

# Unicoloniality in *Reticulitermes urbis*: a novel feature in a potentially invasive termite species

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## Abstract

Social insects are among the world's most successful species at invading of new habitats. A good example of this invasive ability is *Reticulitermes* (Rhinotermitidae), a prominent group of subterranean termites. As a result of human intervention, i.e. transportation and creation of urban heat islands, *Reticulitermes* have been able to invade and thrive in cities located in areas where the natural habitat is normally too cold for colonization. They commonly infest man-made structures where they can cause extensive damage.

This study was designed to evaluate the invasiveness of *Reticulitermes urbis* that was probably introduced in France from the Balkans. Invasive potential was assessed on the basis of features typical to invasive social insects, i.e. unicoloniality, low intraspecific aggression, high level of polygyny and colony reproduction by budding. The opportunity to study establishment and spreading processes arose after extensive sampling of an imported *Reticulitermes urbis* population was performed over the entire city of Domène, France (Rhône-Alpes region).

For the first time, genetic analysis showed that the termites belonged to a single 'genetic entity' forming a vast colony covering about seven hectares. The colony was structured as an extended family with separate reproductive centres. We speculate that termites were introduced in a single location from which they gradually budded throughout the old town. Based on the absence of aggression among different nests within the colony, we defined this 'genetic entity' as a supercolony.

**Keywords:** termites, insect introduction, urban invasion, social organization, unicolonial population, supercolony, limited dispersal, inbreeding

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## Introduction

Social insects, i.e. ants, bees, wasps (Hymenoptera) and termites (Isoptera), are among the world's most successful species at invading new habitats (Moller, 1996). Much of the ecological success of social insects has been attributed to task partitioning with some individuals being specialized in reproduction and others in rearing offspring (Sundstrom & Boomsma, 2001). In many settings, imported social insect species such as termites have severely disrupted the ecological system and/or caused significant economic damage (Williams, 1994). Although termites are useful recyclers of organic compounds (i.e. cellulose) in their natural habitat, they are highly destructive pests that feed on wooden components of buildings in urban areas.

The genera *Coptotermes* and *Reticulitermes* (Rhinotermitidae) are prominent groups of subterranean termite pests that commonly cause extensive damage by infesting man-made structures (Gay, 1969; Su & Scheffrahn, 2000). *Reticulitermes* species nest in the soil and feed on dead wood and other cellulosic materials in or near the soil surface (Thorne *et al.*, 1999). As a result of their large colony size, strong ability to penetrate a variety of materials and voracious appetite for a wide range of wood types, *Reticulitermes* species have had a severe economic impact wherever they have appeared. In the United States alone, damage and control of subterranean termites cost several billion dollars per year (Haverty *et al.*, 1991; Forschler & Jenkins, 1999; Su & Scheffrahn, 2000).

Control of *Reticulitermes* species is a major problem due to their complex life history. Because subterranean termites (Rhinotermitidae) are cryptic insects, their breeding structure is still poorly understood. According to the classic description, a single pair of primary (winged) reproductives initiates a colony with a simple family structure (one queen, one king and their offspring). Primary reproductives are frequently supplemented or replaced by secondary reproductives that develop either from brachypterous nymphs or from workers (reviewed in Lainé & Wright, 2003). Since these larvae instars, called neotenics, cannot fly, they must mate in the nest. Colonies with secondary reproductives can constitute extended families that continue to grow and expand. Isolated colony fragments can become autonomous and create new colonies (Thorne *et al.*, 1999). This flexibility in caste differentiation probably facilitates colonization and invasion of new environments by subterranean termites.

As a result of human intervention, i.e. transportation and creation of urban heat islands, *Reticulitermes* species have been able to thrive in cities located in temperate climates where the natural forest habitat is normally too cold. This explains the successful invasion of Paris (France), Rouen (France) or Hamburg (Germany) by *R. santonensis*, of Toronto (Canada) by *R. flavipes* and of Devon (Great Britain) by *R. grassei* (Clément *et al.*, 2001; Lainé, 2002).

Termite infestation in Domène, a small town near Grenoble, France (Isère Department, Rhône-Alpes region) was first reported 35 years ago. Initial surveys undertaken for the city by Bagnères *et al.* (unpublished report) showed that 90% of the old town centre was infested by a new *Reticulitermes* phenotype that was tentatively designated *R. sp.* The same phenotype was subsequently observed by Bagnères and coll. in Bagnacavallo (Bologna) in northern Italy (Uva, 2002; Uva *et al.*, 2004). Since these early reports, numerous colonies with the same phenotype have been

described in Italy, southeast France, Greece and Croatia (Marini & Mantovani, 2002; Uva, 2002; Bagnères *et al.*, 2003; Luchetti *et al.*, 2004; Uva *et al.*, 2004; Luchetti *et al.*, 2007; Leniaud *et al.*, in prep); and *R. sp.* was elevated to the status of a species named *Reticulitermes urbis* based on morphological, chemical and molecular markers (Bagnères *et al.*, 2003). Phylogenetic studies indicate that *R. urbis* is close to *R. balkanensis* that certainly originated from the Balkan peninsula (Marini & Mantovani, 2002; Uva, 2002; Bagnères *et al.*, 2003; Luchetti *et al.*, 2004; Uva *et al.*, 2004). Its occurrence exclusively in urban areas of France and Italy is probably a consequence of human intervention (Bagnères & Luchetti, personal communication).

Although numerous termite species have successfully thrived in non-native areas, only a few, e.g. *Coptotermes formosanus*, can be considered as truly invasive, i.e. exogenous species widely established and locally dominant with severe economic and/or ecological impact (Husseneder *et al.*, 2005). In Europe, *R. santonensis* that may have been imported from North American populations of *R. flavipes* (Bagnères *et al.*, 1990; Clément *et al.*, 2001; Marini & Mantovani, 2002; Austin *et al.*, 2005; Dronnet *et al.*, 2005) can be considered as invasive. Dronnet *et al.* (2005) reported several features of *R. santonensis* colony structure that may have facilitated this invasion, i.e. expensive colony, reduced intraspecific aggression, enhanced polygyny and colony expansion by budding. Similar features have been observed in invasive ant species (Holway *et al.*, 2002; Tsutsui & Suarez, 2003; Corin *et al.*, 2007).

The opportunity for this two-phased study, designed to gain insight into termite establishment and spreading processes, arose when a large sampling program was undertaken on the imported population of *R. urbis* in the city of Domène in the Alps. In the first phase of study, we used behavioural tests to estimate the level of aggression between collection sites. Using the triple mark-recapture technique, we estimated the density of the population and its ability to spread in the ground. In the second phase of study, we investigated colony and population structure and characterized the breeding system using ten microsatellite markers. Genetic markers are powerful tools for inferring colony breeding structure in social insects (Thorne *et al.*, 1999; Ross, 2001), and there have been an increasing number of genetic studies on the colony social organization of termites (Atkinson & Adams, 1997; Thompson & Hebert, 1998; Goodisman & Crozier, 2002), especially subterranean termites (Clément, 1981; Bulmer *et al.*, 2001; Clément *et al.*, 2001; Husseneder & Grace, 2001; Vargo, 2003a, b; Vargo *et al.*, 2003; DeHeer & Vargo, 2004; Dronnet *et al.*, 2005). Presuming that *R. urbis* was an invasive termite species, we were particularly interested in determining if it exhibited the invasive features described by other authors.

## Material and methods

### *Specimen collection and description of the location*

Termite samples were collected from 34 sites using artificial feeding stations placed throughout the city of Domène, France. Twenty-nine collection sites were used for genetic study. Behavioural tests were carried out in four of these collection sites and in five other sites ( $n=9$ ) (fig. 1). The infested area was estimated to cover 6.8 hectares and appeared to comprise a single population. This population

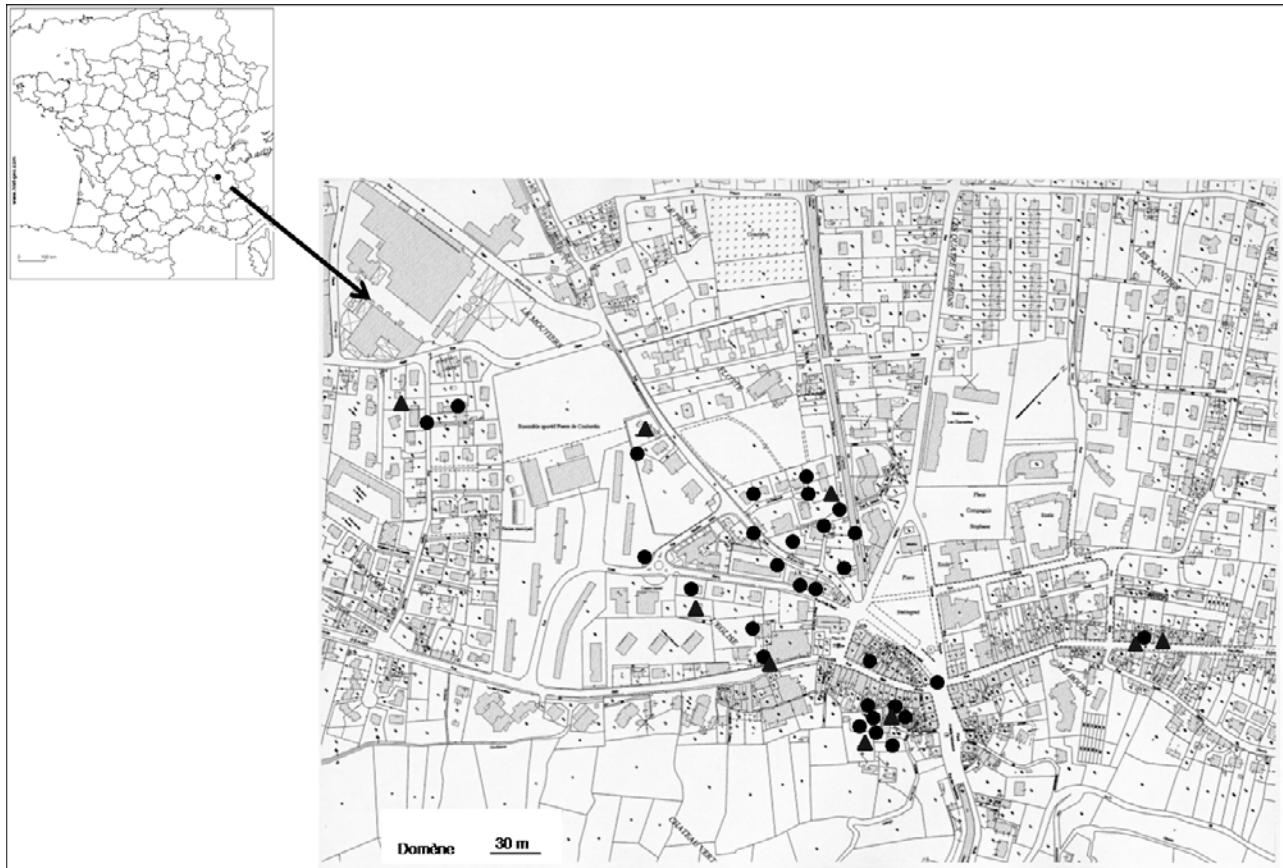


Fig. 1. Map of Domène showing location of the collection points for samples used for (●) genetic analysis and (▲) behavioural tests.

was so well delimited that we could not find infested tree stumps or houses outside the area. Recently, a site infested by *R. urbis* was found in Grenoble (6 km east of Domène), but this point could not be added to the present data set due to insufficient sampling.

#### *Behavioural tests*

To take into account possible seasonal variations in aggressive behaviour as observed by Clément (1978), samples were collected after swarming in the summer of 1999 and during swarming in the spring of 2000. Given the limited number of termites, a total of 20 series of five assays confronting 20 workers from paired nine collection points were carried out. The Ag index between two collection points was calculated according to the following formula,  $Ag = (M + (m/2)) \times 2.5$ , where  $M$  is the average number of dead workers and  $m$  is the average number of injured workers. As previously described (Clément, 1986), Ag index can range from 0 (no aggression) to 100 (all termites dead after 24 h).

#### *Estimation of population density*

Population density was estimated using the triple mark-recapture (TMR) technique that has been used previously for non-destructive study of animal populations (Lebreton

*et al.*, 1993) and insect colonies (Easey & Holt, 1989; Su & Scheffrahn, 1993; Su *et al.*, 1993; Paulmier *et al.*, 1997; Evans *et al.*, 1998). Estimation was carried out in a 150 m<sup>2</sup> area near the old town centre in Domène (fig. 2). This area was chosen because it was the most heavily damaged, thus suggesting that the termite population was old and stable. A network of 20 wooden stakes and TMR stations consisting of a perforated Falcon tube (length = 11 cm,  $\phi$  = 3 cm) with a piece of wet cardboard inside were installed.

The TMR technique involves three cycles of mark and recaptures (Lindberg & Rexstad, 2002). During the first cycle, termites collected from TMR stations were counted, stained with Nile Blue for four days (Bagnères, 1989) and finally released through the same station from which they were collected. During the next two cycles, termites collected from stations containing marked termites were counted and stained again before being released through the same stations. At the end of the three cycles over a five-week period, the size ( $N$ ) of the foraging population and associated standard deviation (SD) were estimated according to the formula of Begon (1979).

#### *Molecular genetics*

A total of 20 individuals (mainly workers) from each of the 29 collection points were placed in 95% ethanol and stored at 4°C. Total DNA from the whole body was obtained





Table 1. Mean intraspecific aggression (Ag) index values measured between *R. urbis* workers collected at different points in Domène after swarming in summer and before swarming in springtime. Five replicates of 20 workers per collection point were confronted (200 workers per series) and Ag index was measured according to the method of Clément (1986). Ag index can range from 0 (no aggression) to 100 (all termites dead after 24 h). The column headings correspond to represent property codes used in the city's official register.

Collection point	D044	D528	D552	D576	E178	E219	E221	E353	E370	
D044			0.5					1.8		<b>Springtime 2000</b> Mean Ag = 1.8 SD = 1.1
D528										
D552	0.8	4.3		0.8	1.0			2.5	1.0	
D576					1.5				3.5	
E178								3.3	3.3	
E219			2.5	5.0						
E221										
E353	0.8		4.0		2.0		3.0		1.3	
E370								2.5		
<b>Summer 1999</b> Mean Ag = 2.8 SD = 1.5										

by comparing the genotypes observed in workers within the colonies with the genotypes expected for each family type using standard criteria for termites (Bulmer *et al.*, 2001; Goodisman & Crozier, 2002; Vargo, 2003a,b; Vargo *et al.*, 2003; DeHeer & Vargo, 2004). Colonies were considered as simple families if worker genotypes were consistent with those expected for a single pair of parents and if the ratios of the observed genotypes did not differ significantly from the expected Mendelian ratios as determined by G-testing. An overall G-value was obtained for each colony by summing all the locus-specific G-values. Colonies were considered as extended families if worker genotypes were not consistent with a single pair of reproductives (e.g. one or more loci with five or more genotypic classes or three classes of homozygotes) or if worker genotypes were consistent with the presence of a single pair of reproductives but the observed frequencies of the genotypes deviated significantly from the values expected for simple families ( $p < 0.05$ , G-test).

*Analysis of isolation by distance*

Isolation by distance was calculated in a two-step analysis. First, the pairwise  $F_{ST}$  for all collection point pairs was computed. Second, the correlation coefficient between  $F_{ST}/(1 - F_{ST})$  and  $\ln$  of geographic distances (Slatkin, 1993; Rousset, 1997) was calculated using the Mantel test in the web-based GENEPOP (Raymond & Rousset, 1995). The correlation between geographic and genetic distances was calculated according to the Spearman's rank correlation (i.e. rho), since Spearman's rank correlation coefficient does not require the assumption that the relationship between the variables is linear. The significance of the correlation (rho) was assessed with permutation test. A significance threshold of  $p < 0.05$  was used to reject the null hypothesis (i.e. 'geographic and genetic distances are not significantly correlated').

**Results**

*Behavioural testing*

The mean Ag index was 1.8 (SD = 1.1) before swarming in the spring and was 2.8 (SD = 1.5) after swarming in the summer (table 1). Termites from one station would accept

termites from another station. No significant difference was observed between collection times (*t*-test  $p$ -value = 0.13).

*Triple mark-recapture technique*

Based on TMR, the foraging population was about 330,000 individuals per 150 m<sup>2</sup>, with an average density of 2200 termites m<sup>-2</sup> (SD = 250). Density was probably underestimated since sampling from the feeding stations may have missed a large part of the population. Details of TMR analysis are shown in table 2 and fig. 3. Tunnelling capacity was also high. Termites were able to cover up to 50 m within one month. This was the maximum distance between two interconnected collection points.

*Genetic analysis*

There were no more than four alleles per microsatellite locus and only one COII haplotype in the entire city of Domène (based on analysis of the 29 collection points). This extremely low genetic diversity suggests that reproductives were closely related.

G-test of differentiation between paired collections points showed that none of the samples from the 29 stations in Domène were genetically differentiated, i.e. displayed significantly different microsatellite genotypic frequencies ( $p > 0.00017$ ). This finding indicates that all workers belonged to the same colony, i.e. a unicolonial population covering an area of almost seven hectares.

More than four genotypes were observed in the population. This finding is inconsistent with the presence of a single pair of reproductives and suggests that the colony formed an extended family with several reproductives. This assumption is further supported by the fact that each collection point usually had a range of genotypes (i.e. more than four genotypes or non-mendelian distribution of the genotypes frequencies) consistent with an extended family.

*Isolation by distance*

Analysis showed significant isolation by distance between collection points in Domène. A significant positive correlation was observed between the geographical distance and genetic differentiation of sample pairs ( $F_{st}$ ) (fig. 4;  $n = 29$ ,

Table 2. Estimation of termite population density using the triple mark-recapture technique. The estimated population size in Domène, France was 329,000 individuals over a total surface of 150 m<sup>2</sup>. The average density was 2200 termites m<sup>-2</sup> (SD=250). The letters in the trap column refer to fig. 3. The numbers in parenthesis indicate the number of marked termites in each cycle in relation to the total number of termites (workers and/or soldiers) collected.

Cycle	Trap	Release (Cycle <sup>-1</sup> )	Number of individuals marked			Total		
			Workers	Soldiers	Workers	Soldiers		
1	A B C	3461	1046	(11)	6	(0)	1052	(11)
2	A B C F	931	820	(8)	5	(2)	825	(10)
3	A B C F H	958	1386	(13)	16	(0)	1402	(13)
Total		5350	3252	(32)	27	(2)	3279	(34)

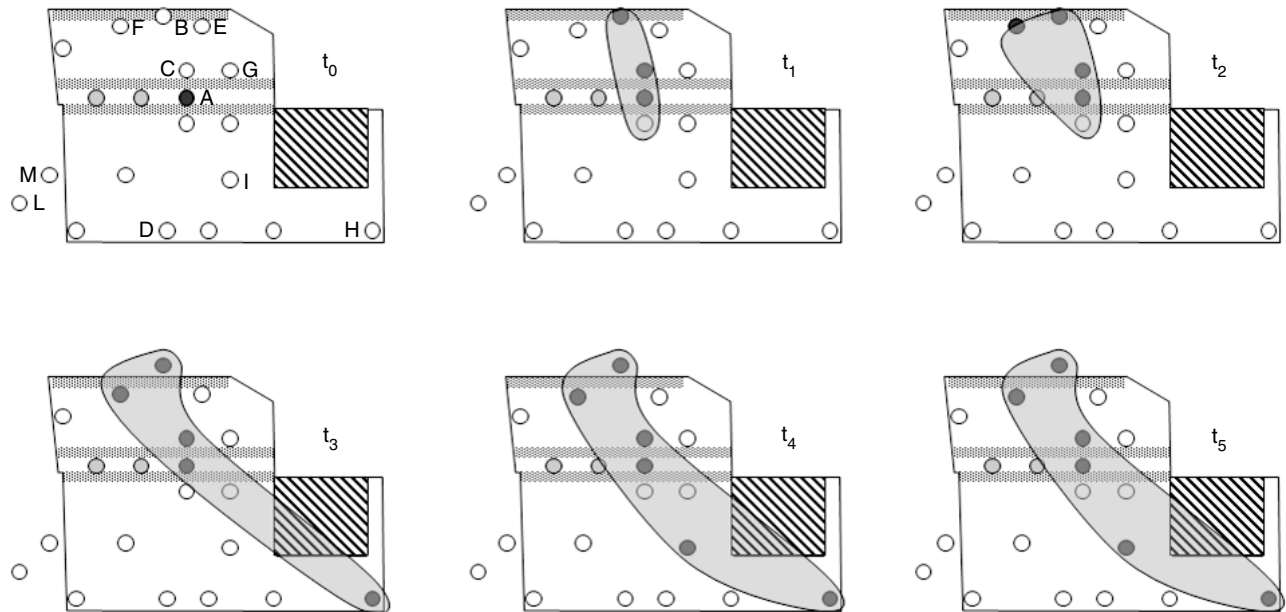


Fig. 3. Termite progression during the measurement of population density using the triple mark-recapture technique (summer 1999). A 'termite trap' contains unmarked termites, a 'blue termite trap' contains marked termites and a 'termites free trap' contains no termites. The time between t<sub>0</sub> and t<sub>5</sub> is five weeks. Letters correspond to the trap name (□, wall; ▨, built; ○, termite trap; ●, blue termite trap; ○, termites free trap).

$r=0.31$ , Mantel test  $p<0.0001$ ). This finding indicates high viscosity and nonrandom mating in the colony. These features are characteristic of dispersion by budding.

### Discussion

*Reticulitermes urbis* probably originated from the Balkans (Marini & Mantovani, 2002; Uva, 2002; Bagnères *et al.*, 2003; Luchetti *et al.*, 2004; Uva *et al.*, 2004). Except for one forest located not far from an urban area in southern France (Sophia Antipolis) (Uva *et al.*, 2004), *R. urbis* has never been identified in a natural setting in France or Italy. The hypothesis that *R. urbis* was introduced into western Europe by human activity (importation of infested materials) is further supported by recent genetic data showing lower genetic diversity and fewer COII haplotypes in France and Italy than in Balkan areas (Leniaud *et al.*, in prep.).

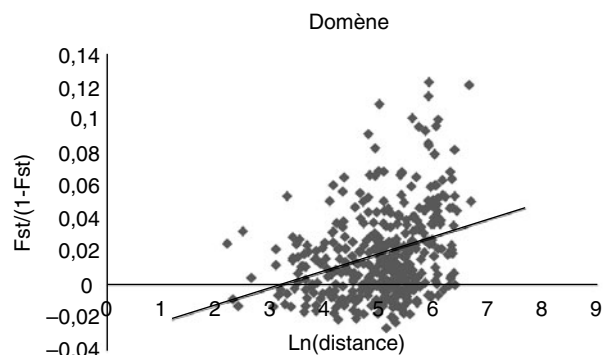


Fig. 4. Results of isolation-by-distance analysis in Domène. The relationship between pairwise estimates of  $F_{ST}/(1-F_{ST})$  and geographical distance (m) is shown for paired collection points ( $r=0.31$ , Mantel test  $p$ -value  $<0.0001$ ).

Table 3. Termite population density measured in various locations and species using the triple mark-recapture technique.

Location	Species	Density (termites m <sup>-2</sup> )	Reference
Domène (Isère, France)	<i>Reticulitermes urbis</i>	2200	This study (summer 1999)
Bagnacavallo (Italy)	<i>R. urbis</i>	1100	Ferrari <i>et al.</i> , 1998
Bourges (Cher, France)	<i>R. santonensis</i>	300	Paulmier <i>et al.</i> , 1997
Paris (Ile de France, France)	<i>R. santonensis</i>	1200	Paulmier <i>et al.</i> , 1997

In this study, colony or population size was estimated only by the indirect TMR technique. Excavation is, in general, not practical since the nests are spread out over such an extensive foraging area that it would be too destructive (Husseneder *et al.*, 2005). The termite density in Domène was about 2200 termites m<sup>-2</sup>. Previous studies using the TMR for estimation of population density of the same species and of *R. santonensis* (table 3: Paulmier *et al.*, 1997; Ferrari *et al.*, 1998) indicated much lower densities. Physical conditions, mediated by abiotic factors, have been shown to increase the abundance of pest insects in North America (Swetnam & Lynch, 1993; Williams & Liebholt, 1995). The foraging population was estimated at about 329,000 individuals, but this estimation could be an under-estimation since individuals may be distributed unequally within the entire foraging area (Evans *et al.*, 1999). The dispersion capacity of the termites in Domène was about 50 m per month. This dispersion rate is in agreement with previous studies, i.e. 45–50 m for the same species in Bagnacavallo (Ferrari *et al.*, 1998), 40 m for *R. santonensis* in Paris (Paulmier *et al.*, 1997) and 7–70 m for *R. flavipes* in Florida (Su *et al.*, 1993).

Termites from different collection points in Domène showed no aggressive behaviour. Individuals tolerated each other even when collection points were mixed. Based on this finding, it can be assumed that interchange between individuals was free. Similar findings were reported by Ferrari *et al.* (1998) for *R. urbis* in Bagnacavallo (Italy). Absence of aggression may promote the colonization process by allowing exchange of food and individuals. Non-aggressive behaviour may also facilitate coping with stressful conditions, e.g. isolation of a small group of individuals and disturbances caused by human intervention. As in several studies on the Argentine ant, reduced intraspecific aggression and absence of competitors could contribute to the elevated population densities directly responsible for the widespread success of invaders (Holway *et al.*, 1998) and in particular of *R. urbis*.

In this study, genetic analysis showed that the termites in Domène belonged to a single genetic entity. The low genetic diversity, no more than four microsatellite alleles (compared with the higher diversity in Balkan areas: Leniaud *et al.*, in prep) and the single COII haplotype in the population, suggests that this extended family colony may have descended from closely related females or even from a single pair of reproductives. Based on these findings, it seems probable that the presence of *R. urbis* in Domène resulted from a limited number of introduction events. Indeed, it may even be possible that the invasion originated from a single location. These findings would mean that a small number of termites were sufficient to infest the entire city in less than 50 years. This is consistent with one previous

study (Pichon *et al.*, 2007), showing that only 30 *R. santonensis* workers were sufficient to produce a new generation via formation of neotenics. In a recent study, Zayed *et al.* (2007) showed that the solitary bee, *Lasioglossum leucozonium*, invaded North America through the introduction of a very small number of propagules. *R. santonensis*, which was probably imported from the USA, is known to create vast colonies in introduced ranges and exhibits lower genetic diversity in introduced ranges than in the original area of distribution (Dronnet *et al.*, 2005). This is, however, the first study documenting a unicolonial population in termites, i.e. a single entity covering 6.8 ha that developed in a previously unoccupied range less than 40 years after introduction.

Although we have no genetic information from the native range of *R. urbis*, we could speculate that, as observed in Argentine ants (Tsutsui *et al.*, 2000; Giraud *et al.*, 2002; Corin *et al.*, 2007), a population genetic bottleneck during or subsequent to the introduction may have promoted the formation of this unicolonial population. Tsutsui *et al.* (2000), Holtzer *et al.* (2006) and Thomas *et al.* (2006) defined a supercolony as a group of nests exhibiting no intraspecific aggression. Given the absence of aggression, the nests in Domène fit this definition, despite the possible existence of several 'physical units', i.e. groups of foragers and/or members of satellite nests isolated from the rest of the colony. These units develop neotenics from existing workers and sometimes become independent nests. Field analyses and TMR in Domène confirmed the difficulty of defining nest limits. This study found significant isolation by distance among the collection points within the extensive colony in Domène. This finding indicates that the workers were not genetically homogeneous and suggests that there are spatially separated reproductive centres among which exchange of termites is limited. In a recent study on *Coptotermes formosanus*, Husseneder *et al.* (2005) found some genetic differentiation in a large extended family, suggesting the presence of separate reproductive centres. Isolation by distance would be expected if dispersal over the spatial scale was relatively limited as a result of the short range of mating flights and/or frequent colony reproduction by budding. Termites in the supercolony in Domène may have been introduced in a single location from which they gradually propagated all over the old town by budding and/or human dispersion. Colony budding is an advantageous process that may provide several adaptive benefits. By producing nearby colony buds, colony budding can lead to polydomous colonies (having multiple nest sites), increased kin recognition and cooperation, fusion of colony boundaries and ultimately formation of supercolonies (Abbott, 2006).

Recent studies on invasive social insect populations have demonstrated that introduction is accompanied or followed



quickly by dramatic changes in behaviour, social organization, reproductive biology and population genetics. The best documented models for such changes remain the invasive ants (*Linepithema humile*, *Wasmannia auropunctata*, *Solenopsis invicta* etc.), but similar findings have been observed in other social insects including *Vespula germanica*, *Bombus terrestris* and *Apis mellifera scutellata* (reviewed in Moller, 1996; Chapman & Bourke, 2001; Schneider *et al.*, 2003). In a recent genetic study on *R. santonensis*, Dronnet *et al.* (2005) found several similarities with *L. humile* that forms non-aggressive populations in its introduced range. In North America, *R. flavipes* colonies are small and well delimited with variable levels of aggression (Bulmer & Traniello, 2002; DeHeer & Vargo, 2004), and Vargo (2003a,b) found that *R. flavipes* colonies exhibit a variable and flexible breeding system with simple and extended families headed by a small number of inbred neotenic. In contrast, in France, Dronnet *et al.* (2005) found that 100% of the *R. santonensis* colonies were spatially extensive, headed by numerous neotenic with separate reproductive centres and restricted movement of neotenic and workers.

A termite colony structure involving extended families with many neotenic reproductives and non-aggression would facilitate colonization in urban areas. Human activity could also enhance the spread of this type of colony. These findings have important implications for control of *R. urbis* using termiticides transmitted by contact or food exchange (trophallaxis). Acceptance of a contaminated termite in another nest would promote propagation of the toxin. However, treating only a small area of a large supercolony would probably be insufficient, since termites can move freely from one nest to another and, thus, can quickly recolonize treated parcels. Based on this assumption, a wide-scale termite eradication program using baiting techniques was initiated in the old-town centre of Domène in 2004 and in the rest of the city in 2005. In 2007, 95% of the 6.8 contaminated hectares were already free of termites. The unicoloniality of the Domène population was certainly a factor in these spectacular results, since this single introduced population probably had similar sensitivity to the IgR toxin.

Since there are currently no studies available on the social organization in *R. urbis* colonies in their native setting, we cannot know whether introduction events affected colony structure in this species and played any role in its invasive success. Further investigation on the behaviour and genetic structure of populations in the native range will be needed. Comparative studies with other introduced populations of this species will also be helpful to identify common features in colony breeding structure and to determine what role they may play in invasion success.

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