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Relationship between invasion success and colony breeding structure in a subterranean termite

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Abstract

Factors promoting the establishment and colonization success of introduced populations in new environments constitute an important issue in biological invasions. In this context, the respective role of pre-adaptation and evolutionary changes during the invasion process is a key question that requires particular attention. This study compared the colony breeding structure (i.e. number and relatedness among reproductives within colonies) in native and introduced populations of the subterranean pest termite, *Reticulitermes flavipes*. We generated and analysed a data set of both microsatellite and mtDNA loci on termite samples collected in three introduced populations, one in France and two in Chile, and in the putative source population of French and Chilean infestations that has recently been identified in New Orleans, LA. We also provided a synthesis combining our results with those of previous studies to obtain a global picture of the variation in breeding structure in this species. Whereas most native US populations are mainly composed of colonies headed by monogamous pairs of primary reproductives, all introduced populations exhibit a particular colony breeding structure that is characterized by hundreds of inbreeding reproductives (neotenics) and by a propensity of colonies to fuse, a pattern shared uniquely with the population of New Orleans. These characteristics are comparable to those of many invasive ants and are discussed to play an important role during the invasion process. Our finding that the New Orleans population exhibits the same breeding structure as its related introduced populations suggests that this native population is pre-adapted to invade new ranges.

Keywords: breeding structure, invasion genetics, microsatellites, neoteny, *Reticulitermes flavipes*, sociality

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Introduction

Biological invasions involve the introduction of species in regions where they were previously absent, followed by population growth and range expansion (Sax *et al.* 2005). The negative ecological and economic impacts of many invasions generated strong research interest, although a general understanding of how introduced populations become established and invade their new environments is still lacking (Novak 2007; Bock *et al.* this issue). In particular, the ecological and genetic factors promoting the colonization success of introduced populations in new environments constitute a crucial but yet unresolved issue (Baker & Stebbins 1965; Sakai *et al.* 2001; Lee 2002; Facon *et al.* 2006). Certain introduced populations possess phenotypic and life history traits that pre-adapt them to successful colonization of the invaded habitats (Kolar & Lodge 2001; Pysek & Richardson 2007). In contrast, some populations are not

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necessarily pre-adapted to the invaded habitats at the time of introduction, but evolve phenotypic traits that facilitate their survival, reproduction and range expansion (Wares *et al.* 2005; Dlugosch & Parker 2008a; Colautti & Barrett 2013; Vandepitte *et al.* 2014). Despite the fact that founding event(s) often reduce the genetic diversity in introduced populations, there is nevertheless growing evidence that introduced populations contain enough additive genetic variance to efficiently respond to selection and adapt to the ecological factors present in the invaded habitats (Dlugosch & Parker 2008b; Rollins *et al.* 2013; Moran & Alexander 2014). Natural selection is often considered as the principal evolutionary force acting on introduced populations (Moran & Alexander 2014), but other mechanisms such as genetic drift or admixture can also cause important genetic and phenotypic changes (Suarez *et al.* 2008). Although the study of biological invasions is clearly important from an applied perspective to mitigate impacts and prevent future introductions, Baker & Stebbins (1965) were among the first authors to develop the idea that invasive species provide unique model systems for understanding micro-evolutionary mechanisms and for identifying the ecological factors that shape complex phenotypic traits (Barrett this issue).

The evolution of introduced populations, more especially adaptive evolution, has recently been considered an important component leading to invasiveness (Colautti & Barrett 2013; Blows & McGuigan this issue), although empirical evidence of adaptations in invading populations at a molecular level remains very limited (Vandepitte *et al.* 2014; Hodgins *et al.* this issue). To detect evolutionary change during invasions, comparative analyses of source versus introduced populations have been conducted. So far, most of these comparative studies included measures of neutral genetic variation, phenotypic variation and, more rarely, quantitative genetic variation and phenotypic plasticity (Geng *et al.* 2007; Suarez *et al.* 2008). These studies principally used invasive plants as model systems (Bossdorf *et al.* 2005; van Kleunen *et al.* this issue), although other taxa among which fungi (Gladieux *et al.* this issue) or insects, and more particularly invasive social insects, have proved useful.

Social insects count among the most successful invaders, often causing severe damage to local communities and ecosystems (Moller 1996). The colonization success of social insects has been largely attributed to their complex and flexible social organization (Moller 1996; Chapman & Bourke 2001; Holway *et al.* 2002). There has been considerable empirical work on various social Hymenoptera including wasps (*Vespula* spp.) (Donovan *et al.* 1992; Kasper *et al.* 2008; Hanna *et al.* 2014), bumble bees (*Bombus terrestris*) (Buttermore 1997; Nagamitsu & Yamagishi 2009) and some of the most damaging invasive ants: *Anoplolepis gracilipes*, *Linepithema humile*, *Solenopsis invicta*, *Lasius neglectus*, *Pheidole megacephala* and *Wasmannia auropunctata* (Morel *et al.* 1990; Vanloon *et al.* 1990; Ross *et al.* 1996; Espadaler & Rey 2001; Holway *et al.* 2002; Tsutsui & Suarez 2003; Le Breton *et al.* 2004; Fournier *et al.* 2009; Blight *et al.* 2012). Interestingly, the introduction of social insects is often accompanied by important modifications of their breeding structure (i.e. the number and relatedness of reproductives within social units) and mode of dispersal allowing colonies to rapidly grow and attain high densities and occupy large spatial expanses. Invasive social insects provide promising model systems for studying evolutionary changes that occur during invasions as well as for identifying ecological factors that shape the evolution of breeding structures.

This study aims to compare the colony breeding structure of native and introduced populations of an invasive pest termite, *Reticulitermes flavipes* (Rhinotermitidae), in order to test the hypothesis that the evolution of the breeding structure following an introduction event is not limited to the social Hymenoptera but also occurs in the Isoptera. *R. flavipes* is a subterranean termite that lives in forest ecosystems and in urban areas where it can cause significant damage to human-built wooden structures. Originating from the US, this species is widespread in the eastern part of the US from Texas to Nebraska in the west and from Florida to Massachusetts in the east. This species has been accidently introduced by humans to several areas both in the Americas and in Europe. In the Americas, *R. flavipes* has been found in California, the Bahamas, Canada, Chile and Uruguay (Ripa & Castro 2000; Austin *et al.* 2002, 2005; Su *et al.* 2006; Scaduto *et al.* 2012). In Europe, *R. flavipes* is today widespread throughout France and has been locally found in Austria (Vienna), Germany (Hamburg) and Italy (Varese) (Kollar 1837; Weidner 1937; Ghesini *et al.* 2010). Recently, a global genetic analysis using *R. flavipes* samples collected in the native and introduced ranges revealed that the most likely source of French populations is located in the region of New Orleans, Louisiana (Perdereau *et al.* 2013). This study also suggests that French introductions occurred directly from Louisiana and that the first introduction(s) started during the 18th century at the earliest (i.e. period of first trade between New Orleans and France). In addition, French populations showed lower genetic diversity than US populations, suggesting that founding event(s) resulted in genetic bottlenecks (Perdereau *et al.* 2013). Finally, this global analysis indicates that the population of New Orleans might also be the source of Chilean populations (Perdereau *et al.* 2013), a hypothesis that recently has received support by additional data (E. L. Vargo, unpublished data).

Like most subterranean termites, *R. flavipes* has cryptic nesting habits and forms complex colonies with diffuse nests and multiple feeding sites connected by underground tunnels. Colonies are typically founded by monogamous couples of primary reproductives (alates) that pair together following a nuptial flight. Such colonies composed of primaries and their nonreproductive offspring are classified as 'simple families.' As colonies grow, secondary reproductives (neotenics) can differentiate among the offspring of primaries either from nymphs or, more rarely, from workers (Buchli 1958). In their natal colonies, neotenics replace or supplement primaries in reproduction. Because neotenics do not fly, they stay and mate in their natal colony resulting in high degrees of inbreeding. Colonies constituted partially or exclusively by the offspring of neotenics are called 'extended families.' In addition, colonies can occasionally form 'mixed families', which contain offspring of more than two unrelated reproductives. In *Reticulitermes* species, it has been shown that mixed families can result from fusion between two or more colonies (DeHeer & Vargo 2004, 2008; Perdereau *et al.* 2010a). The colony breeding structure thereafter defined as 'the proportion of simple, extended and mixed families within populations as well as by the estimated number of functional neotenics within colonies' strongly varies within and among *Reticulitermes* species, although the factors underlying such variation remain poorly known (Vargo & Husseneder 2009; Vargo *et al.* 2013).

Studies conducted on *R. flavipes* reveal that the colony breeding structure strongly varies between introduced French and native US populations. A first difference is that mixed families are much more frequent in French populations than in the US populations (Vargo & Husseneder 2009; Perdereau *et al.* 2010a). A second major difference between French and US populations concerns the number of functional neotenics heading colonies. All French colonies studied so far contained numerous (hundreds) neotenics, while native populations in the US show high variability with some populations comprised of mostly colonies headed by monogamous pairs of primary reproductives (simple families) and other populations comprised of mainly extended-family and mixed-family colonies (Vargo & Husseneder 2009, 2011). A high number of neotenics in French colonies seems to be consistent with their extremely large spatial expansiveness, which commonly exceeds several hundred metres. These two colony-level traits of introduced French populations may have contributed to the colonization success of *R. flavipes* in France by helping them to become established and reproduce right after their introduction, and by increasing their spread by human-mediated dispersal. However, more empirical data remain necessary to test this hypothesis.

The first objective of this study was to test the hypothesis that the two colony-level traits of French populations (i.e. the propensity to fuse and maintain large numbers of functional neotenics) are general for introduced populations. All three introduced populations studied so far were collected in France (Dronnet *et al.* 2005; Perdereau *et al.* 2010a). Here, we analysed the colony breeding structure in three additional introduced populations, one sampled in France (forest of Olonne-sur-mer) and two in Chile (regions of Valparaiso and Santiago cities). The second objective of this study was to test the hypothesis that the two colony-level traits in introduced populations have been acquired after the introduction from their introduced range. To test this hypothesis, we analysed the colony breeding structure near New Orleans, Louisiana, the putative source population of French and Chilean infestations (Perdereau *et al.* 2013; E. L. Vargo, unpublished data). In addition, we combined our results with those of previous studies to get a clearer picture of the level of variation in colony breeding structure in *R. flavipes* and to infer the possible role of this attribute in the colonization success of this invasive species.

Materials and methods

Sample collection

Termite samples were collected from wood fragments, tree stumps or in-ground monitoring stations consisting of at least 19 workers per collection point. In New Orleans, Louisiana, termite samples were collected at 21 points (L1 to L21; Fig. 1A). In France, samples were collected at 20 points (O1 to O20; Fig. 1B) in the forest of Olonne-sur-mer in Vendée. Two zones of the forest separated by 6 kilometres were surveyed, and samples were collected at 10 points for each zone. In Chile, termite samples were collected at 10 points in the region of Valparaiso (V1 to V10; Fig. 1C) and 10 points in the region of Santiago (S1 to S10; Fig. 1C). All termite samples were collected in 2009 (see Table S1, Supporting information for details). After collection, samples were preserved in 90% ethanol prior to DNA extraction.

Molecular procedures

DNA from individual specimens was extracted using standard phenol–chloroform purification (Sambrook *et al.* 1989) or the Wizard® Genomic Purification Kit (Promega). In total, 1200 workers were genotyped at seven microsatellite loci: *Rf24-2*, *Rf21-1*, *Rf11-1*, *Rf6-1*, *Rf1-3*, *RS15* and *RS1*. Polymerase chain reactions were conducted following the method developed by Vargo (2000) and Dronnet *et al.* (2004). PCR products were

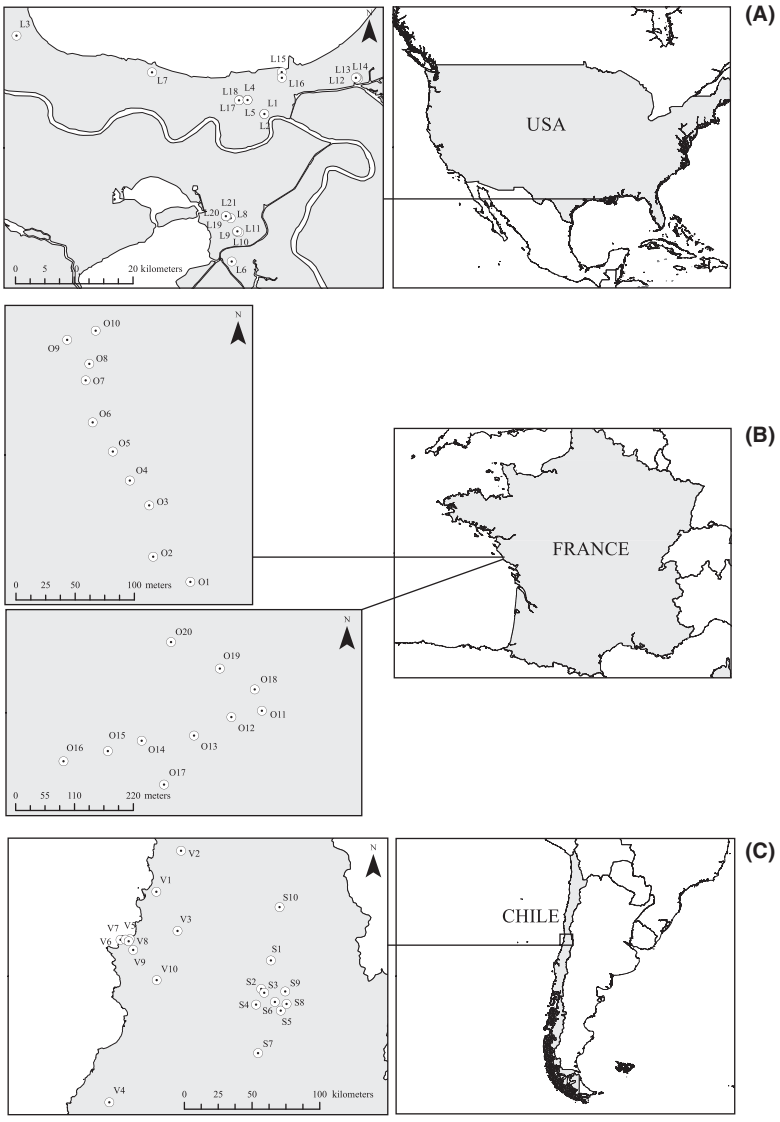

Fig. 1 Locations of the three study populations of *R. flavipes* within native and introduced ranges: (A) Locations of the 21 samples collected in New Orleans, Louisiana, USA (native range). (B) Locations of the 20 samples collected in Olonne-sur-mer, France (introduced range). (C) Locations of the 20 samples collected in Valparaiso and Santiago regions, Chile (introduced range).

separated by electrophoresis on a 6% polyacrylamide gel in a LI-COR 4000L sequencer. Alleles were scored using GENE PROFILER 4.03 (Scanalytics, Inc, Fairfax, VA, USA). For French samples, locus *Rf24-2* was discarded because it was difficult to score and had spurious bands.

A portion of the cytochrome oxidase II (COII) gene (680 bp) of the mitochondrial DNA was amplified and sequenced from 109 individuals using the primers B-tLys (50-GTTTAA GAGACCATTACTTA-30) (Simon *et al.* 1994) and modified A-tLeu (50-CAGATAAGTGC-ATTG GATTT-30) (Miura *et al.* 2000). PCR amplification was performed with Multiplex PCR Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. PCR templates were purified and then sequenced. For Chilean samples, purified PCR templates were sequenced using an automated ABI 3100-Avant sequencer (Applied Biosystems). For the Louisiana and French samples, templates were sequenced by Genoscreen platform (http://www.genoscreen.fr) using BigDye 3.1 and a 96-capillary Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems). Sequences were aligned using MEGA v.4 package (Tamura *et al.* 2007) and Geneious v.6.1.8 (Biomatters) before further inspection and manual editing.

Colony boundaries

Microsatellite analyses were carried out to determine whether different collection points belonged to the same colony. Genotypic frequencies were compared for all pairs of collection points using a log-likelihood (*G*)-based differentiation test from GENEPOP on the Web (Raymond & Rousset 1995). The overall significance was determined using Fisher's combined probability test, with a Bonferroni correction for multiple compari-

sons. Samples from two collection points were considered to belong to different colonies if genotypic differentiation was statistically significant (Vargo 2003b; DeHeer & Vargo 2004; Dronnet *et al.* 2005). *G*-tests have proven useful and are widely used to delineate colonies of social insects (Vargo & Husseneder 2011).

Breeding structure of colonies

GENEPOP on the Web (Raymond & Rousset 1995) was used to test the deviation from Hardy–Weinberg equilibrium for each population and was also used to infer the breeding structure. Genotypic disequilibrium was estimated using *F*stat 2.9.3.2 (Goudet 1995) to avoid any problems that might occur from nonindependent genotypes in the data set. The breeding structure of each colony was then determined by classifying them among the three family types (i.e. simple, extended and mixed families) as previously defined. To do this, the number and frequency of alleles and genotypes observed in colonies were compared to theoretical predictions according to standard criteria for the three family types (Vargo 2000; Bulmer *et al.* 2001; DeHeer & Vargo 2004; Vargo & Carlson 2006). Colonies were classified as simple families when worker genotypes were consistent with direct offspring of a single pair of reproductives and when the observed frequencies did not differ significantly from those expected under Mendelian segregation of alleles from two parents. Significance was determined by a *G*-test ($P < 0.05$) combined across all loci. Colonies were considered extended families when there were no more than four alleles at any one locus and when worker genotypes were not consistent with a single pair of reproductives (e.g. more than four genotypes at a locus or three or more homozygote genotypes). Colonies were considered mixed families when more than four alleles were found at one or more loci, a pattern that is consistent with offspring produced by more than two unrelated reproductives.

F-statistics and relatedness estimates

The colony-level *F*-statistics (Weir & Cockerham 1984) and coefficient of relatedness (*r*) (Queller & Goodnight 1989) were estimated using *F*stat 2.9.3.2 (Goudet 1995). Results were compared to the simulated termite breeding structure models proposed by Thorne *et al.* (1999) and Bulmer *et al.* (2001), where the various components of variation are classified as individual (I), colony (C) and total (T). In these models, F_{IT} is the coefficient of inbreeding for individuals relative to the total population; F_{CT} is the estimated genetic differentiation between colonies; and F_{IC} is the coefficient of inbreeding for individuals within colonies and provides information on the number of reproductives and relatedness among them. The value of F_{IC} theoretically approaches zero as the number of functional neotenics within colonies increases. This parameter value also can become positive if genotyped individuals come from genetically differentiated colonies either due to colony fusion or sharing of foraging tunnels (Thorne *et al.* 1999). The significance of the *F*-statistics was assessed by bootstrapping over loci (1000 replications) with a probability $\alpha = 0.05$. The same software was used to determine the number of alleles per loci, the allelic richness and gene diversity (Nei 1987) within each population.

Isolation by distance within populations

The analysis was performed on the four populations used in this study (New Orleans NO, Olonne-sur-mer O, Santiago S and Valparaiso V) and on the three other introduced populations previously studied in France (Paris P, Ile d'Oléron Saumonard IS and Ile d'Oléron Saint Trojan IT) (Dronnet *et al.* 2005; Perdereau *et al.* 2010a). Following the notation of Thorne *et al.* (1999) and Bulmer *et al.* (2001), each colony was treated as a population where F_{CT} estimates the level of genetic differentiation among colonies. The correlation coefficients between $F_{CT}/(1\text{-}F_{CT})$ and natural logarithm of geographic distance (Slatkin 1993; Rousset 1997) were obtained using the Isolde option implemented in GENEPOP on the Web.

Results

Genetic diversity within populations

Comparisons between populations showed that New Orleans, the putative source population of French infestations, had nearly 3-4 times the average number of alleles (*N*a) and more than twice the allelic richness (*R*s) than the three introduced populations (Table 1). There was no significant difference in allele number or allelic richness among introduced populations. Concerning the mitochondrial data (Table 2), there was only a single haplotype in the Chilean samples (haplotype BM), present in both the Valparaiso and Santiago populations, and one haplotype among the Olonne-sur-mer samples (haplotype A), whereas the New Orleans population had 11 haplotypes (haplotypes A, E, K, H, AC, AD, AE, AF, AH, AI and AJ). All these haplotypes were previously described in Perdereau *et al.* (2013) (Table 2).

Colony boundaries and breeding structure

None of the loci showed consistent patterns of genotypic disequilibrium. Genotypic differentiation tests for

the New Orleans population grouped the 21 collection points into 20 colonies (significant *G*-tests between pairs of collection points, $P < 0.0003$). For the population of Olonne-sur-mer, the 20 collection points grouped into 2 large colonies, one in the northern part of Olonne forest and the other in the south. For both Chilean populations, Valparaiso and Santiago, genotypic differentiation tests identified each of the 20 collection points as a unique colony (G-test differentiation between pairs of collection points, all $P < 0.001$), revealing 10 colonies in Santiago region and 10 colonies in Valparaiso region (Table 2). The number of collection points assigned per colony drastically differed between the population of Olonne-sur-mer and the three other studied populations (New Orleans, Valparaiso and Santiago). This difference most likely is due to the different spatial scale used in sampling Olonne-sur-mer, which was performed over several hundred metres, whereas sampling in New Orleans, Valparaiso and Santiago was conducted over several kilometres (Fig. 1). The two colonies delimited in Olonne-sur-mer were spatially expansive, extending several hundred metres in length. Two colonies represent a weak sample size to consider Olonne-sur-mer as a 'standard' population. Nevertheless, each colony comprised numerous collection points (respectively, 10 collection points) as it is sometimes the case in social insects, especially in those forming unicolonial populations (Giraud *et al.* 2002).

The number of genotypes per colony indicated that none of the 42 colonies identified in the four studied populations was classified as a simple family. All colonies had more than four genotypes for at least one locus, a result rejecting the hypothesis that colony workers were produced by a single pair of reproductives (Max *N*g, Table 2). An analysis of the number of alleles per colony indicated that 20 colonies of the 42 studied (48%) were mixed families, that is had more than four alleles at one or more loci (Table 2). Among these mixed-family colonies, 10 were from New Orleans, 5 from Santiago and 3 from Valparaiso in addition to the 2 identified colonies in Olonne-sur-mer. The number of alleles at the informative loci to infer mixed families varied from 5 to 9 alleles. These results showed that these colonies were headed by more than two unrelated reproductives. Such a colony genetic structure has been shown to result from fusion of two or more mature colonies (DeHeer & Vargo 2004, 2008; Perdereau *et al.* 2010a). For mixed families in Olonne-sur-mer and New Orleans populations, 5-25 individuals per colonies were sequenced to provide possible information on the maternal relationship between cohabiting colonies. However, we did not find more than one haplotype within mixed-family colonies. The remaining 22 other colonies (10 from New Orleans, 5 from Santiago and 7 from Valparaiso) had no more than four alleles at any one locus and were therefore considered extended families (Table 2).

F-statistics and relatedness estimations

The *F*-statistics and relatedness estimates for the extended-family colonies of the New Orleans population were consistent with those expected for a theoretical population composed of extended-family colonies having hundreds of neotenics (200 females and 100 males) that had inbred for three generations (case B-*v*), Table 3. In mixed-family colonies of the same population, the *F*-statistics and relatedness estimates were consistent with the theoretical breeding structure case F-*ii* (Table 3). Interestingly, results obtained with the two mixed-family colonies found in the Olonne-sur-mer population were also in accordance with the same two breeding structure models (i.e. cases B-v and F-*ii*). Case F-*ii* describes a theoretical population composed of mixed families headed by two primary queens and one primary king (pleometrosis) with the presence of ten neotenics that had interbred for three generations. Relatedness (*r*) within mixed-family colonies was not significantly different from 0.5, as expected for this type of family structure with the presence of unrelated or distantly related individuals.

Concerning the two Chilean populations, *F*-statistics and relatedness estimates for the extended-family colonies were not consistent with any theoretical cases of colonies with breeding among neotenics (i.e. B-*i* to B-*v*). Instead, these values were close to the expectations for a population of mixed families. In mixed families, however, the estimated values of relatedness conformed to those expected for this family type ($r = 0.108$ for Santiago population, $r = 0.178$ for Valparaiso population). *F*-statistics estimates for mixed families and extended families from Santiago and Valparaiso populations were intermediate between two cases (cases E-*i* and F-*iii*, Table 3). Case E-*i* indicates workers from unrelated nests mix at collection sites, while case F-*iii* designates pleometrosis headed by five primary queens and five primary kings with 10 secondary reproductives that had interbred for three generations. The estimated values of F_{IC} observed in the Chilean populations were particularly high compared to the other *Reticulitermes* populations studied (Vargo & Husseneder 2009). Such unusual values are most probably caused by a Wahlund effect, which can occur when colonies fuse and either they are in the early stages of the fusion process or because there is no interbreeding between reproductives in the two (or more) original colonies.

Table 1 Variability at seven microsatellite loci in *R. flavipes* populations from Louisiana, France and Chile. The number of alleles (*Na*), allelic richness (R_S) and gene diversity (H_S) were calculated from the entire set of samples

	Native population			Introduced populations								
	New Orleans, Louisiana, USA			Olonne-sur-mer, France			Santiago, Chile			Valparaiso, Chile		
Locus	*Na*	R_S	H_S	*Na*	R_S	H_S	*Na*	R_S	H_S	*Na*	R_S	H_S
*Rf*24-2	33	14.9	0.541	—	—	—	6	4.6	0.536	9	5	0.599
*Rf*21-1	40	16.6	0.645	5	4	0.450	12	8.3	0.696	11	6.7	0.680
*Rs*15	11	6.2	0.528	6	5.3	0.361	6	3.1	0.249	5	4.1	0.529
*Rs*1	18	11.3	0.665	6	5.4	0.632	8	5.8	0.528	7	4.2	0.605
*Rf*11-1	9	6.6	0.568	5	4.9	0.480	7	5.6	0.690	6	4.9	0.592
*Rf*1-3	9	6.5	0.539	2	2	0.272	4	3.7	0.504	4	4	0.582
*Rf*6-1	15	10.7	0.665	7	6.8	0.460	6	5	0.710	5	4.6	0.714
Mean ± SD Overall	19.3 ± 12.4 (a)	10.4 ± 4.2 (a)	0.593	5.2 ± 1.7 (b)	4.7 ± 1.6 (b)	0.442	7 ± 2.5 (b)	5.2 ± 1.7 (b)	0.559	6.7 ± 2.5 (b)	4.8 ± 0.9 (b)	0.614

Letters inside parentheses indicate significant differences between populations (Kruskal–Wallis tests, nonparametric ANOVA, *Na* $\chi^2 = 13.8$, d.f. = 3, $P < 0.01$; R_S $\chi^2 = 11.6$, d.f. = 3, $P < 0.01$).

Tests between native and introduced populations (Mann–Whitney tests): *Na* of New Orleans population = 19.3 *versus* *Na* of Olonne-sur-mer population = 5.2, *Na* of Santiago population = 7 and *Na* of Valparaiso population = 6.7; respectively, Mann–Whitney tests, $U = 0$, $P < 0.01$, $U = 3$, $P < 0.01$ and $U = 3.5$, $P < 0.01$.

R_S of New Orleans population = 10.4 *versus* R_S of Olonne-sur-mer population = 4.7, R_S = 5.2 of Santiago population and R_S of Valparaiso population = 4.8; respectively, Mann–Whitney tests, $U = 3$, $P < 0.01$, $U = 3$, $P < 0.01$ and $U = 3$, $P < 0.01$.

Tests between introduced populations (Mann–Whitney tests): *Na* of Olonne-sur-mer population= 5.2 versus *Na* of Santiago population = 7, *Na* of Olonne-sur-mer population= 5.2 versus *Na* of Valparaiso population = 6.7 and *Na* of Santiago population = 7 versus *Na* of Valparaiso population = 6.7; respectively, Mann–Whitney tests, $U = 11.5$, $P > 0.05$, $U = 15.5$, $P > 0.05$ and $U = 21.5$, $P > 0.05$.

R_S of Olonne-sur-mer population = 4.7 *versus* R_S of Santiago population = 5.2, R_S of Olonne-sur-mer population = 4.7 *versus* R_S of Valparaiso population = 4.8 and R_S of Santiago population = 5.2 *versus* R_S of Valparaiso population = 4.8; respectively, Mann–Whitney tests, $U = 19$, $P > 0.05$, $U = 18$, $P > 0.05$ and $U = 21$, $P > 0.05$.

Table 2 Genetic data of colonies from native (New Orleans, Louisiana) and introduced populations (France and Chile) populations of *R. flavipes*. Microsatellite analyses: type of family structure found for each colony, numbers of workers analysed in each colony (N_C), maximum numbers of genotypes found at any one locus in each colony (Max *N*g), maximum numbers of alleles found at any one locus in each colony (Max *N*a), average number of alleles over all loci (*N*a) and the number of loci with more than 4 alleles (*N* loci 4 +). Mitochondrial analyses: mitochondrial haplotype per colony referenced in Perdereau *et al*. (2013) (Haplotype) and GenBank Accession nos

		Microsatellite analyses					COII	
Colony	Family structure	N_C	Max *N*g	Max *N*a	*N*a	*N* loci 4+	Haplotype	Accession no.
New Orleans, Louisiana population								
Col L1-L2	Mixed	38	8	6	3.14	1	AC	JQ280639/JQ280640
Col L3	Extended	18	6	4	3.57	0	AD	JQ280641
Col L4	Mixed	18	8	5	3.29	1	AE	JQ290642
Col L5	Mixed	17	10	5	3.86	1	K	JQ280643
Col L6	Mixed	19	7	6	3.86	1	AC	JQ280644
Col L7	Extended	19	9	4	3.29	0	A	JQ280645
Col L8	Mixed	18	9	6	2.43	1	H	JQ280646
Col L9	Extended	16	6	3	2.71	0	AJ	JQ280647
Col L10	Extended	18	9	4	3.29	0	E	JQ280648
Col L11	Mixed	18	9	7	5.29	4	E	JQ280649
Col L12	Extended	17	10	4	3.29	0	E	JQ280650
Col L13	Extended	19	8	4	3.00	0	AC	JQ280651
Col L14	Mixed	18	13	9	5.14	4	H	JQ280652
Col L15	Mixed	19	8	5	3.57	2	AF	JQ280653
Col L16	Extended	19	9	4	2.86	0	AE	JQ280654
Col L17	Mixed	17	7	5	2.86	1	AH	JQ280656
Col L18	Mixed	19	6	6	3.57	1	A	JQ280657
Col L19	Extended	17	8	4	3.57	0	H	JQ280658
Col L20	Extended	18	8	4	2.86	0	AI	JQ280659
Col L21	Extended	17	8	4	3.00	0	E	JQ280660
French population								
Olonne-sur-mer								
Col O1-O10	Mixed	197	10	5	4.33	2	A	JQ280590
Col O11-O20	Mixed	183	14	6	3.83	3	A	JQ280590
Chile populations								
Santiago region								
Col S1	Mixed	20	13	8	4.57	2	—	—
Col S2	Mixed	20	9	6	4.29	3	BM	JQ280708
Col S3	Extended	20	8	4	2.71	0	—	—
Col S4	Mixed	20	10	6	4.14	2	BM	JQ280708
Col S5	Extended	20	8	4	3.71	0	—	—
Col S6	Extended	20	6	4	3.57	0	BM	JQ280708
Col S7	Extended	20	7	4	2.71	0	—	—
Col S8	Extended	20	9	4	3.86	0	—	—
Col S9	Mixed	20	8	5	3.57	1	—	—
Col S10	Mixed	20	11	6	4.57	3	BM	JQ280708
Valparaiso region								
Col V1	Extended	20	8	4	3.43	0	—	—
Col V2	Extended	20	9	4	4.00	0	BM	JQ280708
Col V3	Mixed	20	16	8	4.86	3	—	—
Col V4	Extended	20	5	3	2.86	0	BM	JQ280708
Col V5	Mixed	20	12	5	4.57	3	BM	JQ280708
Col V6	Extended	20	9	4	4.14	0	BM	JQ280708
Col V7	Extended	20	7	4	3.14	0	—	—
Col V8	Extended	20	8	4	3.71	0	—	—
Col V9	Extended	20	8	4	3.43	0	—	—
Col V10	Mixed	20	9	7	5.14	4	BM	JQ280708

Table 3 *F*-statistics classified as individual (I), colony (C) and total (T) and relatedness coefficients (*r*) for worker nestmates of *R. flavipes* colonies from Louisiana, Chile and France populations and values expected for some possible breeding systems of termites as derived from computer simulations by Thorne *et al*. (1999) and Bulmer *et al*. (2001). Confidence intervals of 95% are shown. For the simulated breeding systems, X represents the number of generations of production of replacement reproductives within a colony, N_f and N_m represent the number of replacement females and males produced per generation, and p is the proportion of workers coming from one of the two nests

Colony	F_{IT}	F_{CT}	F_{IC}	r
Experiment results				
New Orleans, Louisiana				
New Orleans population				
Extended families (n = 10)	0.380 ± 0.060	0.339 ± 0.035	0.060 ± 0.050	0.493 ± 0.033
Mixed families (n = 10)	0.342 ± 0.037	0.307 ± 0.026	0.050 ± 0.034	0.458 ± 0.030
All (n = 20)	0.362 ± 0.046	0.325 ± 0.029	0.055 ± 0.039	0.477 ± 0.030
France				
Olonne-sur-mer population				
All Mixed families (n = 2)	0.335 ± 0.074	0.318 ± 0.070	0.025 ± 0.055	0.480 ± 0.084
Chile				
Santiago population				
Extended families (n = 7)	0.422 ± 0.078	0.114 ± 0.029	0.347 ± 0.088	0.161 ± 0.041
Mixed families (n = 3)	0.485 ± 0.064	0.132 ± 0.023	0.406 ± 0.073	0.178 ± 0.032
All (n = 10)	0.491 ± 0.057	0.177 ± 0.034	0.381 ± 0.065	0.238 ± 0.043
Valparaiso population				
Extended families (n = 5)	0.540 ± 0.047	0.199 ± 0.033	0.426 ± 0.053	0.259 ± 0.039
Mixed families (n = 5)	0.403 ± 0.070	0.075 ± 0.028	0.354 ± 0.070	0.108 ± 0.037
All (n = 10)	0.495 ± 0.045	0.156 ± 0.022	0.402 ± 0.051	0.209 ± 0.028
Simulated breeding system				
(A) Colonies headed by monogamous reproductive pairs	0	0.25	−0.33	0.50
(B) Colonies with breeding among neotenics				
i $N_f = N_m = 1$, $X = 1$	0.33	0.42	−0.14	0.62
ii $N_f = N_m = 1$, $X = 3$	0.57	0.65	−0.22	0.82
iii $N_f = 2$, $N_m = 1$, $X = 3$	0.52	0.59	−0.17	0.78
iv $N_f = N_m = 10$, $X = 3$	0.37	0.38	−0.02	0.56
v $N_f = 200$, $N_m = 100$, $X = 3$	0.34	0.34	0.00	0.51
(C) Nest budding with interconnected daughter nests				
i $N_f = N_m = 1$, $X = 3$, $P = 0.5$	0.66	0.56	0.22	0.68
ii $N_f = N_m = 100$, $X = 3$, $P = 0.9$	0.43	0.41	0.03	0.58
(D) Inbreeding, then mixing of unrelated workers at collection sites				
$N_f = N_m = 10$, $X = 3$, $P = 0.8$	0.37	0.25	0.15	0.36
(E) Workers from unrelated nests mix at collection sites				
i $N_f = N_m = 1$, $X = 1$, $P = 0.5$	0.33	0.20	0.17	0.29
ii $N_f = N_m = 1$, $X = 3$, $P = 0.9$	0.57	0.43	0.25	0.55
(F) Pleometrosis				
i headed by 2 queens and one king	0	0.19	−0.23	0.38
ii headed by 2 queens and one king, then $N_f = N_m = 10$, $X = 3$	0.27	0.29	−0.03	0.45
iii headed by 5 queens and 5 kings, then $N_f = N_m = 10$, $X = 3$	0.10	0.12	−0.02	0.22

Isolation by distance within populations

The relationship between pairwise estimates of $F_{CT}/(1-F_{CT})$ and geographical distances between colonies within each population (New Orleans NO, Olonne-sur-mer O, Santiago S, Valparaiso V, Paris P, Ile d'Oléron Saumonard IS and Ile d'Oléron Saint Trojan IT) was computed (Fig. 2). No significant correlation has been found between the levels of genetic differentiation and geographical distance among colonies in the source population of New Orleans and introduced populations (Fig. 2) (NO: Mantel test: $r = 0.0004$, $P = 0.49$; IS: Mantel

test: $r = 0.147$, $P = 0.014$; IT: Mantel test: $r = 0.013$, $P = 0.92$; P: Mantel test: $r = 0.025$, $P = 0.33$; S: Mantel test: $r = 0.112$, $P = 0.07$; V: Mantel test: $r = 0.129$, $P = 0.12$). Olonne-sur-mer population containing only two colonies, the correlation between $F_{CT}/(1\text{-}F_{CT})$ and logarithm of geographic distances was not computed. Overall, the results showed that different population-level spatial scales used in our sampling do not bias comparisons between populations.

Comparison of the breeding structure in native and introduced populations of R. flavipes

All available results on the colony breeding structure in native and introduced populations of *R. flavipes* have been synthesized in Fig. 3 and Table 4. This synthesis was based on this study and previous studies that used similar microsatellite-based approaches to study colonies and their family types as well as to estimate the number of reproductives within populations (Bulmer *et al.* 2001; Vargo 2003a,b; DeHeer & Vargo 2004; Dronnet *et al.* 2005; Vargo & Carlson 2006; DeHeer & Kamble 2008; Parman & Vargo 2008; Perdereau *et al.* 2010a; Ab Majid *et al.* 2013; Vargo *et al.* 2013). This synthesis includes 11 native populations from seven states in the US, and six introduced populations, four in France and two in Chile.

Our analysis revealed two main types of populations according to their colony breeding structure (Fig. 3). Some populations (type 1) are composed of a majority of simple families (>50%), with some extended families headed by a few neotenics (<10) and none or a few mixed families (≤15%). Eight US populations are classified in this first population type (Fig. 3). Other populations (type 2) are exclusively composed of extended and/or mixed families headed by numerous neotenics (>100). All six introduced populations and the New Orleans population, the putative source population of French infestations, were classified in this second type. Two US populations (i.e. MF Massachusetts and LN Nebraska) are intermediate between types 1 and 2. These populations possess a majority of extended and/or mixed families, which are, at least for the Massachusetts population, headed by hundreds of neotenics. In contrast with type 2, however, these two populations have a substantial proportion of simple-family colonies.

Discussion

Although the colonization success of invasive species fundamentally relies on a match between biological traits of the invader and ecological factors of the invaded habitat (Facon *et al.* 2006), there is no doubt that certain expressed phenotypes in the introduced

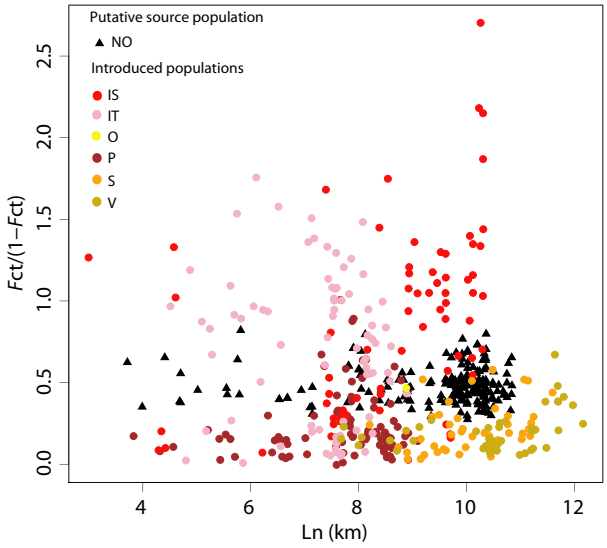

Fig. 2 Isolation by distance analysis for seven *R. flavipes* populations using microsatellite data. The relationship between pairwise estimates of $F_{CT}/(1\text{-}F_{CT})$ and geographical distance between colonies within each population (New Orleans NO, Olonne-sur-mer O, Santiago S, Valparaiso V, Paris P, Ile d'Oléron Saumonard IS and Ile d'Oléron Saint Trojan IT) is shown. The correlation coefficients were not significant for any populations (NO: Mantel test: $r = 0.0004$, $P = 0.49$; IS: Mantel test: $r = 0.147$, $P = 0.014$; IT: Mantel test: $r = 0.013$, $P = 0.92$; P: Mantel test: $r = 0.025$, $P = 0.33$; S: Mantel test: $r = 0.112$, $P = 0.07$; V: Mantel test: $r = 0.129$, $P = 0.12$).

populations increase the probability of invasion success. The factors promoting the establishment and colonization of the North American termite *R. flavipes* in other regions of the world are currently unknown. So far, only a few studies aimed to analyse introduced populations (Perdereau *et al.* 2010a,b, 2011, 2013) in order to compare them to native US populations. These studies revealed that introduced populations differ from native populations in several colony-level traits including a propensity of colonies to fuse in mixed families and to contain numerous functional neotenics. Until now, however, only three introduced populations, all from France, were studied. The present study revealed that these two colony-level traits previously found in the three French populations (Dronnet *et al.* 2005; Perdereau *et al.* 2010a) also occurred in the infestation of Olonne-sur-mer (France) and in the two studied introduced populations in Chile (Valparaiso and Santiago). Our genetic analyses indeed show that these populations are composed of extended and/or mixed families, which are all headed by hundreds of neotenics (type 2, Fig. 3). Mixed families have been documented to result from colony fusion in *R. flavipes* (DeHeer & Vargo 2004, 2008). Although we found a high proportion of mixed families in these populations (100% in Olonne-sur-mer,

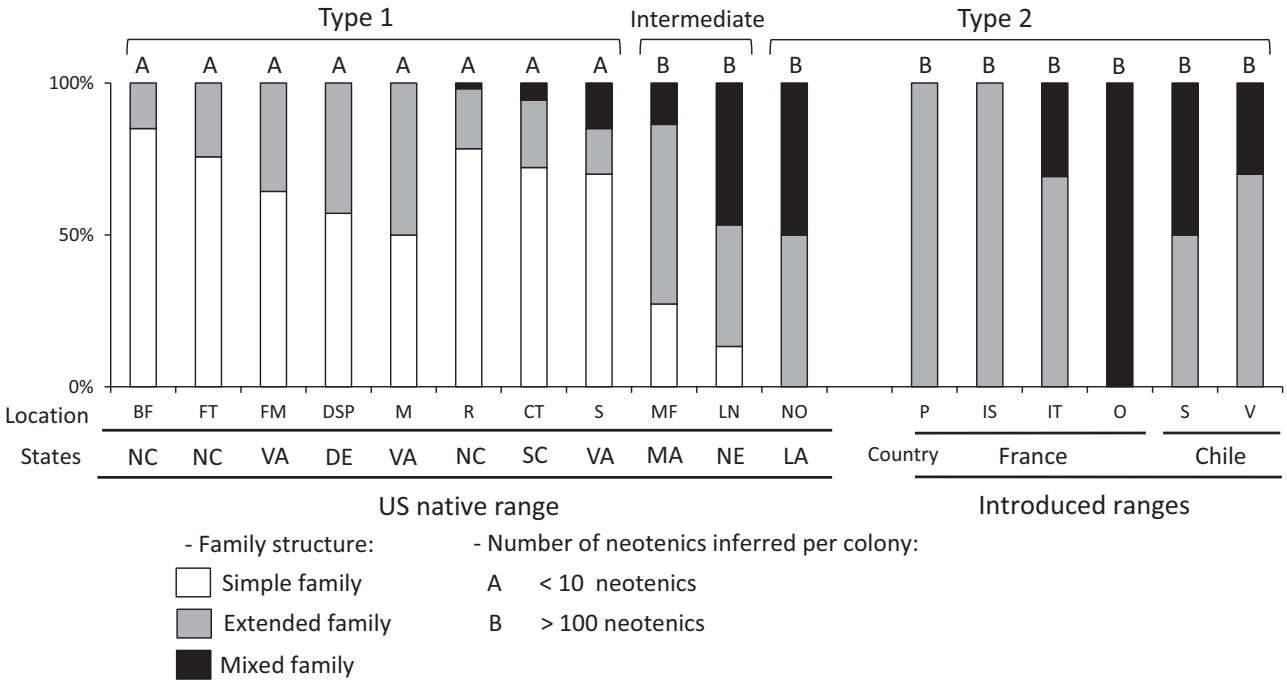

Fig. 3 Synthesis of breeding system in native and introduced populations of *R. flavipes*. The comparison of colony breeding structure is based on the proportion of the family structure and the number of active neotenics inferred per colony. The graph was based on present data and previous studies (Bulmer *et al.* 2001; Vargo 2003a,b; DeHeer & Vargo 2004; Dronnet *et al.* 2005; Vargo & Carlson 2006; DeHeer & Kamble 2008; Parman & Vargo 2008; Perdereau *et al.* 2010a; Ab Majid *et al.* 2013; Vargo *et al.* 2013). Detailed data are given in Table 4. Native range US: North Carolina: Bladen Forest BF (n = 20), Fletcher FT (n = 37) and Raleigh R (n = 319); South Carolina: Charles Towne Landing State Historic Site CT (n = 18); Virginia: Fenwick Mines FM (n = 14), Mason Neck State Park M (n = 18) and Suffolk S (n = 20); Delaware: Delaware State Parks (n = 28); Massachusetts: Middlesex Fells MF (n = 22); Nebraska: Lincoln Wilderness Park LN (n = 15); and Louisiana: New Orleans NO (n = 20). Introduced ranges: France: Paris P (n = 14), Ile d'Oléron Saumonard IS (n = 12), Ile d'Oléron St Trojan IT (n = 13) and Olonne-sur-Mer O (n = 2); Chile: Santiago S (n = 10) and Valparaíso V (n = 10). Class A indicates fewer than 10 neotenics per colony, and class B indicates greater than 100 neotenics per colony.

50% in Santiago, 30% in Valparaiso) (Fig. 3), this may well be an underestimate because the low genetic diversity in introduced populations makes it more difficult to detect mixed-family colonies, as previously discussed by Perdereau *et al.* (2010a). The number of alleles per locus is indeed the only measure that can distinguish mixed families from extended families by means of microsatellite loci. This probably explains why Dronnet *et al.* (2005) did not detect any fusion in Paris and Ile d'Oléron Saumonard populations (Fig. 3), which exhibited the lowest average number of alleles found in any *R. flavipes* populations.

The production of a large number of reproductives within colonies and a high fusion frequency among colonies both contribute to the formation of populous and spatially expansive colonies. For instance, each of the two mixed-family colonies identified in Olonne-sur-mer (France) consisted of at least 10 foraging nests (logs or laying trunks) that are separated by 10-60 metres (Fig. 1B) and connected by underground tunnels. These expansive colonies seem to be very common in French populations where they have been previously characterized in both forests and cities (Dronnet *et al.* 2005; Perdereau *et al.* 2010a). Such a social organization can be compared to a particular social form found in ants referred to as 'unicoloniality', which is defined by the absence of colony boundaries between nests, which can exchange workers, brood and fertile queens, and by the production of many queens (polygyny)(Hölldobler & Wilson 1990; Helanterä *et al.* 2009). For example, in the well-studied Argentine ant, *Linepithema humile*, introduced populations form one geographically large super-colony, genetically and chemically uniform without intraspecific aggression (Tsutsui *et al.* 2000; Giraud *et al.* 2002; Brandt *et al.* 2009; Blight *et al.* 2012). The homogenization of chemical recognition cues (cuticle hydrocarbons) associated with unicoloniality in introduced populations has often been attributed to a reduction in genetic diversity induced by founding events (bottlenecks) (Tsutsui *et al.* 2000; Suarez *et al.* 2008), although natural selection has also been proposed to drive this social trait (Giraud *et al.* 2002). In *L. humile*,

Table 4 Synthesis of data of colony breeding systems in native and introduced populations of *R. flavipes*

	Location	Name of population	Mean allele number	Number of colonies	Per cent of simple families	Per cent of extended families	F_{IC}	Number of neotenics	Per cent of mixed families	*r*	References
Native range USA											
Massachusetts	Middlesex Fells	MF	—	22	27.3%	59.1%	0.097	>100	13.6%	NR	Bulmer *et al.* (2001)
Delaware	Lum's Pond and White Clay Creek State Parks	DSP	10.4 ± 6.6	28	57.1%	42.9%	−0.150	<10	0	—	Vargo *et al.* (2013)
Virginia	Mason Neck State Park	M	9.4 ± 4.2	18	50%	50%	−0.220	<10	0	—	Vargo *et al.* (2013)
	Fenwick Mines	FM	8.3 ± 4.1	14	64.3%	35.7%	−0.02	<10	0	—	Vargo *et al.* (2013)
	Suffolk	S	8.5 ± 4.7	20	70%	15%	−0.280	<10	15%	0.330	Vargo *et al.* (2013)
North Carolina	Raleigh	R	11.4 ± 9.2	319	78.4%	19.7%	−0.209	<10	1.9%	0.327	Vargo (2003a,b); DeHeer & Vargo (2004); Vargo & Carlson (2006); Parman & Vargo (2008)
	Fletcher	FT	10.8 ± 8.8	37	75.7%	24.3%	−0.080	<10	0	—	Vargo *et al.* (2013)
	Bladen Forest	BF	10.5 ± 6.5	20	85%	15%	−0.290	<10	0	—	Vargo *et al.* (2013)
South Carolina	Charles Towne Landing State Historic Site	CT	11 ± 5.8	18	72.2%	22.2%	−0.14	<10	5.6%	NR	Vargo *et al.* (2006)
Nebraska	Lincoln	LN	3.1 ± 0.8	15	13.3%	40%	0.159	>100	46.7%	0.421	DeHeer & Kamble (2008); Ab Majid *et al.* (2013)
Louisiana	New Orleans	NO	19.3 ± 12.4	20	0	50%	0.060	>100	50%	0.460	This study
Introduced range											
France	Paris	P	3.1 ± 1.4	14	0	100%	0.032	>100	0	—	Dronnet *et al.* (2005)
	Ile d'Oléron Saumonard	IS	4.7 ± 1.9	12	0	100%	−0.001	>100	0	—	Dronnet *et al.* (2005)
	Ile d'Oléron St Trojan	IT	4.9 ± 1.5	13	0	69.2%	0.052	>100	30.8%	0.177	Perdereau *et al.* (2011)
	Olonne-sur-mer	O	5.2 ± 1.7	2	0	0	—	>100	100%	0.480	This study
Chile	Santiago region	S	7 ± 2.5	10	0	50%	0.347	>100	50%	0.178	This study
	Valparaiso region	V	6.7 ± 2.5	10	0	70%	0.426	>100	30%	0.209	This study

unicoloniality has been demonstrated to provide major ecological advantages for colonization (Tsutsui *et al.* 2000; Giraud *et al.* 2002), for exploitation of resources (Holway & Case 2001) and for competition with other species (Holway 1999). Interestingly, the mechanisms generating unicolonial structure seem comparable between the introduced populations of the Argentine ant and the introduced French and Chilean populations of *R. flavipes* to attain high densities and interspecific dominance in invaded habitats. In French introduced populations of *R. flavipes*, previous studies revealed a lack of aggression between colonies (Clément & Bagnères 1998; Perdereau *et al.* 2011), a strong homogeneity in the chemical recognition cues (cuticular hydrocarbons) (Perdereau *et al.* 2010b) and reduced genetic diversity (Perdereau *et al.* 2013). Similar to the Argentine ants (Vasquez & Silverman 2008), these characteristics have been cited as a possible explanation for the propensity of colonies to fuse in introduced populations (Perdereau *et al.* 2011).

The colony breeding structure found in the population of New Orleans, the putative source population of French and Chilean infestations (Perdereau *et al.* 2013; E. L. Vargo, unpublished data), is very similar to that of all studied French and Chilean introduced populations (referred as 'type 2', Fig. 3). This result was unexpected as no native population had been found previously to exhibit this breeding structure. Although the colony breeding structure significantly varies among native US populations of *R. flavipes* (Vargo *et al.* 2013), the New Orleans population is unique in the native range because it is the only one studied so far that is exclusively composed of extended and mixed-family colonies containing numerous neotenics (Fig. 3). Four alternative hypotheses could explain this general pattern. The first hypothesis is that the unique type 2 breeding structure first evolved in the New Orleans population and subsequently was transmitted to introduced populations derived from this population. A second hypothesis is that the New Orleans population and the introduced populations independently evolved the type 2 breeding structure. This scenario appears less parsimonious than the first one because it requires at least two evolutionary transitions in the breeding structure. More precisely, three transitions are required when considering that the introduction of *R. flavipes* in France and Chile occurred independently, whereas two transitions are required if these two introductions did not occur independently (i.e. Chilean populations would be in that case founded by French introductions or *vice versa*). A third hypothesis considers a complex scenario composed by three main steps. First, the introduction of *R. flavipes* in France and Chile would have independently caused the evolution of type 2 breeding structure in French and Chilean populations. Second, repeated trades between France (and/or Chile) and New Orleans would have allowed the re-introduction of French (and/or Chilean) colonies back to New Orleans. Third, the re-introduced populations in the region of New Orleans would have then competed and replaced native populations. This third hypothesis seems very unlikely. If this scenario had indeed occurred, one would expect to find low levels of genetic diversity in the New Orleans population as the result of multiple sequential events of introduction, re-introduction, rapid expansion and population replacement. However, the results obtained here (Table 4) revealed that the population of New Orleans exhibits a high level of genetic diversity based on microsatellite data, which is higher or at least comparable to the diversity levels found in the other native populations. In addition, this third hypothesis requires many more events than the other two and therefore is less parsimonious. These first three hypotheses postulate that attributes of the breeding structure are heritable, although there is currently no empirical evidence to support this characteristic. A fourth hypothesis assumes that the colony capacities to differentiate neotenics and to fuse are only expressed in response to one or several specific environmental factor(s) (i.e. phenotypic plasticity) (Lande this issue). A similar breeding structure between the source and the introduced populations would therefore be due to the presence of this key factor(s) in the habitats of New Orleans, French and Chilean populations. This hypothesis consequently implies the absence of the key factor(s) in the habitats of the other native US populations. Testing these four hypotheses requires further knowledge of the different traits associated with colony breeding structure in *R. flavipes*, including the genetic and ecological factors underlying their variation and their levels of heritability within populations. Nonetheless, among the four hypotheses, the first one seems to be the most likely. According to this hypothesis, invasive colonies of French and Chilean populations nowadays produce numerous neotenics and frequently fuse because the first pioneering introduced colonies imported from New Orleans already expressed these traits and transmitted them to each generation until the present.

The type 2 breeding structure found in the studied introduced populations may improve the ability to successfully establish in the introduced ranges. High number of functional neotenics is known to boost the reproductive rate of colonies and therefore their growth (Myles 1999). Furthermore, the absence of costs related to territoriality and aggression in introduced populations allows colony members to devote more energy to other activities such as colony growth, foraging and collection of resources (Holway *et al.* 1998; Holway 1999). On the

other hand, mating among closely related neotenics within colonies leads to high levels of inbreeding, which can result in inbreeding depression in *R. flavipes* (DeHeer & Vargo 2006). However, the deleterious effects of inbreeding might be counterbalanced by colony fusion, a phenomenon that can 'refresh' the allelic diversity of colonies by mixing families. Finally, it is possible that the capacity of many colony members (i.e. nymphs and potentially workers) to differentiate into neotenics improves long-distance human-mediated dispersal events. Just a few 'competent' individuals present in transported materials, such as soil or wood, can allow new colonies to become successfully established in introduced areas (Pichon *et al.* 2007). Together, these advantages may also explain why *R. flavipes* infestations are common and populous around urban areas and have successfully colonized large cities such as Paris and Bordeaux in France (Dronnet *et al.* 2005) or Santiago and Valparaiso in Chile. A recent review concerning invasive termites, which belong to two distinct families – Rhinotermitidae, especially *Reticulitermes* spp., and Kalotermitidae – reveals that almost all invasive populations share a tendency to differentiate neotenics (Evans *et al.* 2013). A notable exception to this rule, however, has been reported in the Formosan subterranean termite, *Coptotermes formosanus*, which is probably the most widespread invasive termite species worldwide. Introduced populations of the US, Hawaii and Japan indeed are composed of a majority of simple families, and a minority of extended families headed by few neotenics (Vargo & Husseneder 2009, 2011; Husseneder *et al.* 2012).

Phylogeographic studies suggested that populations of the south-eastern part of the US, especially around New Orleans, have served as the source of invasive populations in other regions of the world (Ascunce *et al.* 2011; Perdereau *et al.* 2013). The propensity for introduction from this area to other continents could be attributed to their geographical location in the US. The south-eastern region of the US has indeed been considered as an important centre of human transportation that is connected by trade to a wide range of other locations around the world. Species living near such a 'transport hub' are therefore particularly prone to be transported and introduced elsewhere (Floerl *et al.* 2009). Recently, a phylogeographic study on the invasive fire ant, *Solenopsis invicta*, showed that after its introduction in the south-eastern region of the US, invasive populations spread to the rest of the world (Ascunce *et al.* 2011; Yang *et al.* 2012). In addition, several invasive ants have been proved or suspected to start their expansion range in the US from the south-eastern region. One of the first introduction records of Argentine ants was New Orleans, Louisiana, and the first detection of fire ants was recorded in Mobile, Alabama (Barber 1916; Tschinkel 2006). Therefore, the introduction success of termite propagules from New Orleans to other regions of the world could be attributed to its geographical location.

The evolutionary forces that drove the type 2 breeding structure in the New Orleans population are currently unknown. Two major hypotheses could explain this phenomenon. The first one is that the population of New Orleans experienced recent demographic events that caused genetic bottlenecks, which in turn would have induced the production of numerous neotenics within colonies and a high propensity for colony fusion. Among demographic events that could cause such bottlenecks, the New Orleans population could have suffered a catastrophic event (such as Hurricane Katrina) leading to a reduction in population size and/or because this area may have been recently founded by introduction(s) from surrounding populations. However, the high level of genetic diversity based on microsatellite data we found in the New Orleans population (Table 4) is not consistent with the predictions of this hypothesis. The second hypothesis is that certain environmental factors specifically present in the region of New Orleans (and absent in the habitat of the other native populations) has promoted the production of numerous neotenics and a high propensity for colony fusion. This hypothesis considers the type 2 breeding structure as an adaptation: some specific environmental factors of the New Orleans habitat would produce a selective regime favouring colonies that express the type 2 breeding structure. One possible selective factor that could have favoured the production of numerous neotenics within colonies and decreased competition among colonies is high food resources that are possibly more available in urban areas than natural forest areas. In his review, Myles (1999) speculated that there is a positive relationship between the amount of food available and the level of neotenic differentiation. However, several studies suggest that the breeding structure does not differ between *R. flavipes* colonies collected in urban and natural (forest) areas (Vargo 2003a; Dronnet *et al.* 2005; Parman & Vargo 2008). Other environmental factors have recently been suggested to influence colony breeding structure in *Reticulitermes*. For instance, Vargo *et al.* (2013) established a strong association between several abiotic factors and the colony breeding structure, both in *R. flavipes* and in a European species of the same genus, *R. grassei*. Among these factors, soil moisture was found to be positively associated with greater levels of inbreeding within colonies that result from breeding among numerous related neotenics. Such an association between soil moisture and the number of neotenics could explain why a large number of neotenics are systematically produced in the population of

New Orleans, a region where soils are known to be particularly wet. Testing the role of selection and other possible forces in the evolution of breeding structure of *Reticulitermes* termites remains a central research goal that will require further investigation into population genomics and ecology.

To conclude, the present study revealed a particular colony breeding structure present in all studied introduced populations of the subterranean termite *R. flavipes*. Only the source population in the native range exhibits such a breeding structure, suggesting that this particular trait may have pre-adapted transported colonies originating from the population of New Orleans to invade successfully. This together with the heavy international transportation in and out of the New Orleans area may have laid the foundation for the introduction and establishment of this pest to new areas. With notable exceptions, this breeding structure and its associated social organization appear rather widespread in invasive termites and, most remarkably, it is analogous to the social organization encountered in many invasive ants. This study suggests that *R. flavipes* provides a promising model system for studying the genetic and environmental factors underlying the observed variation in colony breeding structure and their role in invasion success.

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References

Ab Majid AH, Kamble ST, Miller NJ (2013) Colony genetic structure of Reticulitermes flavipes (Kollar) from Natural Populations in Nebraska. *Journal of Entomological Science*, **48**, 222–233.

Ascunce MS, Yang CC, Oakey J *et al.* (2011) Global invasion history of the fire ant *Solenopsis invicta*. *Science*, **331**, 1066–1068.

Austin JW, Szalanski AL, Uva P, Bagnères AG, Kence A (2002) A comparative genetic analysis of the subterranean termite genus *Reticulitermes* (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America*, **95**, 753–760.

Austin JW, Szalanski AL, Scheffrahn RH, Messenger MT (2005) Genetic variation of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) in North America applying the mitochondrial rRNA 16S gene. *Annals of the Entomological Society of America*, **98**, 980–988.

Baker HG, Stebbins GL (1965) *The Genetics of Colonizing Species*. Academic Press Inc, New York, USA.

Barber TC (1916) The Argentine ant: distribution and control in the United States. USDA Bureau of Entomology Bulletin.

Barrett SCH (this issue) Foundations of invasion genetics: the Baker and Stebbins legacy. *Molecular Ecology*, doi: 10.1111/mec.13014.

Blight O, Berville L, Vogel V *et al.* (2012) Variation in the level of aggression, chemical and genetic distance among three supercolonies of the Argentine ant in Europe. *Molecular Ecology*, **21**, 4106–4121.

Blows MW, McGuigan K (this issue) The distribution of genetic variance across phenotypic space and the response to selection. *Molecular Ecology*, doi: 10.1111/mec.13023.

Bock DG, Caseys C, Cousens RD *et al.* (this issue) What we still don't know about invasion genetics. *Molecular Ecology*, doi: 10.1111/mec.13032.

Bossdorf O, Auge H, Lafuma L *et al.* (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, **144**, 1–11.

Brandt M, Van Wilgenburg E, Tsutsui ND (2009) Global-scale analyses of chemical ecology and population genetics in the invasive Argentine ant. *Molecular Ecology*, **18**, 997–1005.

Buchli H (1958) L'origine des castes et les potentialités ontogéniques des termites européens du genre *Reticulitermes*. *Annales des Sciences Naturelles. Zoologie et Biologie Animale*, **11**, 263–429.

Bulmer MS, Adams ES, Traniello JFA (2001) Variation in colony structure in the subterranean termite *Reticulitermes flavipes*. *Behavioral Ecology and Sociobiology*, **49**, 236–243.

Buttermore RE (1997) Observations of successful *Bombus terrestris* (L.) (Hymenoptera: Apidae) colonies in southern Tasmania. *Australian Journal of Entomology*, **36**, 251–254.

Chapman RE, Bourke AFG (2001) The influence of sociality on the conservation biology of social insects. *Ecology Letters*, **4**, 650–662.

Clément J-L, Bagnères A-G (1998) Nestmate recognition in termites. In: *Pheromone Communication in Social Insects. Ants, Wasps, Bees, and Termites* (eds Vander Meer RK, Breed MD, Espelie KE, Winston ML), pp. 126–155. Westwiew Press, Boulder, Colorado.

Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science*, **342**, 364–366.

DeHeer CJ, Kamble ST (2008) Colony genetic organization, fusion and inbreeding in *Reticulitermes flavipes* from the Midwestern U.S. *Sociobiology*, **51**, 307–325.

DeHeer CJ, Vargo EL (2004) Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. *Molecular Ecology*, **13**, 431–441.

DeHeer CJ, Vargo EL (2006) An indirect test of inbreeding depression in the termites *Reticulitermes flavipes* and *Reticulitermes virginicus*. *Behavioral Ecology and Sociobiology*, **59**, 753–761.

DeHeer CJ, Vargo EL (2008) Strong mitochondrial DNA similarity but low relatedness at microsatellite loci among families within fused colonies of the termite *Reticulitermes flavipes*. *Insectes Sociaux*, **55**, 190–199.

Dlugosch KM, Parker IM (2008a) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Dlugosch KM, Parker IM (2008b) Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters*, **11**, 701–709.

Donovan BJ, Howie AME, Schroeder NC, Wallace AR, Read PEC (1992) Comparative characteristics of nests of *Vespula-germanica* (F) and *Vespula-vulgaris* (L) (Hymenoptera, Vespinae) from Christchurch-City, New-Zealand. *New Zealand Journal of Zoology*, **19**, 61–71.

Dronnet S, Bagnères A-G, Juba T, Vargo EL (2004) Polymorphic microsatellite loci in the European subterranean termite, *Reticulitermes santonensis* Feytaud. *Molecular Ecology Notes*, **4**, 127–129.

Dronnet S, Chapuisat M, Vargo EL, Lohou C, Bagnères A-G (2005) Genetic analysis of the breeding system of an invasive subterranean termite, *Reticulitermes santonensis*, in urban and natural habitats. *Molecular Ecology*, **14**, 1311–1320.

Espadaler X, Rey S (2001) Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera, Formicidae). *Insectes Sociaux*, **48**, 159–164.

Evans TA, Forschler BT, Grace JK (2013) Biology of invasive termites: a worldwide review. *Annual Review of Entomology*, **58**, 455–474.

Facon B, Genton BJ, Shykoff J *et al.* (2006) A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution*, **21**, 130–135.

Floerl O, Inglis GJ, Dey K, Smith A (2009) The importance of transport hubs in stepping-stone invasions. *Journal of Applied Ecology*, **46**, 37–45.

Fournier D, Biseau JC, Aron S (2009) Genetics, behaviour and chemical recognition of the invading ant *Pheidole megacephala*. *Molecular Ecology*, **18**, 186–199.

Geng YP, Pan XY, Xu CY *et al.* (2007) Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biological Invasions*, **9**, 245–256.

Ghesini S, Messenger MT, Pilon N, Marini M (2010) First report of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) in Italy. *Florida Entomologist*, **93**, 327–328.

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

Gladieux P, Feurtey A, Hood ME *et al.* (this issue) The population biology of fungal invasions. *Molecular Ecology*, doi: 10.1111/mec.13028.

Goudet J (1995) FSTAT (vers 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Hanna C, Cook ED, Thompson AR *et al.* (2014) Colony social structure in native and invasive populations of the social wasp *Vespula pensylvanica*. *Biological Invasions*, **16**, 283–294.

Helanterä H, Strassmann JE, Carrillo J, Queller DC (2009) Unicolonial ants: where do they come from, what are they and where are they going? *Trends in Ecology & Evolution*, **24**, 341–349.

Hodgins KA, Bock DG, Hahn MA *et al.* (this issue) Comparative genomics in the Asteraceae reveals little evidence for parallel evolutionary change in invasive taxa. *Molecular Ecology*, doi: 10.1111/mec.13026.

Hölldobler B, Wilson EO (1990) *The Ants*. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.

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

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

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

Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ (2002) The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics*, **33**, 181–233.

Husseneder C, Simms DM, Delatte JR *et al.* (2012) Genetic diversity and colony breeding structure in native and introduced ranges of the Formosan subterranean termite, *Coptotermes formosanus*. *Biological Invasions*, **14**, 419–437.

Kasper ML, Reeson AF, Austin AD (2008) Colony characteristics of *Vespula germanica* (F.) (Hymenoptera, Vespidae) in a Mediterranean climate (southern Australia). *Australian Journal of Entomology*, **47**, 265–274.

van Kleunen M, Dawson W, Maurel N (this issue) Characteristics of successful alien plants. *Molecular Ecology*, doi: 10.1111/mec.13013.

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, **16**, 199–204.

Kollar V (1837) Naturgeschichte des schädlichen Insekten. *Verb Landwirtsch Ges Wien*, **5**, 411–413.

Lande R (this issue) Evolution of phenotypic plasticity in colonizing species. *Molecular Ecology*, doi: 10.1111/mec.13037.

Le Breton J, Delabie JHC, Chazeau J, Dejean A, Jourdan H (2004) Experimental evidence of large-scale unicoloniality in the tramp ant *Wasmannia auropunctata* (Roger). *Journal of Insect Behavior*, **17**, 263–271.

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.

Miura T, Roisin Y, Matsumoto T (2000) Molecular phylogeny and biogeography of the nasute termite genus *Nasutitermes* (Isoptera: Termitidae) in the Pacific tropics. *Molecular Phylogenetics and Evolution*, **17**, 1–10.

Moller H (1996) Lessons for invasion theory from social insects. *Biological Conservation*, **78**, 125–142.

Moran EV, Alexander JM (2014) Evolutionary responses to global change: lessons from invasive species. *Ecology Letters*, **17**, 637–649.

Morel L, Vander Meer RK, Lofgren CS (1990) Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (Hymenoptera, Formicidae). *Annals of the Entomological Society of America*, **83**, 642–647.

Myles TG (1999) Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology*, **33**, 1–43.

Nagamitsu T, Yamagishi H (2009) Nest density, genetic structure, and triploid workers in exotic *Bombus terrestris* populations colonized Japan. *Apidologie*, **40**, 429–440.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New-York.

Novak SJ (2007) The role of evolution in the invasion process. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3671–3672.

Parman V, Vargo EL (2008) Population density, species abundance, and breeding structure of subterranean termite colo-

nies in and around infested houses in central North Carolina. *Journal of Economic Entomology*, **101**, 1349–1359.

Perdereau E, Bagnères A-G, Dupont S, Dedeine F (2010a) High occurrence of colony fusion in a European population of the American termite *Reticulitermes flavipes*. *Insectes Sociaux*, **57**, 393–402.

Perdereau E, Dedeine F, Christidès J-P, Bagnères A-G (2010b) Variations in worker cuticular hydrocarbons and soldier isoprenoid defensive secretions within and among introduced and native populations of the subterranean termite, *Reticulitermes flavipes*. *Journal of Chemical Ecology*, **36**, 1189–1198.

Perdereau E, Dedeine F, Christidès J-P, Dupont S, Bagnères A-G (2011) Competition between invasive and indigenous species: an insular case study of subterranean termites. *Biological Invasions*, **13**, 1457–1470.

Perdereau E, Bagnères A-G, Bankhead-Dronnet S *et al.* (2013) Global genetic analysis reveals the putative native source of the invasive termite, *Reticulitermes flavipes*, in France. *Molecular Ecology*, **22**, 1105–1119.

Pichon A, Kutnik M, Leniaud L *et al.* (2007) Development of experimentally orphaned termite worker colonies of two *Reticulitermes* species (Isoptera: Rhinotermitidae). *Sociobiology*, **50**, 1015–1034.

Pysek P, Richardson DM (2007) Traits associated with invasiveness in alien plants: where do we stand? In: *Biological Invasions* (ed. Nentwig W). Springer-Verlag, Berlin, Germany.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

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

Ripa R, Castro L (2000) Presencia de la termita subterranea *Reticulitermes santonensis* de Feytaud (Isoptera: Rhinotermitidae) en la comuna de Quillota. In XXII Chilean Congress of Entomology, Valdivia.

Rollins LA, Moles AT, Lam S *et al.* (2013) High genetic diversity is not essential for successful introduction. *Ecology and Evolution*, **3**, 4501–4517.

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

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Sambrook J, Fritcsh EF, Maniatis T (1989) *Molecular Cloning*, 2nd edn. Cold Spring Harbor Lab Press, Cold Spring Harbor, New York.

Sax DF, Stachowicz JJ, Gaines SD (2005) *Species Invasions: Insights Into Ecology, Evolution, and Biogeography*. Sinauer, SunderLand, Massachusetts.

Scaduto DA, Garner SR, Leach EL, Thompson GJ (2012) Genetic evidence for multiple invasions of the eastern subterranean termite into Canada. *Environmental Entomology*, **41**, 1680–1686.

Simon C, Frati F, Beckenbach A *et al.* (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

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

Su NY, Ye WM, Ripa R, Scheffrahn RH, Giblin-Davis RM (2006) Identification of Chilean *Reticulitermes* (Isoptera: Rhinotermitidae) inferred from three mitochondrial gene DNA sequences and soldier morphology. *Annals of the Entomological Society of America*, **99**, 352–363.

Suarez AV, Holway DA, Tsutsui ND (2008) Genetics and behavior of a colonizing species: the invasive argentine ant. *American Naturalist*, **172**, S72–S84.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.

Thorne BL, Traniello JFA, Adams ES, Bulmer M (1999) Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera Rhinotermitidae): a review of the evidence from behavioral, ecological and genetic studies. *Ethology Ecology & Evolution*, **11**, 149–169.

Tschinkel WR (2006) *The Fire Ants*. Harvard University Press, Cambridge, MA.

Tsutsui ND, Suarez AV (2003) The colony structure and population biology of invasive ants. *Conservation Biology*, **17**, 48–58.

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

Vandepitte K, De Meyer T, Helsen K *et al.* (2014) Rapid genetic adaptation precedes the spread of an exotic plant species. *Molecular Ecology*, **23**, 2157–2164.

Vanloon AJ, Boomsma JJ, Andrasfalvy A (1990) A new polygynous *Lasius* species (Hymenoptera, Formicidae) from Central-Europe. 1. Description and general biology. *Insectes Sociaux*, **37**, 348–362.

Vargo EL (2000) Polymorphism at trinucleotide microsatellite loci in the subterranean termite *Reticulitermes flavipes*. *Molecular Ecology*, **9**, 817–829.

Vargo EL (2003a) Genetic structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) colonies in an urban habitat and tracking of colonies following treatment with hexaflumuron bait. *Environmental Entomology*, **32**, 1271–1282.

Vargo EL (2003b) Hierarchical analysis of colony and population genetic structure of the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. *Evolution*, **57**, 2805–2818.

Vargo EL, Carlson JR (2006) Comparative study of breeding systems of sympatric subterranean termites (*Reticulitermes flavipes* and *R. hageni*) in Central North Carolina using two classes of molecular genetic markers. *Environmental Entomology*, **35**, 173–187.

Vargo EL, Juba TR, DeHeer CJ (2006) Relative abundance and comparative breeding structure of subterranean termite colonies (*Reticulitermes flavipes, Reticulitermes hageni, Reticulitermes virginicus*, and *Coptotermes formosanus*) in a South Carolina lowcountry site as revealed by molecular markers. *Annals of the Entomological Society of America*, **99**, 1101–1109.

Vargo EL, Husseneder C (2009) Biology of subterranean termites: insights from molecular studies of *Reticulitermes* and *Coptotermes*. *Annual Review of Entomology*, **54**, 379–403.

Vargo EL, Husseneder C (2011) Genetic structure of termite colonies and populations. In: *Biology of Termites: A Modern Synthesis* (eds Bignell D, Roisin Y, Lo N), pp. 321–347. Springer, Heidelberg, Germany.

Vargo EL, Leniaud L, Swoboda LE *et al.* (2013) Clinal variation in colony breeding structure and level of inbreeding in the subterranean termites *Reticulitermes flavipes* and *R. grassei*. *Molecular Ecology*, **22**, 1447–1462.

Vasquez GM, Silverman J (2008) Intraspecific aggression and colony fusion in the Argentine ant. *Animal Behaviour*, **75**, 583–593.

Wares JP, Hughes AR, Grosberg RK (2005) Mechanisms that drive evolutionary change: insights from species introductions and invasions. In: *Species Invasions: Insights into Ecology, Evolution and Biogeography* (eds Sax DF, Stachowicz JJ, Gaines SD), pp. 229–257. Sinauer Associates, Sunderland, Massachusetts.

Weidner H (1937) Termiten in Hamburg. *Z Pflanzenkrankh*, **47**, 593–596.

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

Yang CC, Ascunce MS, Luo LZ *et al.* (2012) Propagule pressure and colony social organization are associated with the successful invasion and rapid range expansion of fire ants in China. *Molecular Ecology*, **21**, 817–833.

E.P., A.G.B., E.L.V. and F.D. conceived and designed the study. E.P., A.G.B., E.L.V. and S.D. collected the samples. A.G.B. applied grant (*TermiCentre*) for the study. E.P., G.B., Y.X., P.L. and S.D. generated the data set. E.P., Y.X., G.B. and P.L. analysed the data set. E.P., E.L.V. and F.D. wrote the first draft of the manuscript. All authors edited and approved the manuscript.

Data accessibility

Microsatellite data are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.jd627. Supplementary data file 1 Alignment of the 97 COII sequences of *R. flavipes* individuals.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Location of samples of *R. flavipes* from New Orleans (Louisiana), Olonne-sur-mer (France), Santiago and Valparaiso (Chile).