Global genetic analysis reveals the putative native source of the invasive termite, Reticulitermes flavipes, in France

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Abstract

Biological invasions are recognized as a major threat to both natural and managed ecosystems. Phylogeographic and population genetic analyses can provide information about the geographical origins and patterns of introduction and explain the causes and mechanisms by which introduced species have become successful invaders. Reticulitermes flavipes is a North American subterranean termite that has been introduced into several areas, including France where introduced populations have become invasive. To identify likely source populations in the USA and to compare the genetic diversity of both native and introduced populations, an extensive molecular genetic study was undertaken using the COII region of mtDNA and 15 microsatellite loci. Our results showed that native northern US populations appeared well differentiated from those of the southern part of the US range. Phylogenetic analysis of both mitochondrial and nuclear markers showed that French populations probably originated from southeastern US populations, and more specifically from Louisiana. All of the mtDNA haplotypes shared between the United States and France were found in Louisiana. Compared to native populations in Louisiana, French populations show lower genetic diversity at both mtDNA and microsatellite markers. These findings are discussed along with the invasion routes of R. flavipes as well as the possible mechanisms by which French populations have evolved after their introduction.

Keywords: invasive species, microsatellites, mtDNA, native source population, termite

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Introduction

Many species have been introduced and expanded into new areas as a result of global trade and human transport. Biological invasions often cause ecological changes such as ecosystem degradation and reduced biodiversity, as well as problems to agriculture and public health (Sax et al. 2005). They have been largely studied to better understand the required conditions for exotic species to become successful invaders. To successfully found an invasive population, the introduced group must pass through three essential stages (i) move to the new locality; (ii) establish a viable, reproductively functional population in a habitat that can differ drastically from the native one for many ecological factors; and (iii) increase demographically and spread spatially in the invaded area (Kolar & Lodge 2001). Numerous studies comparing native and introduced populations for both biological and ecological characteristics have suggested that the ability of an introduced population to become invasive depends on several factors, including particular phenotypic traits of the introduced individuals, certain attributes of the invaded environment and indigenous communities, as well as the complex interactions between the two (Shea & Chesson 2002).
Despite a massive research investment, however, a general understanding of biological invasions has remained elusive (Sax et al. 2005).

A key task that needs to be accomplished in order to fully understand the success of biological invasions as well as to develop effective prevention and management strategies against them is to determine the geographical origin of invasive species. The identification of the source populations of invasive species can indeed provide information on the biology of the invasive species in their native ecological niche; it can facilitate the selection of optimal potential biological control agents and is often a required step to reconstruct the routes of invasions (Caldera et al. 2008; Muirhead et al. 2008; Ascunce et al. 2011). Furthermore, identifying the source population is an essential prerequisite for testing hypotheses concerning the evolution of introduced populations. Many studies revealed significant genetic and phenotypic differentiation between native and introduced populations, which is often interpreted to result from evolutionary events that occurred in introduced populations, either during the settlement phase or thereafter during the colonization process (Wares et al. 2005).

A crucial piece of information for understanding the reasons of invasion success is to determine whether the introduction events affected the level of genetic diversity within introduced populations. The main attribute that determines the adaptive potential of a population is heritable phenotypic variation, which is itself often intimately linked to genetic diversity (Keller & Taylor 2008). Several studies have revealed that genetic diversity in introduced populations can be high when populations are founded with numerous individuals from multiple introduction events, or when populations are founded with individuals from several source populations exhibiting strong genetic differentiation among them (Facon et al. 2006). In some cases, the resulting genetic diversity within introduced populations can even be higher than the original genetic diversity occurring within each of the native populations (Facon et al. 2008). However, there are many examples where introduced populations possess significantly lower genetic diversity compared to their native populations (Dlugosch & Parker 2008a,b). Therefore, the relationship between the genetic diversity and invasive success remains unclear, and more empirical data are needed to address this question.

*Reticulitermes flavipes* (Rhinotermitidae) is a subterranean termite species that lives in forest ecosystems and can cause significant damage to human-built wooden structures. Originating from the United States, this species is widespread from Texas to Iowa in the west and from Florida to Massachusetts in the east. In the United States, it has been introduced and established in other states such as California as well as in several other countries in North and South America (the Bahamas, Canada, Chile and Uruguay) (Ripa & Castro 2000; Austin et al. 2002, 2005a; Su et al. 2006). In Western Europe, *R. flavipes* is widespread throughout France and has been locally found in Austria (in Vienna, where *R. flavipes* was first described by Kollar 1837), Germany (Hamburg) and Italy (Varese) (Weidner 1937; Ghesini et al. 2010). French populations were originally described as a European species, *Reticulitermes santonensis* (Feytaud 1924). However, shortly after its description, Feytaud (1924) suggested that *R. santonensis* might be synonymous with *R. flavipes*. On the basis of morphological, chemical and genetic similarities, *R. santonensis* populations are now indeed considered to be introduced populations of *R. flavipes* (Clement et al. 2001; Austin et al. 2005b). It has been speculated that *R. flavipes* was introduced through the wood shipping trade between North America and Europe during the 17th and the 18th century (Bagnères et al. 1990). In addition, a previous study examining chemical components (mostly cuticle hydrocarbons) in *R. flavipes* revealed that colonies collected in France and Louisiana had very similar chemical profiles compared to the others studied colonies from Florida (Perdereau et al. 2010b). One explanation for such a similitude is that French populations have been founded with termite individuals coming from Louisiana. However, this hypothesis remains to be tested by studying variation patterns at several informative markers. While the geographical origins of the source population(s) are currently unknown, the spatial distribution and population abundance of French populations suggest that it may have been introduced into one or more ports on the Atlantic coast such as Nantes, La Rochelle, Rochefort or Bordeaux (Dronnet et al. 2005; Perdereau et al. 2010a).

We recently showed that French populations of *R. flavipes* differ from North American populations in five important traits (i) Chemical signatures involved in social recognition within and among colonies are highly homogenous in French populations compared to those of native populations (Perdereau et al. 2010b); (ii) The level of intraspecific antagonism between nonnestmate individuals is virtually absent in French populations, whereas it can be quite important within and among native populations (Perdereau et al. 2011); (iii) All French colonies studied so far contain a large number of functional secondary reproductives (i.e. neotenics), whereas in most native populations, only 25% of colonies on average possess a few of those reproductives (Dronnet et al. 2005; Perdereau et al. 2010a); (iv) Fusion among colonies might be quite common in France, whereas such a phenomenon has rarely been characterized.
in native populations (Vargo & Husseneder 2009; Perdereau et al. 2010a; Vargo & Husseneder 2011); and (v) French colonies seem to be more populous and spatially larger than North American colonies (Dronnet et al. 2005; Vargo & Husseneder 2009; Perdereau et al. 2010a; Vargo & Husseneder 2011). The unique phenotypic characteristics of French populations have probably helped them to increase demographically and spread spatially (Perdereau et al. 2010a,b, 2011). However, the evolutionary and ecological bases of such ‘invasive’ phenotypes remain unknown. Two main hypotheses could account for the phenotypic differences observed between native and introduced populations. First, the unknown and unstudied source population(s) exhibits the same characteristics as the French populations so that the introduced populations are very similar to the source native population. Second, French populations have acquired these traits during or after their introduction, either by neutral evolutionary change or by adaptation to their newly encountered ecological conditions. To distinguish between these hypotheses, we first need to determine the source populations from which French populations originated.

The two main objectives of the present study are (i) to determine the geographical origin of the French populations; and (ii) to determine whether French populations have suffered a reduction of genetic diversity after introduction. To achieve these objectives, we built a genetic data set by sequencing a region of mtDNA (cytochrome oxidase II) and by genotyping 15 microsatellite loci in individuals of *R. flavipes* collected in numerous native and introduced areas. Genetic data were analysed using both phylogeographic and population genetic approaches.

**Materials and methods**

**Sample collection**

A total of 209 individuals from different colonies were collected within the native and introduced ranges of *R. flavipes*. In Europe, where this species is introduced, 71 samples were collected: 70 in France and one in Austria (Fig. 1a). The Austrian sample is of particular interest as it came from the Naturhistorisches Museum in Vienna and is an old sample taken from the population in the glasshouses of the Schönbrunn Castle before eradication of this historically important population (Hagen 1858). In addition, five collection points from Chile were also sampled. In the native range (USA), samples were taken from 133 collection points, with particular emphasis on the states of Louisiana and Mississippi (*n* = 63) and Florida (*n* = 35) (Fig. 1b). After collection, all samples were preserved in 90% ethanol prior to DNA extraction. Published sequences of termites originating from other introduced populations (Uruguay, Canada, Germany, California and the Bahamas) and native populations (Nebraska and Florida) were also included in the analyses (see Table S1, Supporting information for details).

**Molecular procedures**

DNA extractions were performed between 2000 and 2010. Genomic DNA was extracted from one worker per collection for all of the 209 termite colonies using standard phenol–chloroform purification (Sambrook et al. 1989). A portion of the cytochrome oxidase II (COII) gene (680 bp) of the mitochondrion was amplified using the primers B-tLys (5′-GTG TAA GAG ACC ATT ACT TA-3′) (Simon et al. 1994) and modified A-tLeu (5′-CAG ATA AGT GCA TTG GAT TT-3′) (Miyura et al. 2000). The reaction mix was in a total volume of 25 μL containing about 10 ng DNA template, 1× Qiagen Q-Solution and 1 μL of each primer. PCR amplification was performed using a Biometra T1 thermocycler with an initial denaturing step at 95 °C (15 min), followed by 35 iterations of the following cycles: 94 °C (30 s), 52 °C (1 min 30) and 72 °C (2 min) and finally an extension step at 72 °C (10 min). After purification using the NucleoSpin Extract® II kit (Macherey-Nagel), the PCR products were sequenced using the Big Dye® Terminator v3.1 cycle sequencing kit, and the sequenced products were analysed using a capillary DNA sequencer (ABI PRISM 3100). Sequences were aligned using the Clustal W algorithm (Thompson et al. 1994) within the BioEdit Sequence Alignment Editor 6.0.7 (Hall 1999) and corrected manually. The sequences were deposited in GenBank under the accession numbers shown in Table S1.

A total of 170 worker individuals (one per colony) were genotyped at 15 microsatellite loci (Rf1-3, Rf6-1, Rf15-2, Rf21-1, Rf24-2, Rf5-10, Rf11-1, RS1, RS10, RS15, RS43, RS68, RS76, RS78, RS85), which were all previously described by Vargo (2000) and by Dronnet et al. (2004). The nuclear genetic diversity was analysed for 51 individuals from France, 35 from Florida, 59 from Mississippi–Louisiana and 25 from the eastern United States (Table S1). Among these 25 individuals, nine are from two previous studies on *R. flavipes* populations from Raleigh (North Carolina) (Vargo 2003) and from Charleston (South Carolina) (Vargo et al. 2006). On the same samples, we determined the genotypes at seven additional microsatellite loci to obtain the genotype of 15 loci. Primer sequences and amplification protocols are given in Vargo (2000) and Dronnet et al. (2004).
To determine the possible origin of *R. flavipes* populations introduced into Europe, the haplotypes and their geographical distributions were compared with those of *R. flavipes* from the native range (Table 1 and Table S1). For these samples, the relationships between haplotypes were represented as a haplotype network obtained using the parsimony method and TCS version 1.13 (Clement et al. 2000). Phylogenetic analyses were performed, and all identical haplotypes were collapsed for tree construction to improve the chances of recovering fewer optimal trees and to reduce computing time. COII sequences were analysed using four different phylogenetic analysis methods: maximum parsimony (MP),
neighbour-joining (NJ), maximum-likelihood (ML) and Bayesian methods of inference (BI). The MP and NJ methods were applied using PHYLO_WIN (Galtier et al. 1996), and the ML method using Phyml (Guindon & Gascuel 2003). MrAIC was used to find an appropriate sequence evolution model for the data (Nylander 2004). BI were carried out using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) running it for 2,500,000 generations. No a priori assumptions about tree topology were made, and analyses were performed using uniform priors.

**Identification of the source population(s)**

To identify the source population(s), we also performed analyses with the Bayesian program STRUCTURE 2.2 (Falush et al. 2003) using both mitochondrial and nuclear data. This software was used to determine the population structure in native (USA) and invasive areas (France) independently, as well as to determine whether R. flavipes from France grouped predominantly with any distinct genetic cluster comprising termites from the native range. Assignments were calculated by assessing probabilities through 1 million MCMC simulations (Paetkau et al. 2004). This software uses a model-based clustering method to infer individual group structure; each individual was assigned to K genetic units, and the optimal value of K was calculated using log likelihood (Pritchard et al. 2000) and delta K (Evanno et al. 2005) methods. All simulations were performed based on the admixture model with 100,000 runs following a burn-in period of 50,000 runs.

**Assignment test**

Bayesian assignment tests implemented in the program GeneClass 2.0 (Piry et al. 2004) using microsatellite data were also performed. The Bayesian method of Rannala & Mountain (1997) and the Monte Carlo sampling algorithms were used to assign or exclude reference populations (Florida, Louisiana–Mississippi or the eastern United States) as possible origins of individuals from France. Assignment tests for the introduced R. flavipes in France were performed at both individual and population levels. We used the genetic clusters found with STRUCTURE analyses to delimit invasive populations in France. All analyses were conducted with 1 million simulations and an alpha level of 0.01 (population rejected if probability is inferior to 0.01).

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**Table 1** MtDNA haplotypes obtained in each location within introduced and native ranges

<table>
<thead>
<tr>
<th>Location</th>
<th>Haplotypes</th>
<th>n</th>
<th>n Hap.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduced range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>A, B, C, D, E, F, G, H, I, J and K</td>
<td>72</td>
<td>13</td>
</tr>
<tr>
<td>Germany</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>AV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>K</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>California</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bahamas</td>
<td>BH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South America</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>BM</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Uruguay</td>
<td>A et BM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North USA</td>
<td>A and BD</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Nebraska</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iowa</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indiana</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>BL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>West Virginia</td>
<td>W, AA and CB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delaware</td>
<td>BT, BX, BY, BZ and CA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>BT, BX, CC, CD, BU and BT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tennessee</td>
<td>AV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>BS, BT, BV, BU and BW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Carolina</td>
<td>BO, BN, BP, BQ and BR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mississippi</td>
<td>A, L, V, W, X, Y, AV and AQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>L and BJ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arkansas</td>
<td>BK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georgia</td>
<td>BI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of individual sampled (n) and number of haplotypes (n Hap.).
Genetic diversity measures and bottleneck test

Haplotype and nucleotide diversities were estimated from mitochondrial data using **ARLEQUIN V 3.0** (Excoffier et al. 2005). Microsatellite data were used to calculate the allelic richness (\(R_A\)) (El Mousadik & Petit 1996), the number of private alleles and the observed and expected heterozygosity (\(H_Q\) and \(H_E\)) for each locality using **GENEPOP** version 1.2 (Raymond & Rousset 1995) and **FSTAT** version 2.9.3.2 (Goudet 1995). These programs were also used for each population to test deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium between all pairs of loci.

Mitochondrial and microsatellite data were used separately to determine the partition of genetic variability among and within putative source populations (Florida and Mississippi–Louisiana) and introduced populations in France. The population structure was determined by analysis of molecular variance (AMOVA) by calculating F-statistics from haplotypes with 10 000 permutations using the **ARLEQUIN** software.

The deviation in observed heterozygosity from that expected under mutation–drift equilibrium was assessed using **BOTTLENECK** version 1.2.02 (Piry et al. 1999) to see whether there was any evidence of recent bottlenecks. The bottleneck tests were performed for the termites introduced into France. Tests were carried out on the genetic clusters previously determined by the **STRUCTURE** software. Because the genetic clusters do not correspond to sampling localities (geographical populations), we also performed analyses with three groups of individuals grouped according to their geographical distribution in France: North population, Middle population and South population. The infinite allele model (IAM), the stepwise mutation model (SMM) and the two-phase model (TPM) were used in the analyses. Finally, we determined whether there was a mode shift in the allele frequency distribution as an additional sign of a recent genetic bottleneck.

Results

Information of molecular markers

mtDNA (COII). In *R. flavipes*, 82 unique haplotypes were identified from the 217 aligned individual-based COII sequences. In the native range, 75 haplotypes were identified (23 from the northern United States and 52 from the southern United States) (Table 1 and Table S1). In the introduced range, 18 haplotypes were found (13 haplotypes in Europe and 5 haplotypes in North and South America outside the natural distribution of *R. flavipes*) (Table 1 and Table S1). With a frequency of 0.21, haplotype A was the most common haplotype comprising 33 individuals from France, 12 individuals from the United States (5 are from Louisiana) and one from Uruguay. Among the 11 haplotypes found in France, three haplotypes (A, H and K) were also found in several localities of the introduced range (Uruguay, Toronto and California), and five haplotypes (A, C, E, H and K) were found in different localities of the native range (Louisiana, Mississippi, Florida, Nebraska, Iowa and Indiana). In total, 50% of all individuals collected in Louisiana shared an identical haplotype with French individuals.

Microsatellites. All 15 microsatellites were polymorphic within all localities. No linkage disequilibrium was observed for any pair of loci after Bonferroni correction. A significant deviation from Hardy–Weinberg equilibrium was observed for at least one locus per locality. When all loci were pooled, a significant deviation from Hardy–Weinberg equilibrium was also detected. Numerous alleles were found in the US native range. The allelic richness (\(R_A\)) and observed heterozygosity (\(H_Q\)) were lower in France compared to Florida, Mississippi–Louisiana and the eastern United States, but only observed heterozygosity comparisons appeared significant between localities (except between Mississippi–Louisiana and France) (Table 2) (Tukey–Kramer multiple comparison test for comparison of observed heterozygosity: Florida vs. Mississippi–Louisiana \(q = 0.379, P < 0.05\); Florida vs. eastern United States, \(q = 0.21, P < 0.05\); Mississippi–Louisiana vs. eastern United States, \(q = 0.590, P < 0.05\); France vs. Mississippi–Louisiana, \(q = 3.551, P < 0.05\); France vs. Florida, \(q = 3.930, P < 0.05\); France vs. eastern United States, \(q = 4.141, P < 0.05\)). A discrepancy between expected (\(H_e\)) and observed (\(H_o\)) heterozygosities was observed with a deficiency of heterozygotes in all regions (Table 2). A deficit in observed heterozygosity is often observed within *Reticulitermes* populations. It is typically attributed to inbreeding, which can result from breeding among related neotenics within colonies and particularly within introduced populations (Dronnet et al. 2004, 2005; Perdereau et al. 2010a). It can also be caused by a Wahlund effect, which results when samples from genetically differentiated populations are combined. If an undetected structure exists in our sampling, this should not change the main conclusions of this study for two reasons. First, analyses based on microsatellites loci were performed at various spatial scales, which should reduce the Wahlund effect. Second, our conclusions were also based on analyses using both microsatellite loci and mtDNA, separately or combined.
Table 2: Estimates of genetic variation (mean ± SD) at 15 microsatellite loci from four locations (Florida, Mississippi–Louisiana, eastern United States and France)

<table>
<thead>
<tr>
<th>Sample location</th>
<th>n</th>
<th>$R_{S}$ ± SD</th>
<th>Private alleles</th>
<th>$H_{O}$ ± SD</th>
<th>$H_{E}$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>35</td>
<td>7.90 ± 4.59</td>
<td>18</td>
<td>0.55 ± 0.23 (b)</td>
<td>0.70 ± 0.25</td>
</tr>
<tr>
<td>Mississippi–Louisiana</td>
<td>59</td>
<td>9.16 ± 5.47</td>
<td>38</td>
<td>0.53 ± 0.18 (ab)</td>
<td>0.76 ± 0.16</td>
</tr>
<tr>
<td>Eastern United States</td>
<td>25</td>
<td>7.36 ± 4.12</td>
<td>18</td>
<td>0.57 ± 0.22 (b)</td>
<td>0.69 ± 0.26</td>
</tr>
<tr>
<td>France</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all samples</td>
<td>51</td>
<td>5.94 ± 3.21</td>
<td>3</td>
<td>0.36 ± 0.14 (a)</td>
<td>0.68 ± 0.18</td>
</tr>
<tr>
<td>genetic cluster 1</td>
<td>24</td>
<td>4.87 ± 2.68</td>
<td>1</td>
<td>0.41 ± 0.19</td>
<td>0.63 ± 0.13</td>
</tr>
<tr>
<td>genetic cluster 2</td>
<td>16</td>
<td>4.20 ± 2.34</td>
<td>0</td>
<td>0.30 ± 0.26</td>
<td>0.55 ± 0.24</td>
</tr>
<tr>
<td>genetic cluster 3</td>
<td>6</td>
<td>3.27 ± 1.34</td>
<td>0</td>
<td>0.39 ± 0.23</td>
<td>0.61 ± 0.24</td>
</tr>
<tr>
<td>genetic cluster 4</td>
<td>5</td>
<td>2.73 ± 1.12</td>
<td>0</td>
<td>0.23 ± 0.21</td>
<td>0.47 ± 0.28</td>
</tr>
</tbody>
</table>

Number of individuals ($n$), allelic richness ($R_S$), number of private alleles and mean observed and expected heterozygosity ($H_O$ and $H_E$). Letters inside the parentheses indicate significant differences between locations for the observed heterozygosity ($Tukey–Kramer$ multiple comparison tests).

**Identification of source populations**

The phylogenetic trees reconstructed by the four methods (MP, NJ, ML and BI) showed congruent topologies and always revealed two major clades within *R. flavipes* species (Fig. 2). One clade comprised northern and southern US samples (clade I). The second clade (clade II) is divided into two subclades: one comprised only of southern US samples (clade II.a) and the second subclade comprised both southern US samples and all the introduced samples (North and South American and European samples) (clade II.b). There were only four exceptions to this general pattern. The haplotypes obtained from three individuals collected in northern states (Nebraska, Iowa and Indiana) were nevertheless regrouped in the subclade II.b with the southern and the introduced samples. The last exception concerned the historical sample from Vienna, which was placed in a sister clade of the trees at the same level of other northern and southern US samples (Table 1 and Fig. 2).

The statistical parsimony network did not connect all COII haplotypes within the 13 mutational steps allowed under a 95% confidence limit set by TCS, excluding the northern US haplotypes. The relationship between haplotypes was therefore reconstructed by a haplotype network with 90% confidence (Fig. 2). The network revealed similar haplotype groups as those observed using phylogenetic methods. The relationship between COII haplotypes revealed one mutational step between French haplotypes and several haplotypes from the southern United States. Haplotype A diverged from four other French haplotypes (B, F, G and J) by one mutational step, and haplotype K diverged from two other French haplotypes (D and I) by two mutational steps.

The Bayesian clustering analysis using STRUCTURE gave the probable estimated values $\ln P(X/K)$ showing three genetic clusters for the native range (Fig. 3a). These clusters correspond approximately to the US sampling localities. The first genetic cluster comprised all samples from the eastern United States (West Virginia, Virginia, Delaware, North and South Carolina), one sample from Florida and one sample from Mississippi–Louisiana (Fig. 3a, yellow cluster). The second genetic cluster contained over 94% of individuals from Florida and 36% of individuals from Mississippi–Louisiana (Fig. 3a, blue cluster). The great majority of these 36% of individuals were collected from north of Lake Pontchartrain and from Mississippi. The third genetic cluster contained 63% of the individuals from Mississippi–Louisiana, predominantly samples collected next to the cities of New Orleans and Baton Rouge, and one individual from Florida (Fig. 3a, red cluster). Thus, we refer to these three clusters as the ‘Eastern US cluster’, the ‘Gulf Coast cluster’ and ‘Southern Louisiana cluster’, respectively. For the invasive range (France), STRUCTURE simulations revealed four genetic clusters (Fig. 3b) which do not correspond to geographical regions: each genetic cluster comprised samples spread out over the entire range in France. STRUCTURE simulations with France and US samples revealed that termites from France grouped predominantly with the southern Louisiana cluster (Fig. 3c). Interestingly, for $K = 2$, STRUCTURE distinguished one genetic unit with individuals from the eastern United States, and all individuals of the Gulf Coast clustered in one group, and all individuals from France and individuals of southern Louisiana into another cluster (Fig. 3d).

The program GeneClass 2.0 computes the probability that the multilocus genotype of each individual of a population belong to each other reference population. We used as reference populations clusters determined by STRUCTURE (i.e. the eastern US cluster, the Gulf Coast
cluster and the southern Louisiana cluster). At the individual level, the probabilities estimated for introduced individuals showed that 48 of 51 of them were assigned to the southern Louisiana cluster, with probability values ranging from 0.043 to 0.637. No introduced individuals were assigned to either the Gulf Coast or eastern US clusters. At the cluster level, none of the four French clusters determined by STRUCTURE were assigned to any reference population.

**Genetic diversity and bottleneck**

AMOVA analyses using mitochondrial and microsatellite data showed that the greatest total variation (87.24% and 92.69%) were explained by differences among individuals within populations (Table S2, Supporting information). The rest of the variation extended from subdivision between populations.

The quantification of the genetic diversity did not reveal any clear bottlenecks (Table S4, Supporting information). Evidence for a recent bottleneck in France was only found using the IAM model with the genetic clusters from STRUCTURE software or geographical populations. Only the population from the middle of France and genetic clusters 3 and 4 showed a shift away from the distribution of allele frequencies. There may have been too few loci used in our analysis to detect evidence of a bottleneck. Alternatively, there may have been a sufficient number of generations elapsed since the introduction to erase signs of a bottleneck.

The comparison of the introduced French and the putative source populations revealed that most alleles and haplotypes observed in France were also present in the southern Louisiana (Fig. 4 and see Table S3, Supporting information). More precisely, 83.8% of microsatellite alleles and 36.4% of mtDNA haplotypes found in France...
were also observed in the southern Louisiana. In addition, 18 microsatellite alleles and two mtDNA haplotypes were found to be unique to southern Louisiana and France. So, the number of common variants suggests that the French and southern Louisiana populations are genetically close. Comparisons of genetic variation between these populations show that variations (haplotype and nucleotide diversity, the allelic richness and mean observed heterozygosity) in the introduced *R. flavipes* termites are significantly lower than that observed in southern Louisiana (Table 3). In France, the haplotype diversity, nucleotide diversity and allelic richness represent, respectively 82%, 60% and 52% of the same parameters observed in southern Louisiana.
Discussion

Where do invasive populations of *R. flavipes* come from?

The first aim of this study was to identify the native source population(s) of the termite *R. flavipes* in its introduced ranges and more specifically in France. Phylogenetic and TCS analyses based on mtDNA revealed a strong spatial genetic structure among native populations, separating northern from southern US populations into two main genetic clusters. Most of the samples collected out of the United States, including those from France, Germany, Canada, Chile, Uruguay and the Bahamas, grouped into the southern US population cluster. Several hypotheses could account for this result. First, the main southern US population could be the source of all these introductions. A second hypothesis is that termites could have been exported from the southern US into some unknown introduced range(s) which served as a bridgehead for introduction event(s) into new areas. Such a scenario has recently been described in the fire ant (*Solenopsis invicta*) which, originating from Argentina (Caldera *et al.* 2008), has been first introduced into the southern United States and secondarily spread from this introduced range to many places of the world (Ascunce *et al.* 2011). Although testing these hypotheses could be crucial to prevent or at least mitigate future introductions of *R. flavipes* around the world, it could require the complete reconstruction of the routes of invasions of this termite species. By identifying southern US populations as the most likely putative source populations of *R. flavipes*, the present study is a first important step in the reconstruction of the invasion routes of this termite pest.

Where do French populations of *R. flavipes* come from?

All of results consistently revealed Louisiana as the likely geographical origin of the French populations. Among the five mtDNA haplotypes shared between the southern US populations and France (i.e. A, C, E, H and K), all occur in Louisiana where they are common. In comparison with Florida, which was also a major source of samples, only two samples had identical haplotypes with one of the French haplotypes. These results are also supported by the STRUCTURE and assignment test analyses, which revealed that samples collected in the southern part of Louisiana are the closest genetically to French samples (Fig. 4). This major conclusion revealing Louisiana as the most likely geographical source of French populations is supported by several experimental and historical facts. In a previous study examining chemical compounds (i.e. cuticular hydrocarbons in workers and defensive compounds in

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introduced range

France

Unpaired t-test

- Test

**Table 3** Genetic diversity comparison (mean ± SE) between the southern Louisiana and France genetic clusters inferred by STRUCTURE

<table>
<thead>
<tr>
<th></th>
<th>mtDNA</th>
<th>Microsatellites</th>
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<tbody>
<tr>
<td></td>
<td>n Hap. diversity Nuc. diversity</td>
<td>n Rs H_O</td>
</tr>
<tr>
<td>Native range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern Louisiana</td>
<td>38 0.885 ± 0.027 0.010 ± 0.005</td>
<td>38 11.468 ± 9.550 0.50 ± 0.170</td>
</tr>
<tr>
<td>Introduced range</td>
<td></td>
<td></td>
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<tr>
<td>France</td>
<td>70 0.722 ± 0.044 0.006 ± 0.003</td>
<td>51 5.940 ± 3.210 0.36 ± 0.140</td>
</tr>
<tr>
<td>Unpaired t - Test</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
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Number of analysed individuals (n), haplotype and nucleotide diversities (Hap. and Nuc.), allelic richness (R_s), and mean observed heterozygosity (H_O). Comparisons were assessed using Unpaired t-test.

soldiers) and comparing them within and among several native and introduced populations, we showed that the French chemical signatures were very similar to the signatures found in Louisiana populations (Perdereau et al. 2010b). Although these chemical compounds by themselves are not necessarily good phylogeographic markers, several previous studies in Reticulitermes termites showed that variation in the chemical signatures corroborated very well with informative genetic data used for phylogeographic analyses (Bagnères & Wicker-Thomas 2010). From an historical point of view, the main result revealing Louisiana as the putative source population also makes sense. During the late 17th and early 18th centuries, Louisiana was part of ‘La Nouvelle France’, and New Orleans was one of the main trading ports to France and Europe. Populations of R. flavipes could have been accidentally introduced to France in agricultural and forestry shipments during this time. The first wood damage caused by termites in France was described in Rochefort and La Rochelle, two major ports known for their historical importance in international trade and transport during the 17th and 18th centuries (Bobe-Moreau 1843; Ad 1853). Furthermore, the ports of Rochefort and La Rochelle, as well as Nantes and Bordeaux, are located in the region of France where R. flavipes is currently the most widespread and abundant. Altogether, our results as well as our current knowledge on the distribution of R. flavipes in France strongly support the following scenario: the first specimens of R. flavipes were introduced in one or several ports of the Atlantic coast where they established and reproduced before spreading spatially to other cities and forests. French populations of R. flavipes would have therefore evolved independently for about 200 years.

**Did French populations of R. flavipes suffer from a reduction of genetic diversity?**

Introduced populations often exhibit a lower genetic diversity compared to their native populations (Dlugosch & Parker 2008a). In agreement with these studies, French populations of R. flavipes have a significantly lower genetic diversity in comparison with their putative source population, that is, southern Louisiana. This was true for haplotype diversity, nucleotide diversity and allelic richness (Table 3). Although it has been recognized that measures of genetic diversity are particularly sensitive to sample size (Muirhead et al. 2008), our results can be considered robust given the large sample sizes of the three main populations we analysed (i.e. Florida, Mississippi–Louisiana and France). However, because our analyses failed in detecting significant bottleneck effect, it remains difficult to determine whether introduced populations passed through sequential bottlenecks or not (Table S4). Consequently, introduced populations in France seem to have suffered a moderate, but significant loss of genetic diversity during introduction and establishment.

The reduction of genetic diversity within introduced populations can be explained by a limited number of introduction events, resulting by a limited number of alleles within French populations. An alternative hypothesis would be that the genetic diversity reduction in France would be due to genetic drift following the introduction event(s) or selective pressures induced by new ecological conditions.

**Did phenotypic traits of R. flavipes evolve after its introduction into France?**

French populations of R. flavipes differ from North American populations for several phenotypic traits that are important components of social organization in Reticulitermes termites (i.e. chemical cues, antagonism behaviour, breeding system and colony fusion). However, whether such phenotypic differences between native and introduced populations resulted from evolutionary events during or after introduction remains an open question. As long as the exact source population remained unknown, we could not reasonably exclude...
that French populations inherited their specific phenotypic characteristics from the source population (Perdereau et al. 2010a,b). Our results strongly suggest that the most likely putative geographical origin of French populations is Louisiana, and more precisely southern region of Louisiana and New Orleans. Therefore, comparing phenotypic traits between Louisiana and French populations will now allow us to conclude whether this termite evolved phenotypically after its introduction or not. Unfortunately, whereas the population biology and genetics of R. flavipes have been extensively studied in many states of the United States for several phenotypic traits such as breeding system and colony fusion (reviewed in Vargo & Hüssener 2009, 2011), those phenotypes have been relatively poorly studied in populations from Louisiana except for the chemical signatures involved in interindividual recognition systems (Perdereau et al. 2010b). The chemical signatures (i.e. profiles of cuticle hydrocarbons and defensive components of soldiers) show substantial variation among Louisiana colonies, whereas the chemical signatures obtained in all French colonies collected in three different populations were virtually identical. This result highlights a possible evolutionary change in the chemical signature of French populations, which became homogenized during or after introduction. This homogenization of chemical signatures, which are well known to be nestmate recognition cues, probably explains the total lack of intraspecific aggression among the French colonies (Perdereau et al. 2011), which are able to fuse, resulting in numerous mixed colonies (Perdereau et al. 2010a). Interestingly, analogous post-introduction changes in social organization has been described in several invasive ant species such as the Argentine ant Linepithema humila (Brandt et al. 2009) or the little fire ant Wasmannia auropunctata (Errard et al. 2005). Such a similar pattern in invasive ants and in the termite R. flavipes suggests an evolutionary convergence of their social structure after introduction.

Did French populations of R. flavipes evolve by neutral forces or do they adapt?

As noted by Keller & Taylor (2008), evolution of phenotypic traits during dispersal, establishment and range expansion of introduced populations may reflect neutral phenotypic changes or adaptive evolution. A positive correlation between chemical and genetic distances in R. flavipes colonies has been demonstrated (Dronnet et al. 2006), suggesting that cuticular hydrocarbon (CHC) variation could have a significant genetic basis. Therefore, the observed homogeneity in the CHC profiles among French colonies allowing colony fusion might result from the reduction in genetic diversity in French populations following introduction, as evoked in several invasive ants (Tsutsui et al. 2003; Fournier et al. 2009; Orivel et al. 2009). Contrasting with such an explanation, however, studies on the Formosan subterranean termite, Coptotermes formosanus, showed that the invasion success of this species cannot be attributed to a breakdown in recognition or to a shift in the breeding structure of colonies in introduced populations, despite these populations exhibiting lower genetic diversity than native populations (Vargo & Hüssener 2009, 2011). Dlugosch & Parker (2008b) have suggested that, given the prevalence of successful introduced populations showing reduced genetic diversity, the disadvantages associated with such a reduction may not be so great and may have been overestimated. The genetic diversity present in the introduced populations would be sufficient to allow an adaptive response to the new selective pressures in the introduced range. Therefore, different environmental factors, as, for example, the release from intraspecific competition (Giraud et al. 2002), might also potentially be responsible for the adaptation of R. flavipes populations in France.

Conclusion

This study provides evidence that the invasive populations of R. flavipes come from the southern United States and that the most likely putative source population of French populations hails from Louisiana. Our findings represent a first step for the reconstruction of the invasion routes of R. flavipes; they also suggest that most of the French introductions occurred directly from Louisiana without intermediate ranges. The identification of the geographical origin of French populations of R. flavipes will now allow us to study in more detail the origin and maintenance of ‘invasive phenotypes’ that may evolve after introduction to a new range, such as the capacity of colonies to produce numerous functional secondary reproductives (neotenics). They will also allow investigations of whether these evolutionary changes resulted from neutral evolution or from adaptation to novel conditions and whether the acquired characteristics can help explain the invasion success of this important pest termite species.

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References


Hagen HA (1858) Monographic der Termiten, II. Linea Entomologica, 12, 1–461.


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Data accessibility
COII sequences: GenBank accession numbers JQ280538-JQ280746, see Table S1 for the sampling location.
Microsatellite data: DRYAD entry doi:10.5061/dryad.1t4n5

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

Data S1 Alignment of the 217 COII sequences.

Table S1 Origin of samples of Reticulitermes flavipes from native and introduced ranges.

Table S2 Results of analyses of molecular variance of R. flavipes based on mtDNA and microsatellite data.

Table S3 MtDNA haplotype and microsatellite allele frequencies within the Southern Louisiana and France (clusters determined by STRUCTURE).

Table S4 Probability values for bottleneck tests in using genetic clusters determined by STRUCTURE analyses and geographical populations in France.