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EXPANSION STRATEGIES OF A NORTH AMERICAN TERMITE SPECIES INTRODUCED IN FRANCE (ISOPTERA: RHINOTERMITIDAE)

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Abstract *Reticulitermes santonensis* (Rhinotermitidae) is a subterranean termite that infests urban areas in France where it causes serious economic damages. It is largely accepted that French populations of *R. santonensis* have been founded by the introduction of a North American species, *R. flavipes*. Understanding the expansion strategies of an introduced species in a new environment is important to improve its population control. In this study, we performed a comparative analysis of the social structure of *R. santonensis* with an endemic species, *R. grassei*. All samples were collected on the island of Oléron where the two species live in sympatry. We are presenting three main results: (1) Using behaviour tests, we show that colonies of *R. santonensis* often fuse during intraspecific encounters, and seem to dominate colonies of *R. grassei*. By contrast, *R. grassei* colonies show a strong intraspecific aggressiveness and consequently do not fuse. (2) Chemical analysis of the cuticular hydrocarbons indicates that *R. santonensis* colonies possess a chemical homogeneity, whereas the chemical signature of *R. grassei* colonies presents important variations within populations. (3) Preliminary genetic analyses reveal that *R. santonensis* colonies are vast and possess more than two reproductives. These results show that the social structure of native and introduced species strongly differs, and suggest that *R. santonensis* presents some advantages over *R. grassei* to spread in its environment.
Key Words Biological invasion, *Reticulitermes*, chemical signature

INTRODUCTION

In termites, the genera *Coptotermes* and *Reticulitermes* are prominent groups of subterranean termite pests (Rhinotermitidae) that cause extensive damages in human habitats (Su and Scheffrahn, 2000). *Reticulitermes santonensis* (Feytaud) is a subterranean termite that invades urban areas in France (22 departments) and causes important damages in large cities such as Paris and Bordeaux. Its limited distribution in natural environments and its essentially urban presence suggest that *R. santonensis* is particularly well adapted to reach and spread within urban areas. It is now largely accepted that *R. santonensis* was introduced from North America two or three hundred years ago from one or several populations of *R. flavipes* (Kollar) (Bagnères et al., 1990); (Dronnet, 2004). The arrival of a new species in an environment often leads to biological modifications that allow introduced populations to become more dominant than the endemic species (Chapman and Bourke, 2001). In social insects, the successful spread of invasive species mainly depends on their social structure (Holway et al., 2002). In Hymenoptera, such modifications are well documented, while they remain relatively unknown in Isoptera. In order to limit the expansion of introduced termites, we must understand the biological modifications they may have undergone after introduction, and determine whether such modifications enhance the ability to spread in their new environment.

We have located an insular environment (Oléron island, Charente-Maritime) where *R. santonensis* lives in sympatry with a native termite species of Europe, *R. grassei* (Clément). The main goal of our project is to lead a multi-disciplinary study comparing the population biology, social structure and competition level of the two species in this particular environment. In the present study, we will focus on three characteristics of the social structure of colonies: colonial behaviour, chemical signature, and breeding system. (1) Performing several behavioural tests, we determined colonial aggressiveness at both intra- and inter-specific levels. (2) Using Gas Chromatography (GC), we determined and compared the chemical signature (hydrocarbons present on the insect cuticle) of colonies from the two termite species. The chemical signature of species

depends on the qualitative differences, and the signature of each colony depends mainly on the relative proportions of some cuticular hydrocarbons that compose the specific mixture (Howard et al., 1982; Bagnères et al., 1991; Clément and Bagnères, 1998). The chemical signature plays a major role in both intra- and inter-colony recognition. (3) By genotyping five microsatellite loci, we identified boundaries of colonies and infer their breeding system.

MATERIALS AND METHODS

Sample Collection

All colony samples were collected in a forest of pines (1898 Ha with 80% of *Pinus maritima*) of the Oléron island (Charente-Maritime, France), where *R. santonensis* and *R. grassei* live in sympatry. We collected 50 samples of *R. santonensis* and 63 samples of *R. grassei* from wood fragments or wood stumps. For each collection point, we collected twenty workers, which served for gas chromatographic (GC) analysis of cuticular hydrocarbons, before to be used for DNA analyses. Behavioural tests were performed on four colonies (two for each species) that were brought to the laboratory and maintained in their wood fragment until the experiment started.

Behavioural Tests

Behavioural tests were performed using a system of micro-nests connected to a neutral arena with a piece of wood. 50 termites from a colony of *R. santonensis* or *R. grassei* were mixed with 50 termites of another colony of one of these two species, after acclimatization of 24 hours in their respective micro-nest (2 series of intra-specific tests and 4 series of inter-specific tests). Five replicates for each experiment were carried out (500 termites were used for each series of test) and encounters between individuals from the same colony were considered as controls. Colonies of termites were differentiated by a coloration using two filter papers impregnated with Nile blue (200 ppm). During behavioural tests, repartition in the arena and number of dead individuals were counted during 24 hours. The differences between the proportions of dead termites at the end of experiments were tested using a Kruskall-Wallis or a Mann-Whitney *U test.* All the analyses were performed with the software RGui v.2.2.0 (Ihaka and Gentleman, 1996).

Gas Chromatographic Analysis of Cuticular Hydrocarbons

Cuticular hydrocarbons were extracted by rising 20 individuals in 500 µl of pentane for 5 min. Following extraction, termites were placed in 95% ethanol for later genetic analysis. After evaporation of pentane, extracted hydrocarbons were redissolved in 200 µl pentane and 10 µl of 10^{-7} g/ml of *n*-eicosane were used as internal standard. Samples were analyzed on a Delsi 300 GC with flame ionization detector (FID) equipped with a fused silica capillary column CP Sil 5 (WCOT) Chrompack (ID 0.25 mm × 25 m). Injection mode was splitless (15 sec) and the carrier gas was Helium. Temperature programming was from 70°C to 150°C at 30°C/min and 150°C (isothermal 5 min) to 320°C at 5°C/min. Compound identification was based on previous analyses of the cuticular hydrocarbons by coupled GC-MS (Bagnères et al., 1990 and 1991). To allow analysis of the 113 hydrocarbon profiles, areas of peaks were integrated by GC software then relative proportions of each peak were calculated. To visualize chemical relationships among every point collected, relative proportions were subjected to principal component analysis (PCA) using the STATGRAPHICS software v.4.0 (StatPoint, Inc., Herndon, VA, USA) and UNIWIN PLUS v.3.0 (SIGMA PLUS, Levallois-Perret, France).

Microsatellite Analysis

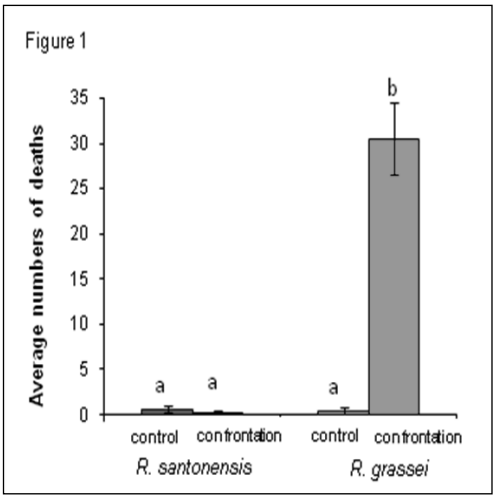
Genomic DNA was extracted from twenty individuals from 9 collection points belonging to *R. santonensis* species using standard phenol-chloroform purification (Sambrook et al., 1989). Five microsatellite markers previously developed for *R. flavipes* (Vargo, 2000) or for *R. santonensis* (Dronnet et al., 2004) were used: Rf11-1, Rf1-3, RS15, RS10 and RS85. Polymerase chain reaction (PCR) amplifications were performed as described in Dronnet et al. (2004). PCR products were separated by electrophoresis on 6% polyacrylamide gels run on a LI-COR 4000 sequencer. Analysis of gels was performed using GeneProfiler™ (Scanalytics, Inc.). Allele size was determined by comparison with standards. *R. grassei* sequencing analyses results are in

process. We first examined whether or not the collection points belonged to the same colony. We compared the genotypic frequencies between all pairs of collection points by test differentiation using the program GENEPOP on the Web (Raymond and Rousset, 1995). A Bonferroni correction was applied to account for multiple comparisons. Once colony boundaries were defined, we investigated the breeding system of each colony using the same computer program. We classified colonies as simple or extended families by comparing the observed genotypes of workers within colonies with the genotypes expected in these types of societies by using standard criteria for termites (Bulmer et al., 2001); (Vargo, 2000); (DeHeer and Vargo, 2004) : 1) Colonies are classified as 'simple families' when workers had genotypes consistent with being the direct offspring of one pair of reproductives, and when the observed frequencies of the genotypes did not differ significantly from those expected under Mendelian segregation of alleles from two parents. 2) Or colonies are considered as 'extended' families when the genotype distributions within colonies were not consistent with being produced by a single pair of reproductives (e.g. more than four genotypes at a locus or three or more homozygote genotypes), or genotype frequencies deviating significantly from those expected in simple families.

RESULTS AND DISCUSSION

Behavioural Tests

After intra-specific pairings, the number of dead termites was scored for each replicate (Figure 1). In *R. santonensis*, the number of dead individuals from inter-colony and intra-colony confrontations was not significantly different (Kruskal-Wallis test, $p > 0.05$). On the contrary, we noted an agonistic behaviour between *R. grassei* colonies. We counted on average 31.5 dead individuals from inter-colony confrontations, which differed significantly from the number of dead individuals counted from intra-colony confrontations (Kruskal-Wallis test, $p < 0.001$). Examination of inter-specific pairings provided significant differences in the number of dead individuals between the two termite species : In *R. grassei*, the number of dead individuals was greater than in *R. santonensis* (31.5 ± 2.87 dead vs 17.25 ± 1.72, p-value < 0.001 Mann-Whitney test) (Figure 2). A social insect colony is defined as "open" when members tolerate conspecific individuals from another colony, and "closed" when members of this colony act aggressively toward members of neighbouring colonies (Wallace, 1963). Previous studies have shown that *R. santonensis* colonies are all open whereas in *R. grassei* and other European species, colonies are open or closed according to the season and the location of the colonies (Clément, 1986); (Bagnères et al., 1990). Ours results corroborate these previous studies and show that, even collected within a same area, neighbouring colonies of *R. grassei* and *R. santonensis* are mutually aggressive. Furthermore, our results revealed for the fisrt time that *R. santonensis* systematically dominate *R. grassei* in inter-species confrontations, suggesting that *R. santonensis* is a better competitor than *R. grassei*.



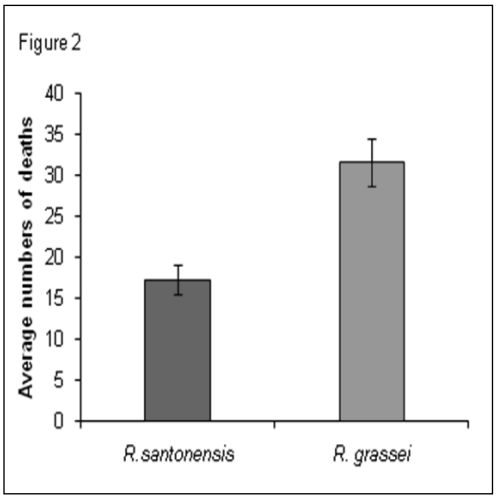


Figure 1. Average number (± DS) of dead individuals of *R. santonensis* and *R. grassei* after intra-specific confrontations (a, b: significantly different modalities; $p < 0{,}001$, Kruskal-Wallis test). **Figure 2.** Average number (± DS) of dead individuals of *R. santonensis* and *R. grassei* after inter-specific confrontations (17.25 ± 1.72 vs. 31.5 ± 2.87, p-value < 0.001, Mann-Whitney test)

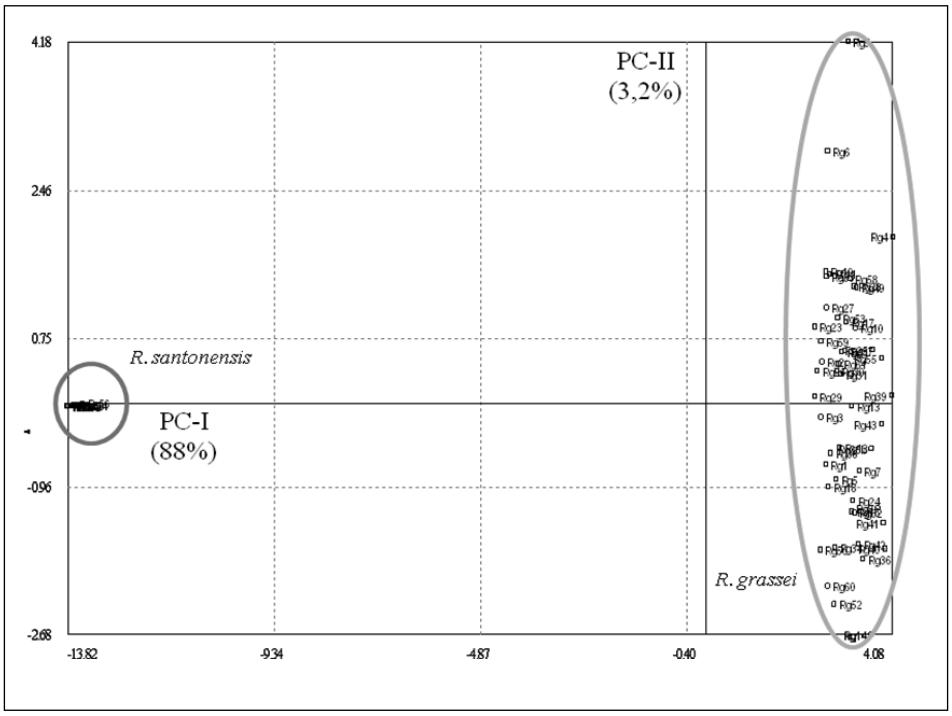


Figure 3. Principal component analysis (PCA) of the cuticular hydrocarbons profiles of *R. santonensis* (left) and *R. grassei* (right).

Gas Chromatographic Analysis of Cuticular Hydrocarbons

Principal component analysis between collection points revealed that the two first axes (PC-I and PC-II) accounted for 91.2% of the variation (Figure 3). The first axis, PC-I, explains the major part of the chemical variation (88%), separating the two species (Figure 3). The second axis accounts for the chemical variation between collection points for each species. In the multivariate plot, the distinction between collection points was relatively clear for *R. grassei*. In many social insects, colony members are able to recognize nestmates and distinguish them from other conspecific colonies or members of other species. The strong chemical variability of *R. grassei* seems to be one of the factors explaining the intraspecific aggression occurring in this species. Conversely, the chemical homogeneity of *R. santonensis* would permit tolerance between conspecific colonies, which could allow colonial fusion.

Microsatellite Analysis

All microsatellite loci were polymorphic in *R. santonensis*, with 2 to 5 alleles per locus (Table 1). We identified three colonies. The first colony regrouped two collection points extended about 40 m^2. The second colony contained three collection points, representing a total surface of about 3500 m^2. The third colony was composed by the last four collection points, and may extend more than 11 000 m^2. These three colonies were included in the analysis of the breeding system. Results showed that all colonies possessed more than 4 genotypes for at least one locus (Table 1), clearly indicating that each of the colonies possesses more than two functional reproductives. Our results are in accordance with previous studies on the breeding system of *R. santonensis* (Dronnet, 2004; Dronnet et al., 2005), which showed that all colonies are extended families (i.e., colonies lead by multiple related reproductives which are the descendants of the original founding pair) (DeHeer and Vargo, 2004). However, more loci would need to be genotyped and analysed.

Table 1. Number total of alleles, number of alleles, number of genotypes and expected heterozygosity (He) per colony (C) at each locus in the *R. santonensis* investigated.

Locus	Number total of alleles	Number of alleles per colony			Number of genotypes			He		
		C1	C2	C3	C1	C2	C3	C1	C 2	C3
Rf 1-3	5	3	3	3	5	5	4	0.58	0.44	0.36
Rf 11-1	2	1	2	2	1	3	3	0	0.51	0.52
Rs 10	4	2	4	4	2	8	9	0.24	0.66	0.71
Rs 15	4	3	3	4	4	4	8	0.26	0.48	0.47
Rs 85	4	2	3	3	2	3	3	0.22	0.21	0.17
Mean (±SD)		2.2 ±0.87	3 ±0.71	3.2 ±0.87	**Overall**			0.26	0.46	0.45

Together, these preliminary results demonstrate that the two species, *R. santonensis* and *R. grassei*, present important differences in their social structure, and suggest that *R. santonensis* colonies might be more efficient than those of *R. grassei* for invading their environment. The capacity of *R. santonensis* for fusion and its dominance over *R. grassei* during inter-specific encounters may cause strong local dynamics and favour its territorial expansion. Such peaceful behaviour between conspecific colonies of *R. santonensis* has already been observed in invasive ants, like the Argentine ant (*Linepithema humile*) and the Fire ant (*Solenopsis invicta*), which form vast and dominant colonies (Tsutsui et al., 2000); (Holway et al., 2002). Furthermore, a lack of available discriminative cues on the cuticle, reflected by chemical similarity among *R. santonensis* colonies, prohibits an effective differentiation. This phenomenon is a key for the invasive success of introduced species. The introduced populations of the invasive ant *Wasmannia auropunctata* show a chemical uniformity allowing them to become ecologically dominant (Errard et al., 2005). Cuticular hydrocarbon variations may depend on both genetic and environmental factors (Dronnet et al., 2006); (Foitzik et al., 2007). The two species, *R. grassei* and *R. santonensis*, living in the same ecosystem and having the same diet (pine wood) are probably subjected to similar environmental conditions. Thus, the loss of chemical variation from *R. santonensis* may be explained by the loss of genetic variation that occurred during the introduction event(s) (Dronnet et al., 2006). Such a phenomenon has been studied on invasive ants in which authors have concluded that the loss of genetic diversity associated with founder effects impedes nestmate recognition (Holway et al., 1998); (Tsutsui and Suarez, 2003).

The presence of secondary reproductives has often been cited as a main condition for colony foundation by budding, whereby workers and replacement reproductives (neotenics) initiate new colonies close to their natal nest (Thorne et al., 1999). Previous studies revealed that 100% of the French colonies of *R. santonensis* (synonym to *R. flavipes*) possess functional neotenics whereas only 25% of the North American colonies possess such reproductives. The reason(s) for such modification in the introduced populations of *R. santonensis* is unknown. However, it is believed that the capability of the French colonies to produce neotenics favours their local dynamics, and increases their chance to reach and spread within urban areas.

In conclusion, these studies demonstrate that two species living in the same ecosystem and subjected to the same environmental factors possess different expansion strategies. Furthermore, the invasive characteristics of *R. santonensis*: its high number of secondary reproductives, capacity to merge, superiority in inter-specific confrontations, and its chemical homogeneity, are trump cards facilitating its territorial expansion in comparison with the endemic species. In addition, the same characteristics would allow *R. santonensis* to reach and develop effectively in human habitats.

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