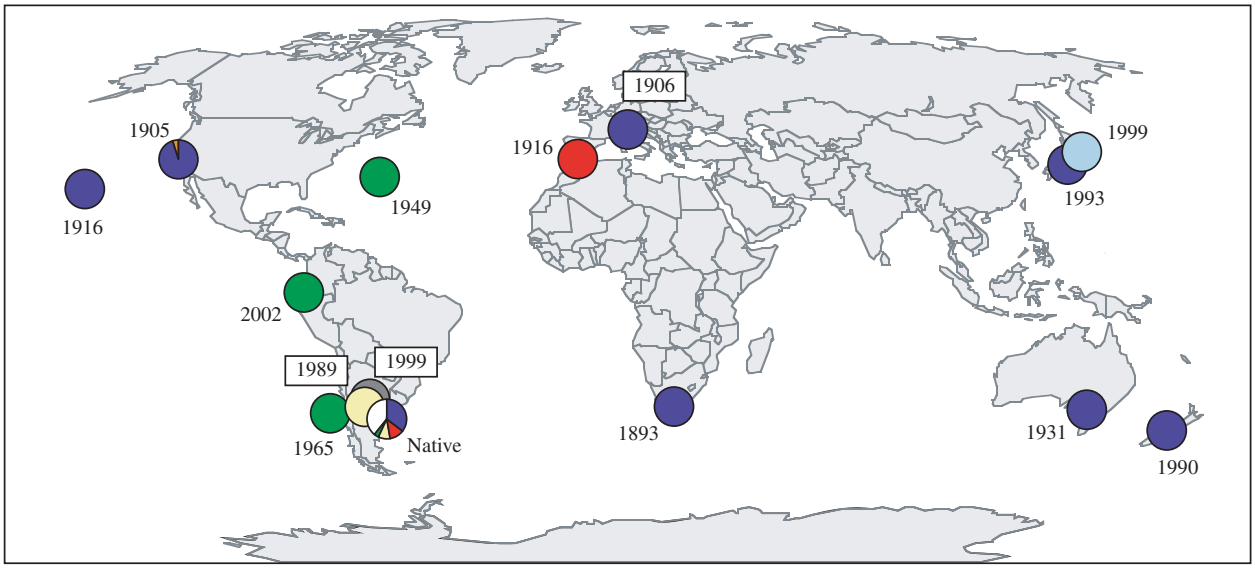

Figure 2 Geographic distribution of *Linepithema humile* supercolonies studied. Haplotypes found in introduced supercolonies are represented by the same colours as in Fig. 1, and the pie diagrams show the frequencies observed. The total proportion of the 11 haplotypes exclusively found in native supercolonies is shown in white. The estimated year of introduction is from our records (J.S.P.) for the Argentinean supercolonies Tucumán and La Rioja, and follows Okaue *et al.* (2007) for Japan S1–2 and Wetterer *et al.* (2009) for all other supercolonies.

or (2) one supercolony possessing a high proportion of all the alleles present in the other supercolony, which is the expected pattern if one introduced supercolony is the source of the other (pairs with the 5% highest share of alleles).

These criteria were met for California and Hawaii as well as for Australia and New Zealand. The genetic differentiation between California and Hawaii was F_{ST} = 0.19 and 86% of the alleles found in Hawaii were also present in California. Similarly, the genetic differentiation between Australia and New Zealand was low (F_{ST} = 0.11), and 84% of the alleles found in New Zealand were also present in Australia. These data suggest that the New Zealand population might reflect a secondary introduction from Australia and the Hawaiian supercolony a secondary introduction from California. The observed genetic differentiation and proportion of alleles shared between the other introduced supercolonies harbouring the H1 haplotype does not allow us to determine whether they originate from secondary introductions or from separate introductions from the native range.

The Bayesian analysis performed with Structure confirmed the close relationship between the Australian and New Zealand supercolonies and suggests that the Japanese S2 supercolony might also be related to these two supercolonies. The most probable number of genetic groups identified for supercolonies sharing haplotype H1 (without South Africa) was K = 4. In the ten runs, the Australian, New Zealand and the Japanese S2 supercolonies constituted the first genetic group (average proportion of membership of these supercolonies to the first genetic group ± SD; Australia: 0.85 ± 0.00, New Zealand: 0.91 ± 0.00 and Japan S2: 0.88 ± 0.00). The remaining supercolonies corresponded to independent genetic groups (European Main: 0.89 ± 0.00, California: 0.94 ± 0.00 and Hawaii: 0.93 ± 0.00).

The final haplotype, H3, was found in Bermuda, Chile and Ecuador. The F_{ST} value was low between Bermuda and Ecuador and they shared many identical alleles raising the possibility of a secondary introduction between these two supercolonies. The genetic differentiation of these two supercolonies and the one from Chile was higher, suggesting that the Chilean supercolony could have originated from yet another independent primary introduction from Argentina. This assumption is supported by the Structure analysis over the supercolonies sharing the haplotype H3 that separated the dataset in two distinct genetic groups (K = 2). All the individuals of the Ecuadorian and Bermudan supercolonies were assigned to the same genetic group (average proportion of membership for Ecuadorian individuals mean ± SD = 0.97 ± 0.07 and Bermuda: 0.99 ± 0.02), while the Chilean supercolony was assigned to the second group with an average probability of 1.00 ± 0.00.

Comparison between native and introduced supercolonies

The genetic diversity was not drastically different between native and introduced supercolonies. The majority of both native (19/25) and introduced (13/14) supercolonies contained a single mitochondrial haplotype (Fig. 1). The introduced supercolonies possessed a significantly lower genetic diversity at nuclear markers than native ones both when quantified as allelic richness (k' = 3.23 vs. 4.83; $P < 0.0002$) and expected heterozygosity (H_{exp} = 0.527 vs. 0.719; $P < 0.0002$). However,

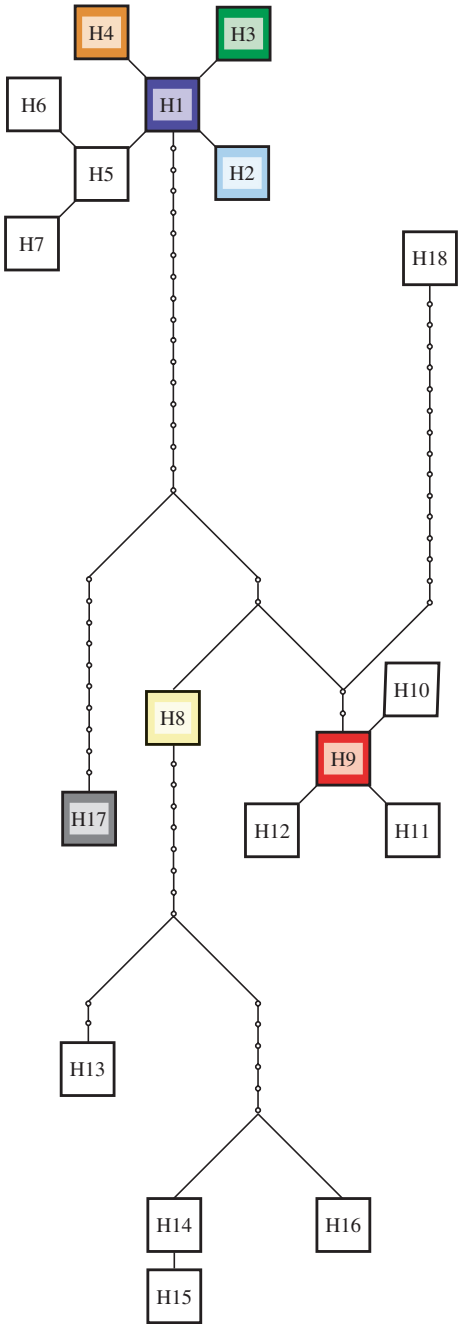

Figure 3 Minimum spanning network based on TCS analysis representing the relationships between the 17 mitochondrial DNA (mtDNA) haplotypes detected in *Linepithema humile*. Each square represents a haplotype. Solid lines connecting haplotypes show hypothesized single base pair mutations. Haplotypes found in introduced supercolonies are represented by the same colours as in Fig. 1.

because the diversity varied considerably within each group, there was a wide overlap with 51% and 33% of all supercolonies falling within the overlapping range of diversity when estimated as k' and H_{exp}, respectively (Fig. 5). In particular, 10 of the 14 introduced supercolonies had higher allelic richness than the least diverse native supercolony (Otamendi S3; Fig. 5). The analyses of genetic diversity were based on five microsatellite loci because the native supercolonies were analysed for these only. However, the ranking order of introduced supercolonies stayed very similar for both measures of genetic diversity when the analyses were performed with all 13 loci (k': Spearman $r_s = 0.912$; H_{exp}: $r_s = 0.749$; $P < 0.0001$ for both).

DISCUSSION

Primary introductions of the Argentine ant

This study investigated the worldwide expansion of the Argentine ant. The clustering method implemented in BAPS revealed that the 113 nests collected in the 40 localities from the introduced ranged clustered in 14 distinct genetic groups. Two lines of evidence suggest that these groups correspond to distinct supercolonies. First, previous studies in both the native (Tsutsui & Case, 2001; Pedersen *et al.*, 2006; Vogel *et al.*, 2009) and introduced range (Tsutsui & Case, 2001; Giraud *et al.*, 2002; Tsutsui *et al.*, 2003; Jaquiéry *et al.*, 2005; Thomas *et al.*, 2006; Corin *et al.*, 2007a,b) have shown that genetically similar nests belong to the same supercolonies. Second, and most importantly, previous studies revealed that the same genetic method clustered nests in perfect agreement with aggression data in 18 localities of both the native and introduced range (Giraud *et al.*, 2002; Pedersen *et al.*, 2006; Vogel *et al.*, 2009).

On the basis of the information provided by the mitochondrial and microsatellite markers, a minimum of seven independent introductions out of the native range were identified (Fig. 2). Four supercolonies (Japan S1, La Rioja, Catalonia and Tucumán) each had a mitochondrial haplotype that was not found in any of the other introduced supercolonies and thus most likely represent independent introductions from the native range.

Seven other introduced supercolonies (Australia, New Zealand, California, Hawaii, Main Europe, South Africa and Japan S2) shared another haplotype. An analysis of the nuclear markers revealed relatively low genetic differentiation among six of these supercolonies but a high differentiation between this group and South Africa signifying that the later supercolony most likely represents a separate primary introduction from South America as also suggested by Tsutsui *et al.* (2001). The low genetic differentiation between the six other supercolonies suggests a possible common origin, which is also indicated by a recent study showing that ants from the Australian, the Californian, the Hawaiian and the European Main supercolonies are similar both genetically and at their cuticular hydrocarbon profiles (Brandt *et al.*, 2009). Furthermore, Sunamura *et al.* (2009a) found that the European Main and the Californian supercolonies were both non-aggressive towards the large supercolony discovered in Japan (represented by Japan S2 in the present study). A detailed analysis of these six supercolonies based on the genetic diversity, the proportion of shared alleles and the Bayesian clustering methods implemented in STRUCTURE support the

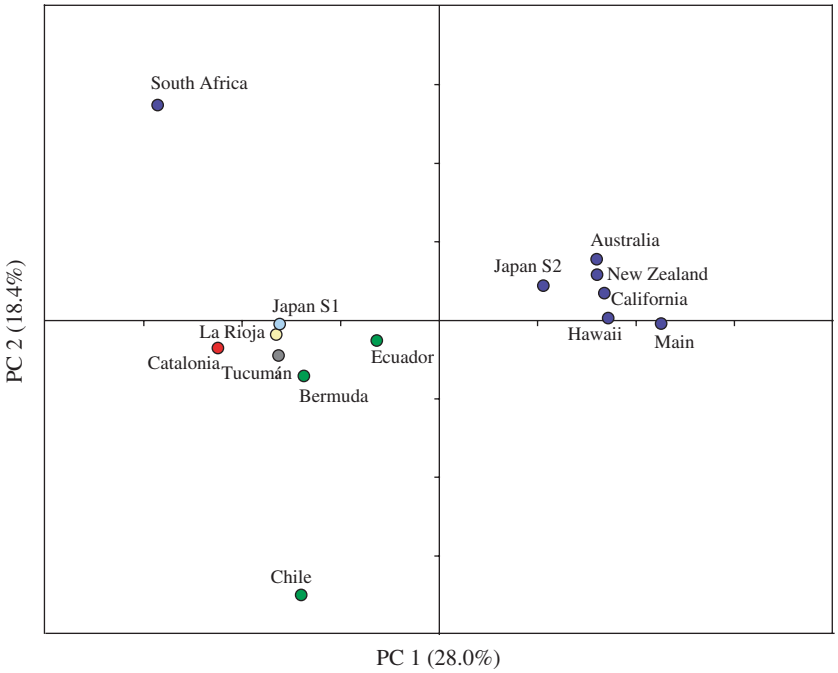

Figure 4 Principal Component Analysis of microsatellite allele frequencies of the *Linepithema humile* introduced supercolonies. Marker colours represent haplotypes found (see Fig. 1). Percentage of variance explained is given in brackets.

view of at least two secondary introductions events, one from Australia to New Zealand, as also suggested by Corin *et al.* (2007b), and the other one from California to Hawaii. The geographic proximity of New Zealand to Australia and of Hawaii to California makes these introduction pathways plausible. Also consistent with this scenario, the Argentine ant was discovered considerably later in New Zealand and Hawaii than in their putative source area on the mainland (Fig. 2).

The seventh introduction event comprises the supercolonies in Chile, Ecuador and Bermuda, which shared another unique haplotype. The Chilean supercolony was slightly more differentiated from the Ecuadorian and Bermudian supercolonies than was the differentiation between the two later ones. However, the introduction history of these three supercolonies is difficult to reconstruct given the lack of more discriminative genetic information and the limited records on dates of introduction and commercial relationships between these countries.

The estimate of at least seven primary introductions of *L. humile* from the species' native range in Argentina is obviously an underestimate, since our method is conservative and because the sampling is not exhaustive. Indeed, regional studies suggest the occurrence of four supercolonies in Japan (Hirata *et al.*, 2008; Sunamura *et al.*, 2009b), five in California (Thomas *et al.*, 2006) and several in the south-eastern United States (Buczkowski *et al.*, 2004).

The origin and development of introduced supercolonies

The distribution of haplotypes in the introduced range is consistent with each introduced supercolony being derived from a single supercolony. Except California, all introduced supercolonies, including the ones that extend over large geographical scale such as the two European supercolonies (which are mutually aggressive) and the Chilean supercolony, each had a single mitochondrial haplotype despite a large number of individuals analysed. Similarly, most of the native supercolonies (19 out of 25) displayed a unique haplotype (see also Vogel *et al.*, 2009 for additional support). In contrast, there was more than one haplotype in all the native localities where several supercolonies were sampled (Otamendi, Corrientes and Boca), reflecting the very high differentiation at both nuclear and mitochondrial markers between closely located native supercolonies (Pedersen *et al.*, 2006; Vogel *et al.*, 2009). Thus, it is very likely that the propagules at the basis of introduced populations typically consist of individuals from a single native supercolony rather than from several supercolonies.

One supercolony from the native range seems to have been particularly successful (see also Brandt *et al.*, 2009; Sunamura *et al.*, 2009a), being the source of extant supercolonies spanning about 6000 km in Europe ('main supercolony', Giraud *et al.*, 2002), 1000 km in California ('large supercolony', Tsutsui *et al.*, 2003), 2800 km in Australia (Suhr *et al.*, 2009) and 900 km in New Zealand (Corin *et al.*, 2007a), and two growing supercolonies in Hawaii and Japan (this study; 'Hiroshima Bay group' or 'main', Hirata *et al.*, 2008; Sunamura *et al.*, 2009a,b). The size and lifespan (> 100 years) of these introduced supercolonies contrast with those of native supercolonies that typically have a size of a few hundred metres (Suarez & Tsutsui, 2008; Vogel *et al.*, 2009) and a longevity of only a few years (Vogel *et al.*, 2009).

These differences between native and introduced supercolonies may stem from the combined effects of several factors.

Table 3 Genetic differentiation at 13 microsatellite loci between introduced supercolonies of *Linepithema humile*. Proportions of shared alleles are given above the diagonal with the percentage of alleles in the row supercolony shared by the column supercolony before the slash and vice versa after the slash. Pairwise F_{ST} values are given below the diagonal. Values in bold indicate supercolony pairs most likely to have a common history (see text for explanation).

Supercolony	Tucumán	La Rioja	Chile	Ecuador	California	Hawaii	Bermuda	Catalonia	Main	Australia	New Zealand	South Africa	Japan S1
La Rioja	0.38***												
	44/16												
Chile	0.52***	0.41***											
	37/20	35/51											
Ecuador	0.58***	0.36***	0.41***										
	33/28	24/56	47/**75**										
California	0.57***	0.38***	0.44***	0.39***									
	41/25	28/48	51/59	66/48									
Hawaii	0.65***	0.41***	0.48***	0.40***	**0.19*****								
	44/32	32/65	51/70	63/54	73/86								
Bermuda	0.51**	0.30***	0.33***	0.20***	0.36***	0.40***							
	52/27	32/47	65/65	94/59	64/55	70/51							
Catalonia	0.51**	0.33***	0.46***	0.44***	0.48***	0.48***	0.38***						
	30/12	34/38	39/31	47/23	50/34	54/31	41/32						
Main	0.60***	0.44***	0.46***	0.50***	0.27***	0.36***	0.48***	0.52***					
	44/24	30/45	49/51	63/41	82/73	81/61	57/59	40/53					
Australia	0.55***	0.39***	0.45***	0.36***	0.18***	0.27***	0.37***	0.48***	0.24***				
	37/22	32/52	55/61	56/39	75/72	86/70	53/59	34/48	71/76				
New Zealand	0.60***	0.41***	0.46***	0.37***	0.28***	0.36***	0.37***	0.46***	0.30***	**0.11*****			
	33/24	23/46	51/70	53/46	64/76	73/73	49/68	28/49	55/73	67/**84**			
South Africa	0.57***	0.43***	0.50***	0.47***	0.47***	0.54***	0.41***	0.48***	0.54***	0.45***	0.50***		
	37/18	32/42	59/53	66/37	57/44	62/40	63/56	34/39	49/42	52/42	70/46		
Japan S1	0.50***	0.31***	0.45***	0.36***	0.43***	0.48***	0.29***	0.41***	0.51***	0.44***	0.45***	0.46***	
	30/17	32/51	39/43	50/34	43/40	49/38	43/47	26/36	39/40	39/38	49/38	42/51	
Japan S2	0.56*	0.37***	0.42***	0.26***	0.24***	0.35***	0.28***	0.44***	0.31***	0.18***	0.15***	0.46***	0.34***
	41/27	32/59	49/61	63/49	66/71	78/71	57/71	31/49	61/73	70/78	73/66	40/56	40/46

Probabilities with Bonferroni corrected significance levels: *nominal $P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

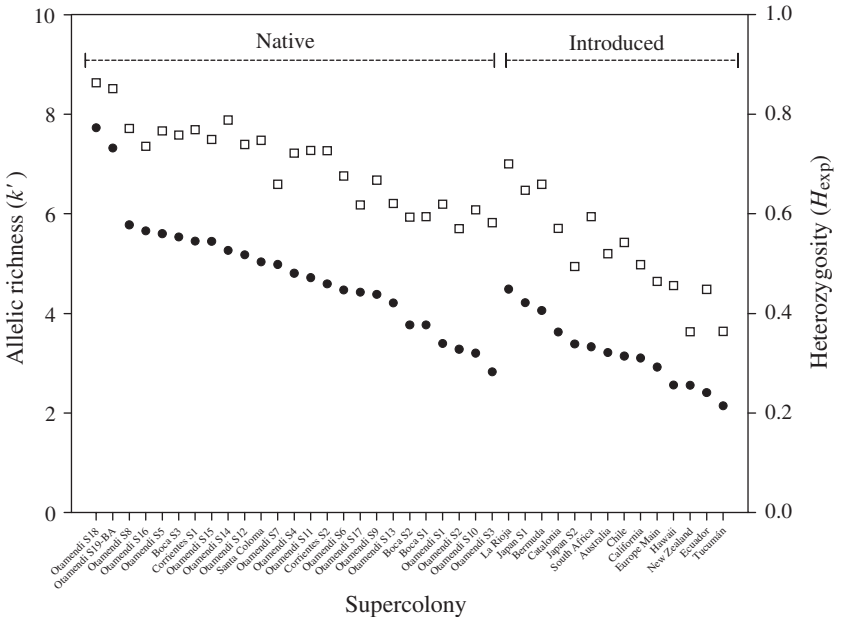

Figure 5 Average genetic diversity at five microsatellite loci estimated as allelic richness (k'; closed circles) and expected heterozygosity (H_{exp}; open squares) in native and introduced supercolonies of *Linepithema humile*. The minimum common sample size for estimating k' was seven individuals. Within each range (native and introduced), supercolonies were ranked after descending estimates of k'.

The first is the release of introduced populations from their native enemies and parasites (Elton, 1958; Reuter *et al.*, 2005; Cremer *et al.*, 2008), so these populations may have a competitive advantage and achieve high densities. The second is the large number of secondary introductions at the regional scale in the introduced range, most likely by human-mediated jump dispersal (Suarez *et al.*, 2001). Finally, the introduction of new supercolonies may be very difficult once a supercolony has colonized most suitable habitats within a region in the introduced range. This is because supercolonies are closed breeding units that are very aggressive towards each other (Buczkowski *et al.*, 2004; Jaquiéry *et al.*, 2005; Thomas *et al.*, 2006), which may prevent both the establishment of newly introduced propagules and/or their contribution to the local gene pool. Consistent with this view, interception data suggest that while introductions from different primary sources commonly occur (e.g. Corin *et al.*, 2007b), these must be only rarely successful given the relatively small number and large sizes of supercolonies in the introduced range (this study; see however Buczkowski *et al.*, 2004; Sunamura *et al.*, 2009a,b).

Loss of genetic diversity

The genetic diversity of native and introduced populations had been compared in several studies (see Suarez *et al.*, 2008 for a review; Brandt *et al.*, 2009). While some of these studies concluded that there had been a strong bottleneck, others concluded that the loss of genetic diversity was not very high. The main reason for the conflicting conclusion of these studies is that they included variable numbers of supercolonies in the sample of reference for native and introduced populations. Furthermore, the large variation in diversity within both ranges (this study) makes estimates of the loss of diversity highly sensitive to what supercolonies are chosen for comparison (figure 1 in Suarez & Tsutsui, 2008). Given that supercolonies are closed breeding units that are highly differentiated both in the native (Tsutsui & Case, 2001; Pedersen *et al.*, 2006; Vogel *et al.*, 2009) and introduced (Tsutsui & Case, 2001; Jaquiéry *et al.*, 2005; Thomas *et al.*, 2006; this study) range it is vital to apply 'supercolony' as the level of analysis in population genetic studies of supercolonial ants, just as one needs to take nest membership into account when conducting population genetic studies of social insects in general (e.g. Ross, 2001).

By using an analysis that explicitly accounted for population structure at the supercolony level, our study revealed that the genetic diversity in the introduced range is significantly lower, with an average of 33% lower allelic richness at highly polymorphic loci than in the native range. Interestingly, this estimate is the same as the average reduction in allelic richness across microsatellite studies of 25 introduced species reviewed by Dlugosch & Parker (2008). However, there was considerable variation in genetic diversity between supercolonies both in the native and introduced range, such that about two-thirds of the introduced supercolonies had higher genetic diversity than the least diverse native supercolony (Fig. 5).

Introduced populations of invasive species are expected to be less genetically diverse than native populations because of a low number of successfully dispersing individuals (Allendorf & Lundquist, 2003). Although such differences is observed in the majority of studies (Dlugosch & Parker, 2008; Puillandre *et al.*, 2008), it is not universal and there is growing evidence for the importance of invasive populations being the result of multiple introductions, often including several native sources (Bossdorf *et al.*, 2005; Suarez *et al.*, 2008; Wilson *et al.*, 2009). In the other invasive ant species forming supercolonies, the social organization and mode of dispersal most likely prevent the mixture of individuals from different origins (Helanterä *et al.*,

2009). Thus, the genetic diversity of supercolonies is expected to be lower in the invasive range compared to the native range. Accordingly, the overall reduction in genetic diversity in the introduced range observed in this study is readily understood as a result of consecutive genetic bottlenecks during the past *c.* 120 years of spread from the species' native range in Argentina (Suarez *et al.*, 2001). A previous estimation by Giraud *et al.* (2002) that the two supercolonies in Europe were initiated after a bottleneck with an effective number of 6–13 queens is confirmed by the more detailed genetic data of this study. The estimate of Giraud *et al.* (2002) was based on the allelic richness of the Santa Coloma supercolony ($k' = 5.04$; present study) in the native range and the main European and Catalonian supercolonies (average $k' = 3.28$) in the introduced range. These estimates are very close to the average allelic richness of all studied native (4.83) and introduced (3.23) supercolonies. Interestingly, in the red imported fire ant *Solenopsis invicta*, the estimated founder group of queens during introduction into the USA is of similar magnitude (9–20 queens; Ross & Shoemaker, 2008).

Conclusion

The success of the Argentine ant as an invading species is mainly explained by its social organization (Human & Gordon, 1996; Holway, 1998; Holway & Suarez, 2004) that is characterized by individuals mixing freely within large supercolonies, which may expand over thousands of km (Tsutsui *et al.*, 2000; Giraud *et al.*, 2002; Corin *et al.*, 2007a; Suhr *et al.*, 2009). Two explanations have been proposed for the occurrence of such large supercolonies in the introduced range. The first is that narrow population bottlenecks have led to reduced intraspecific aggression in the introduced range (Tsutsui *et al.*, 2000; Tsutsui & Case, 2001; Brandt *et al.*, 2009). This hypothesis is based on the observation of lower genetic diversity in the introduced range (Tsutsui *et al.*, 2000; Tsutsui & Case, 2001) and on the diversity of chemical recognition cues being positively correlated with the overall genetic diversity of supercolonies (Brandt *et al.*, 2009). Alternatively, it has been proposed that, except supercolony size and longevity, there is no major difference in the social organization and genetic structure of native and introduced supercolonies (Pedersen *et al.*, 2006; Vogel *et al.*, 2009). This conclusion is based on the finding that social organization and kin structure are very similar in the native and introduced ranges (Pedersen *et al.*, 2006; Vogel *et al.*, 2009). In both the native and introduced range, there is also almost no genetic differentiation between nests within supercolonies and gene flow is very limited or even absent between supercolonies. Thus, both native and introduced supercolonies form closed breeding units that have the potential to expand rapidly and reach high densities. The greater size of introduced supercolonies would thus simply reflect lower parasitic load and interspecific competition in the introduced range. As a result, the supercolonies have a much longer lifespan and can reach a much greater size.

The data of the present study are in full agreement with the second explanation, but not the first. First, there was a large (> 50%) overlap in genetic diversity between native and introduced supercolonies that is comparable to the overlap in diversity of chemical recognition cues previously found between the native and introduced ranges (Brandt *et al.*, 2009). Second, our data show that introduced supercolonies typically contain a single mitochondrial haplotype suggesting that each introduced supercolony is derived from individuals originating from a single native supercolony rather than from the mixing of several supercolonies as a result of a reduction of recognition abilities.

Altogether the demonstration of the lack of a categorical difference in genetic diversity between native and introduced populations adds to other studies (Pedersen *et al.*, 2006; Vogel *et al.*, 2009) showing that, with the exception of supercolony size and longevity, there is no major difference in the social organization and genetic structure of native and introduced supercolonies of Argentine ants. Thus, the invasiveness in the Argentine ant did not evolve recently as a unique and historically contingent event. Rather, native supercolonies of the species have characteristics which make them pre-adapted to invade new and in particular disturbed habitats when given the opportunity (Helanterä *et al.*, 2009; Orivel *et al.*, 2009), and these characteristics may be common to the majority of invasive ants (Passera, 1994; McGlynn, 1999; Aron, 2001; Cremer *et al.*, 2008).

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BIOSKETCHES

This work was part of **Valérie Vogel's** PhD thesis under the supervision of **Laurent Keller** in collaboration with **Jes S. Pedersen**. For her PhD, V. Vogel studied the evolution of unicoloniality in *Linepithema humile* and is now studying the evolution of antibiotic resistance in *Staphylococcus aureus*.

J. S. Pedersen's research focuses mainly on the evolution of invasive supercolonies in ants.

L. Keller works on various aspects of evolutionary ecology such as reproductive skew, sex allocation, caste determination as well as the molecular basis of ageing and behaviour in ants.

Tatiana Giraud and **Michael J. B. Krieger** worked previously on *L. humile* and provided the data of some of the introduced populations. The rest of the data were obtained by V.V. and J.S.P. These two authors also performed the analyses and wrote the paper with L.K.

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