

GLOBAL INVASION HISTORY OF THE TERMITE *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE) AS REVEALED BY THREE CLASSES OF MOLECULAR MARKERS

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Abstract The subterranean termite, *Reticulitermes flavipes*, is native to North America. In its native range, it is widely distributed in the central and eastern U.S.A. where it plays a significant ecological role as a decomposer of cellulose and is a major pest of human built structures. It has been introduced to many locations in the world where it attacks buildings, including France, Chile, Uruguay, Canada and the Bahamas. To understand the invasion history of this species, we sampled termites from throughout its native and introduced range and applied three sets of molecular genetic markers: mitochondrial DNA sequence data (cytochrome oxidase II gene), microsatellite genotypes (15 loci) and single-nucleotide polymorphisms (SNPs; >13,000 loci). All three sets of markers are consistent in pointing toward the source of the French populations being New Orleans, Louisiana, a result also supported by the similarity of the breeding structure of colonies in New Orleans and in France. In addition, the SNP markers suggested that New Orleans was the likely source of several other introduced populations including the Bahamas, Germany, one population in Canada, and possibly the Chilean population. Thus, New Orleans seems to have served as a source of introduced populations around the world on multiple occasions.

Key words Invasive species, mitochondrial DNA, microsatellite markers, single-nucleotide polymorphism

INTRODUCTION

Termites are prominent species in many tropical and temperate ecosystems where they play an important role in carbon cycling by consuming and digesting cellulose. They can also be important pests of human-built structures where they can cause significant damage. Several termite species are invasive and cause damage in their introduced ranges (Evans et al. 2013). The eastern subterranean termite, *Reticulitermes flavipes* (Kollar), is native to much of central and eastern United States where it is an important decomposer of woody material as well as a major wood-destroying insect pest. It has been introduced to many places around the world, including France, Germany, Chile, Uruguay, Canada, and the Bahamas (Evans et al. 2013). It has become a significant pest in many of these areas, especially France, Chile and Canada (Dronnet et al. 2004, Smith et al. 2006, Scaduto et al. 2012).

We have conducted recent studies to try and pinpoint the sources of invasive populations, especially those in France (Perdereau et al. 2013, Perdereau et al. 2015). In this paper, we combine results from newly published work based on microsatellite genotyping and mitochondrial DNA sequencing with unpublished results using single nucleotide polymorphisms to identify potential source populations. Our results suggest that most introduced populations originated from the area around New Orleans, Louisiana.

MATERIALS AND METHODS

Microsatellite genotyping was performed on 170 samples collected from along the East Coast and Gulf Coast of the United States and throughout the invasive range in France. A portion of the cytochrome oxidaseII gene was sequenced from 209 individuals collected from throughout the United States and introduced populations in France, Germany, Chile, Uruguay, and the Bahamas. Details of the locations of these samples are described in Perdereau et al (2013). Single nucleotide polymorphism (SNP) genotyping was carried out on 288 samples, 155 from native throughout the native range and 114 from introduced populations. Nineteen samples of *R. virginicus* Banks were included as an outgroup. Samples were subjected to double-digest restriction-associated DNA sequencing (dd RADseq) on an Illumina HiSeq 4000 using paired end chemistry. We obtained a total of 2.4 trillion reads for an average of 8.5M reads per individual, which provided about 9 X coverage. From these reads, we identified ~13,500 SNPs.

DATA ANALYSIS

Microsatellite genotype data were analyzed using the program Structure (Pritchard et al. 2000). Phylogenetic analysis of the mitochondrial DNA sequence data was performed using four different methods as described by Perdereau et al. (2013). SNPs were identified using the SOAP denovo 2 package (Luo et al. 2012). We then generated a maximum likelihood tree based on pair-wise genetic distance using R studio.

RESULTS AND DISCUSSION

Microsatellite genotypes clustered into three main groups: eastern U.S., Gulf Coast and southern Louisiana. Samples from France clustered with the southern Louisiana group, which consisted primarily of samples from New Orleans. A Bayesian phylogenetic tree and minimum spanning network both showed that samples from introduced populations shared close affinity with samples from the New Orleans area. These results are given in detail by Perdereau et al. (2013).

The maximum parsimony tree generated from the SNP data show several distinct branches; most of the introduced samples were placed on branches with samples from Louisiana. One branch contains all the French samples, one from Uruguay, eight of 12 samples from Canada and a sample from the Bahamas. The only U.S. samples in this branch came from the New Orleans area. The Chilean samples were in a branch that included a sample from Uruguay and one from the Bahamas. There was also a sample from New Orleans included in this branch. There was a branch that consisted exclusively of Louisiana samples except for one sample that was from Germany. The exceptions to the association with Louisiana samples was an individual sample from Easter Island, one from the Bahamas and four from Canada that were included in a branch with samples primarily from the North Central part of the U.S.

Results from three classes of genetic markers point to Louisiana, particularly New Orleans, as the source of introduced populations of *R. flavipes* in several countries. A study of colony breeding structures in native and introduced populations also supports a Louisiana origin for populations in France and Chile. Perdereau et al. (2015) showed that populations in France and Chile consisted of a mixture of colonies headed by multiple inbreeding neotenic (replacement) reproductives (called extended families) and colonies with multiple unrelated reproductives (called mixed families). Whereas populations in the U.S. consist mainly of colonies with simple families (single king and queen) and extended families, the population around New Orleans had colony breeding structures very similar to those in France and Chile.

The fact that many of the introduced populations are associated with different lineages from Louisiana is consistent with multiple separate introductions from Louisiana over a period spanning over 200 years into such places as Canada, France, Chile, Germany, Uruguay and the Bahamas, rather than a single introduction into some area that then served as a bridgehead for introductions into other areas.

CONCLUSIONS

The eastern subterranean termite *R. flavipes* has been introduced to at least eight locations from Europe to South America. Genetic data from three classes of genetic markers point to Louisiana, most likely New Orleans, as the source of nearly all of these infestations. These introductions began in France in 1790 with the most recent being the Bahamas in 1998, and appear to have occurred through separate introduction events. Thus, Louisiana has been the source of serial introductions into other countries. Given the number of port cities within the native range of *R. flavipes*, it suggests something unique about shipments from Louisiana that promotes the spread of this termite.

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