

**COLONY STUDIES of the SUBTERRANEAN TERMITE,  
*RETICULITERMES SANTONENSIS* FEYTAUD (ISOPTERA:  
RHINOTERMITIDAE), in the CITY of PARIS**

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**Abstract** Subterranean termites of the genus *Reticulitermes* play a critical role in decomposition of dead wood in natural ecosystems. But in urban areas they are pests that cause serious damage. *Reticulitermes santonensis* Feytaud is found in distinct areas in Paris (France). More than half the neighborhoods (arrondissements) have this pest in buildings as well as in trees along streets. Our understanding of how colonies and populations are organized is fundamental to controlling these pests in a targeted and efficient manner. Preliminary results are presented regarding the colony genetic structure of *R. santonensis* in Paris, using the technique of molecular marker genotyping. Inferences of colony social organization based on the genotype of workers suggest that all colonies were headed by numerous replacement reproductives inbred for at least three generations. Future work with more colonies over both smaller and bigger spatial scales is necessary to advance our knowledge of *R. santonensis* colony and population structure in urban areas. Its distribution in natural habitats is very small and localized (western France), whereas distant cities are infested in France and in Germany (Hamburg). We will also try to understand how such a species can spread and adapt to urban environments. Another area of study will concern the origin of this species in Europe.

**Key Words** *Reticulitermes* termites colony structure inbreeding relatedness urban infestation

## INTRODUCTION

Subterranean termites of the genus *Reticulitermes* are useful components of natural woodland ecosystems in temperate zones. *Reticulitermes santonensis* Feytaud has a "natural" distribution very localized in southwestern France (Clément et al., 2001). But this species causes serious damage in urban areas, especially in Paris where it has been present for a few decades. The presence of *R. santonensis* in Paris was first reported in 1945 in a private garden in the 16<sup>th</sup> arrondissement (ardt). But it was officially noticed by C. Noirot in 1953 near the original sighting. This termite has spread and currently infests more than half of the arrondissements in the city. Buildings are mainly infested but trees along streets have been observed to be attacked by termites since 1993. Between 1994 and 1995, treatment using baits considerably decreased the presence of termites attacking live trees in a Parisian street (Lohou et al., 1997). However, a better understanding of how colonies and populations are organized is necessary to control these pests in a targeted and efficient manner. Indeed, *Reticulitermes* spp. have a cryptic life and foraging habits that make their social structure difficult to determine. Moreover, *R. santonensis* societies are known to be open, i.e., do not show inter-colonial aggression, so that they may form a vast underground network (Clément, 1986; Clément and Lange, 1984; Clément et al., 1988).

The city of Paris is collaborating with the CNRS (Centre National de la Recherche Scientifique) to support a Ph.D. project to investigate the colony and population genetic structure

of *R. santonensis*. This fundamental research is being conducted with the aim of helping to manage infestations of this species. Using polymorphic genetic markers we now provide a preliminary study of colony structure of *R. santonensis*.

## MATERIALS and METHODS

### Sample Collection

In Paris (France), workers of *R. santonensis* were collected from seven collection points (Figure 1): three in the cellars of buildings of the 8<sup>th</sup> ardt (8A, 8B, 8C); four others on alignment trees while termites foraged under galleries: one in the 17<sup>th</sup> ardt (17A) and three in the 13<sup>th</sup> ardt (13A, 13B, and 13C). Two other urban collections were made, in Créteil CRE7 (Val-de-Marne) under a wood footbridge, and in Joué-les-Tours JO1 (Indre-et-Loire) in a stump, these towns located 12 and 210 km from the center of Paris, respectively. A collection point from the forest of Olonnes OL (Vendée) was also collected in order to have a sampling point far from Paris (400 km).

### Microsatellite Analysis

Genomic DNA was extracted from twenty individuals for each collection point using DNeasy Tissue Kit (Qiagen). Five microsatellite markers (tri-nucleotide) previously developed for *R. flavipes* (Vargo, 2000) were used here: Rf1-3, Rf11-1, Rf11-2, Rf15-2, and Rf6-1. All loci were amplified as described in Vargo (2000). For electrophoresis, the PCR amplified samples were run on 6.5% polyacrylamide gels using a LiCor DNA Sequencer. Analysis of gels was performed using GeneProfiler™ (Scanalytics, Inc.). Allele size was determined by comparison with standards.

### Data Analysis

Relatedness values were calculated using the program Relatedness 5.00 (Queller and Goodnight, 1989). All groups were weighted equally, and the standard error estimates of intracolony relatedness obtained by jackknifing over loci. *F*-statistics were calculated using Genetic Data Analysis version 1.1 (Lewis and Zaykin, 2000).

## RESULTS

### Distribution Of Termites In Paris

A map of infested buildings in Paris is shown in Figure 1. There is a high diversity in the degree of infestation among attacked districts, counted by the number of infested buildings. The 17<sup>th</sup> ardt is the most attacked district with 358 infested buildings reported since 1966. The area of termite infestation is patchy: infested areas are separated by others currently known to be unattacked. Regarding trees in the urban environment, no trees in parks are attacked by termites, unlike trees along streets. The distribution of streets having infested trees overlaps with that of infested buildings (not shown in Figure 1).

### Colony Organization

All microsatellite loci were polymorphic, with 4 to 10 alleles per locus (Table 1). From the ten collection sites, termites collected at the three sampling points of the 8<sup>th</sup> ardt were considered to be part of a single colony (renamed 8ABC), because of a match between worker alleles and genotypes, and a low  $F_{CT}$  value, which measured genetic differentiation between these three collection points (not reported here). In the same way, 13A and 13B were grouped together as 13AB. Therefore, seven colonies could be genetically analyzed. Among them, two (8ABC and 17A) had more than four alleles (respectively 5 and 6 alleles) at the most polymorphic locus Rf6-1. More-

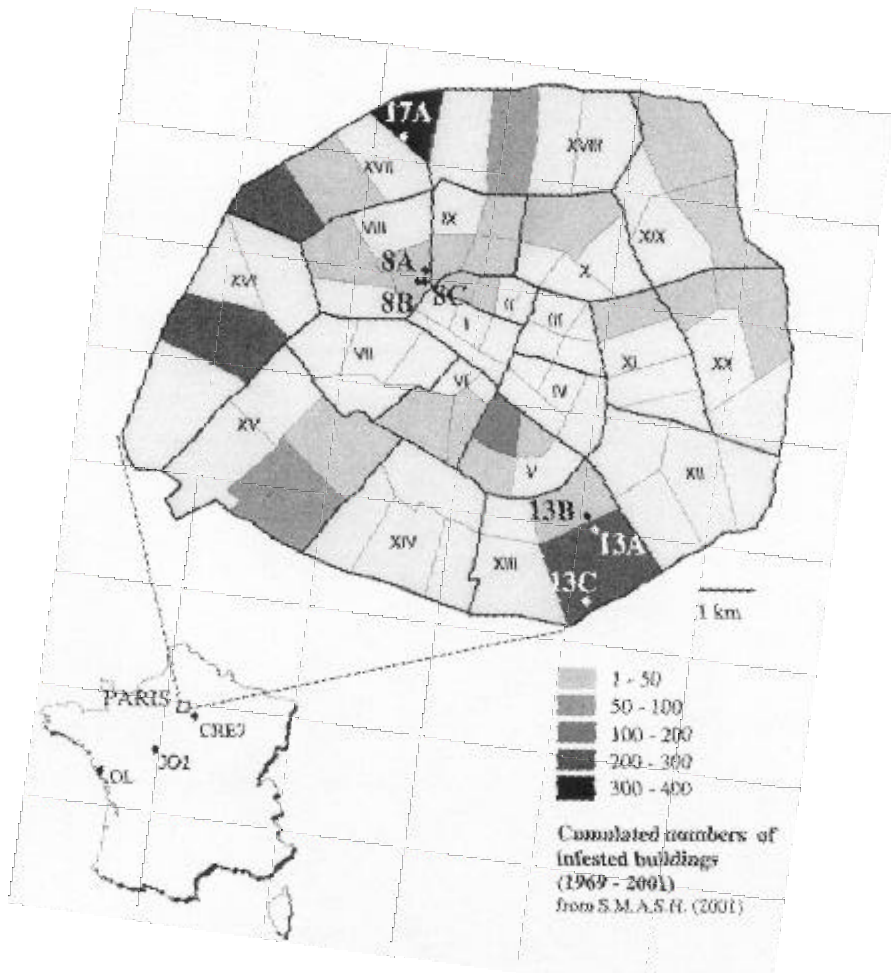


Figure 1. Map of infested buildings in the city of Paris (France). Roman numerals indicate the different arrondissements of Paris. Collection points of *R. santonensis* termites in Paris and in France are shown by diamond-shaped points.

Table 1. Number of alleles at each locus and the frequency of the most common allele in the *R. santonensis* colonies investigated

Locus	Number of alleles	Frequency of the most common allele
Rf1-3	4	0.58
Rf11-1	5	0.46
Rf11-2	4	0.56
Rf15-2	4	0.42
Rf6-1	10	0.23

over, the analysis of genotypes for each colony showed that all colonies were non-Mendelian for one or more loci. An example of such a deviation from expected simple Mendelian families was given for 17A and JO1 (Table 2). Therefore, all colonies investigated were inconsistent with being headed by a single pair of primary reproductives, but five of the seven were consistent with being inbred families descended from simple families.

The coefficient of relatedness among nestmate workers showed high variability (Table 3) among the different colonies: high coefficients of relatedness (0.68 to 0.76) in colonies 8ABC, JO1 and OL, and low values in the other colonies (0.16 to 0.38). Low values of relatedness were associated with high numbers of loci with more than four genotypes per locus (non-Mendelian genotypes).

Measures of  $F$ -statistics and average relatedness were compared with values derived from computer simulations by Bulmer et al. (2001) (Table 4).  $F_{IT}$  is the inbreeding coefficient of the individual relative to the total population,  $F_{CT}$  is the inbreeding coefficient of the colony relative to the total population,  $F_{IC}$  is the inbreeding coefficient of the individual relative to the colony.  $F_{IC}$  were very close to zero, which is indicative of a high number of replacement reproductives. Indeed, neotenic workers were often found during sample collections. The average relatedness value was below 0.5.  $F$ -statistics and  $r$  values corresponded to colonies with breeding among numerous neotenic workers inbred for three generations. Two possible breeding patterns are consistent with the observed values: either only one queen and one unrelated king founded the colony (Table 4, case Biv or Bv) with subsequent production of neotenic workers and cycles of inbreeding, or there was more

Table 2. Microsatellite genotypes of *R. santonensis* workers from colonies 17A and JO1 at all five loci

Locus	Genotypes	
	Colony 17A	Colony JO1
Rf1-3	6 aa, 10 ab, 4 bb	20 bb
Rf11-1	12 aa, 8 ab	1 aa, 5 ac, 1 ad, 4 cc, 6 cd, 3 dd
Rf11-2	2 aa, 3 ab, 4 ac, 6 bb, 3 bc, 2 cc	3 bb, 10 bc, 7 cc
Rf15-2	8 aa, 7 ab, 4 bb	20 cc
Rf6-1	1 ae, 3 bc, 3 be, 6 ce, 1 de, 5 ee, 1 ef	1 bb, 3 bg, 5 bh, 1 gg, 4 gh, 6hh

Table 3. Intracolony relatedness values calculated for each *R. santonensis*

Colony	Relatedness values	Standard errors <sup>a</sup>
8ABC	0.68	0.11
13AB	0.32	0.07
13C	0.16	0.12
17A	0.38	0.32
CRE7	0.23	0.12
JO1	0.68	0.40
OL	0.76	0.18

<sup>a</sup> The Relatedness program (Queller and Goodnight, 1989) calculated standard errors using the jackknife resampling method over loci (Sokal and Rohlf, 1981).

Table 4. Empirical measures of inbreeding (F-statistics), average relatedness (r), and their associated 95% confidence intervals in *R. santonensis*

	$F_{IT}$	$F_{CT}$	$F_{IC}$	r
Actual empirical results				
(i) all collection points (n=10)	0.28 (0.21-0.36)	0.30 (0.26-0.33)	-0.04 (-0.11-0.06)	0.48 (0.40-0.48)
(ii) all colonies (n=7)	0.30 (0.22-0.38)	0.32 (0.28-0.35)	-0.04 (-0.11-0.06)	0.49 (0.42-0.56)
(iii) only Parisian colonies and CRE7 (n=5)	0.23 (0.13-0.39)	0.26 (0.20-0.33)	-0.04 (-0.12-0.12)	0.38 (0.029-0.47)
Simulated Breeding Systems <sup>a</sup>				
(A) Colonies headed by monogamous reproductive pairs				
	0.00	0.25	-0.33	0.50
(B) Colonies with breeding among neotenic				
(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) $N_f = N_m = 10, X=1$	0.33	0.34	-0.01	0.51
(iv) $N_f = N_m = 10, X=3$	0.37	0.38	-0.02	0.56
(v) $N_f = 200, N_m = 100, X=3$	0.33	0.34	-0.00	0.50
(C) A mix of colonies headed by primary reproductives (35%) and neotenic				
$N_f = N_m = 10, X=3$	0.28	0.35	-0.12	0.55
(D) Pleometrosis				
(i) headed by 2 queens and one king	0.00	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
(E) Inbreeding, then mixing of unrelated workers at sampling sites $N_f = N_m = 10, X=3, p=0.8$				
	0.37	0.25	0.15	0.36

<sup>a</sup> The expected values were simulated for breeding systems in *R. flavipes* by Bulmer et al. (2001).  $N_f$  and  $N_m$  represent the number of replacement females and males, respectively, produced per generation. X is the number of generations of production of replacement reproductives within a colony.

than one queen and one unrelated king, an association called pleometrosis, with subsequent production of neotenic and cycles of inbreeding (Table 4, case Dii or Diii). This latter case is suggested for some colonies by the presence of more than four alleles at a locus as seen in colonies 8ABC and 17A.

## DISCUSSION

Our genetic analyses showed evidence for inbreeding with high numbers of replacement reproductives in *R. santonensis* colonies in the urban environment. Reproduction of *Reticulitermes* colonies may occur by two modes: swarming of winged primary reproductives and independent colony founding, and budding in which colonies fragment into daughter colonies (Thorne et al., 1999). Because neotenic reproductives do not fly, they remain in the nest and inbreed. Our results are consistent with most colonies being founded by primary reproductives followed by cycles of inbreeding among neotenic.

This latter breeding strategy contrasted with the one exhibited in natural areas by the same species. Based on enzymatic analyses, Clément (1981) showed that only 57% of colonies of *R. santonensis* from western forests in France indicated much inbreeding, whereas the remainder were headed by a pair of sexual individuals. Therefore the urban environment may lead *R. santonensis* to reproduce more by neotenic than by outbred reproductives only. Compared with urban areas, forests can be considered as a more continuous and homogeneous environment where chances to found new colonies by swarming are greater. By contrast, budding would be more appropriate in towns. This tendency to form more expansive colonies in towns was supported by a triple mark-release-recapture experiment performed on a tree-lined Parisian street (Paulmier et al., 1997), where termites from a colony foraged over an area of 1080 m<sup>2</sup>. Moreover, the non-aggressive intercolonial interactions were generally reported in *R. santonensis*, so that colonies were considered as open (Clément, 1986; Bagnères et al., 1990). Such a characteristic could facilitate colony fusion between different colonies, and therefore the expansion of termites. More samples will be necessary to test this hypothesis in the urban environment.

At the level of population, a trend in genetic differentiation among colonies was suggested by preliminary results (not reported here). Differentiation between the northwestern colonies (8<sup>th</sup> and 17<sup>th</sup> ards) and the southeastern colonies (13<sup>th</sup> ardt and Créteil) appeared with the current data. On the other hand, both Rf11-1 and Rf6-1, had presented two alleles only found in colony JO1. This colony appeared to differ from all other colonies. It will be necessary to confirm these tendencies.

*R. santonensis* distribution in natural areas is very small and localized (western France), whereas distant cities are infested in France and in Germany (Hamburg) (Clément et al., 2001). *R. santonensis* was considered to be associated with habitats affected by human activity (Clément, 1986; Vieau, 2001). *R. santonensis* and *R. grassei* can be found in sympatric zones in Southwestern France, even in the same town, such as Bordeaux. But in urban areas, differences in breeding systems between the two species may explain the more invasive spreading of *R. santonensis*. Future work with more colonies over both smaller and bigger spatial scales will enable us to advance our knowledge of the behavior and adaptation of *R. santonensis* in urban areas. In addition, we will attempt to identify the origin of this species (Bagnères et al., 1990; Vieau, 2001).

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