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Phylogenetic Analyses of Two Mitochondrial Genes and One Nuclear Intron Region Illuminate European Subterranean Termite (Isoptera: Rhinotermitidae) Gene Flow, Taxonomy, and Introduction Dynamics

Tracie M. Jenkins,*[,1] Rob E. Dean,† Robert Verkerk,‡ and Brian T. Forschler§

*Department of Entomology, Redding Building, and †Plant Genetic Resources Conservation Unit, Research Station, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797; ‡Department of Biology, Imperial College of Science, Technology & Medicine, Silwood Park, Ascot, Berkshire SL5 7PY, United Kingdom; and §Department of Entomology, BioScience Building, University of Georgia, Athens, Georgia 30602

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Phylogenetic analyses of multiple DNA sequences were conducted to elucidate gene flow, evolutionary patterns, taxonomy, and the dynamics of two accidental introductions: *Reticulitermes lucifugus grassei* into Devon, United Kingdom and *R. flavipes* into Europe. Two mitochondrial DNA genes totaling 1495 bp and a 380-bp ribosomal intergenic transcribed spacer were sequenced. Neighbor-joining and parsimony analyses revealed that multiple female lineages of *R. lucifugus grassei* were introduced into Devon possibly from southwestern France, where the species was indigenous. The taxonomic status of the European *R. santonensis* as a species separate from the North American *R. flavipes* has been questioned since it was described in 1924. Phylogenetic analyses revealed a close genetic relationship between *R. flavipes* from the United States and *R. santonensis* from France. These analyses, coupled with morphological and chemotaxonomic data, provide strong support for *R. santonensis* and *R. flavipes* being the same species. They also suggested that *R. santonensis* infestations likely resulted from *R. flavipes* being introduced into Europe.

Key Words: **mtDNA; ITS2; gene flow; subterranean termites; *Reticulitermes*.**

INTRODUCTION

Subterranean termites of the genus *Reticulitermes* (Rhinotermitidae) are ecologically and economically important social insects that appear to be environmentally adaptive across a wide and varied geography (Weesner, 1965, 1970). These diplo-diploid insects have a cryptobiotic social structure that makes them difficult to study by conventional field techniques. Thus, gene flow and population structure, phylogenetic patterns and taxonomy, and the dynamics of exotic introductions are poorly understood (Forschler and Jenkins, 2000).

Variation among populations "makes more sense" when compared and interpreted through a phylogenetic framework (Avise, 1994, p. 4). Thus, phylogenetic analyses of DNA sequence markers were recently employed to identify *Reticulitermes* to species and to track intra- and interspecific gene flow (Jenkins *et al.*, 1998, 1999, 2000). Phylogeographic studies of *Reticulitermes* intraspecific matrilineal lines revealed that there was no geographic clustering (Jenkins *et al.*, 1998). Samples from populations across four soil provinces in Georgia, United States and from a population in Toronto, Canada, where the insect was introduced in 1929 (Weesner, 1970), showed no geographic differentiation. The life history of these termites, therefore, appeared to promote dispersal and an interconnection through gene flow. Furthermore, this tendency toward dispersal and adaptation likely facilitated the introduction of *Reticulitermes* into nonindigenous areas in North American (Weesner, 1970) and Europe (Becker, 1981).

The purpose of this study was to verify the taxonomy and evaluate the gene flow and introduction dynamics of two subterranean termite species, *Reticulitermes lucifugus grassei* and *R. flavipes*, by phylogenetic analyses of two mitochondrial genes and one intron region.

R. lucifugus grassei was accidentally brought into the United Kingdom via Saunton, north Devon (R. H. J. Verkerk, personal communication) 30 years ago from southwest France or northern Spain, where it was indigenous (Clément, 1981). The species was verified by A. G. Bagnéres and F. Vieau from cuticular hydrocarbon phenotypes and morphological charac-

[1] To whom correspondence should be addressed. Fax: (770) 229-3323. E-mail: tjenkin@gaes.griffin.peachnet.edu.

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TABLE 1

Sampling Information

Sample[a] code	Species[b]	Sequences/sample size[c] COI	COII	ITS2	Year of collection	Location of collection	Location of culture
UK1	Rlg	5	5	2	1998	Saunton, UK[d]	BRE, UK[e]
UK2	Rlg	1	3	2	1999	Saunton, UK	BRE, UK
UK3	Rlg	1	3	1	1999	Saunton, UK	BRE, UK
UK4	Rlg	1	3	3	1999	Saunton, UK	BRE, UK
UK5	Rlg	1	3	2	1999	Saunton, UK	BRE, UK
UK6	Rlg	1	3	3	1999	Saunton, UK	BRE, UK
F1	Rs	1	3	2	*ca.* 1970[f]	Bordeaux, France	BRE, UK
F2	Rs	1	3	2	*ca.* 1970	Bordeaux, France	BRE, UK
F3	Rs	1	3	2	*ca.* 1970	Bordeaux, France	BRE, UK
F4	Rlg	1	3	2	1998	Royan, France	BRE, UK
UK7	Rlg	1	4	1	1995	Saunton, UK	BRE, UK
UK8	Rlg	1	4	2	1998[g]	Saunton, UK	BRE, UK
F5	Rs	2	9	4	1978	Île d' Olérons, France	UGA[h]
USA1	Rf	1	3	3	1996	Georgia, USA	UGA
USA2	Rf	1	3	1	1995	Georgia, USA	UGA
USA3	Rh	1	3	2	1996	Georgia, USA	UGA
USA4	Rh	1	3	2	1995	Georgia, USA	UGA
USA5	Rv	1	3	2	1996	Georgia, USA	UGA
USA6	Cf	1	8	2	1999	Georgia, USA	UGA
UGA7	Cf	1	2	2	1996	Georgia, USA	UGA
UGA8	Cf	2	2	2	1999	Georgia, USA	UGA
Totals		27	76	44			

[a] Sample code refers to the country in which samples were collected, e.g., UK, United Kingdom; F, France; USA, United States of America.
[b] Rlg, *R. lucifugus grassei*; Rs, *R. santonensis*; Rf, *R. flavipes*; Rh, *R. hageni*; Rv, *R. viginicus*; Cf, *Coptotermes formasanus*; USA1, Rf Strain Rf; USA2, Rf Strain BWS; USA3, Rh strain Andy 1; USA4, RH strain Dukes; USA5, Rv strain 109; USA6, Cf strain Char; USA7, Cf strain CF; USA8, Cf strain WB.
[c] Refers to the number of individual DNA samples. Each sample was sequenced in both directions.
[d] All samples were taken from areas of activity within a *ca.* 2400-m^2 infested, semirural zone in Saunton, North Devon.
[e] BRE, Centre for Timber Technology & Construction, Building Research Laboratory Ltd., Garston, UK.
[f] Exact year and place of collection unknown.
[g] Mixture of UK 1–8.
[h] UGA, cultures maintained in Forschler Laboratory, Department of Entomology, University of Georgia, Athens, GA.

ters, respectively (A. G. Bagnéres, C.N.R.S., Marseille, France, personal communication; Bagnères *et al.*, 1991). *R. flavipes* was introduced into Europe from North America many times (Becker 1970, 1981). This species was morphologically indistinguishable from the European *R. santonensis* (Feytaud, 1924) and the chemotaxonomic phenotypes of the two species were similar (Bagnères *et al.*, 1990).

Two mtDNA genes, cytochrome oxidase subunit I (COI) and subunit II (COII), totaling 1495 bp, and a 380-bp ribosomal internal transcribe spacer (ITS2) region, known to be intra- and interspecifically variable in insects (Campbell *et al.*, 1993; Cornel *et al.*, 1996), were sequenced from individual termites. Nine *R. lucifugus grassei* populations in Devon, United Kingdom, three cultures of *R. santonensis* from Bordeaux, France, one *R. santonensis* population from Île de' Olèrons, France, and populations of three North American *Reticulitermes* species, including the widespread eastern subterranean termite *R. flavipes*, were sampled.

Phenetic and cladistic analyses produced the same phylogeny for each of the three sequence data sets. The Devon infestation formed a monophyletic clade with some sequence differentiation among populations for COI and COII genes. The North American *R. flavipes* formed a monophyletic clade with all *R. santonensis* from France for COI, COII, and ITS2 data sets in rooted and unrooted phylogenetic trees. The gene flow and introduction dynamics of *R. lucifugus grassei* and *R. flavipes* is discussed in light of these results.

MATERIALS AND METHODS

Taxa Sampled

Specimens were identified to species by use of morphometric characters (Snyder, 1954; Weesner, 1965; Nutting, 1990; Scheffrahn and Su, 1994) and, except for the outgroup species, *Coptotermes formosanus*, were *Reticulitermes* spp. Samples submitted for sequencing were numbered blinds. Species identification was clarified after sequence analyses. Table 1 delineates all sampling information. Abdomens from worker

termites preserved in 70 or 100% EtOH were removed prior to DNA analysis according to Liu and Beckenbach (1992) and Jenkins *et al.* (1999).

Data Collection

The polymerase chain reaction (PCR) was used to amplify two regions of the mitochondrial genome and a ribosomal intron: partial COI, 810 bp; complete COII, 685 bp; and the entire ITS2 region, 380 bp. Primer pairs, which were used to PCR amplify and to prime forward and reverse sequencing reactions, include COI forward and reverse primers CIJ2195 (TTGATTYTTTGGTCAYCCWGAAGT) and TL2N3014 (ATGGCAGATTAGTGCAATGG), COII forward and reverse primers TL2J3037 (TCYAWTGCAYTAATCTGCCATATTA) and TKN3785 (GTTAAGAGACCAGT ACTTG), and ITS2 forward primer ITS2F anchored in 5.8s rDNA (TGTGAACTGCA GGACACAT) and reverse primer ITS2R anchored in 28s rDNA (GACTACCCCCTAA ATTTAAGC). COI and COII primers are according to Liu and Beckenbach (1992) with modifications for more universal insect application (Simon *et al.*, 1994). Insect mtDNA was confirmed according to Jenkins *et al.* (1998).

PCR amplification was performed in a standard 25- or 50-μl reaction with from 10 to 50 ng of total genomic DNA, 1 pmol of each primer, 2.0 mM $MgCl_2$, 1.6 mM dNTPs, and 0.06 U/μl *Taq* DNA polymerase. Amplification was accomplished in a Perkin–Elmer Gene Amp PCR system 9600 (Applied Biosystems, Foster City, CA). It included a precycle denaturation at 94°C for 2 min, a postcycle extension at 72°C for 7 min, and 25 cycles of a standard three-step PCR (50°C annealing) for the COI and COII genes and the ITS2 region. Fragments were treated with exonuclease I (10 U/μl) and shrimp alkaline phosphatase (1 U/μl). They were then incubated in a Perkin–Elmer GeneAmp PCR System 9600 first at 37°C for 15 min and then at 80°C for 15 min to remove primers and to inactivate dNTPs left over from the PCR, respectively (Jenkins *et al.*, 1999). DNA (10–20 ng/100 bp PCR product) sequencing reaction was performed with the Dye-Terminator Cycle Sequencing Kit (PE Applied Biosystems) in a GeneAmp 9600 PCR system. Electrophoresis was then accomplished on a 6% polyacrylamide gel. Reactions were fractionated and initial base assignments were made by the ABI 373A automated DNA sequencer system (Applied Biosystems).

Data Analysis

Sequencer 3.1.1 software (Gene Codes Corp., Ann Arbor, MI) was used to edit individual electropherograms and form contigs. There were no differences in DNA sequences among individuals collected from the same site (Table 1). Consensus sequence was assigned for the COI and COII genes and ITS2 intron region from specific collection sites (Table 1) with Sequencer 3.1.1 software. The initial 5′ base position of both the partial COI and the complete COII sequence was correlated with the COI and COII sequence of three species in GenBank. The first, *Drosophila yakuba* (Accession No. 5834829), is considered the standard for mtDNA comparisons (Wolstenholm and Clary, 1985). The second, *Blatella germanica* (Accession No. 572627), is closely related to Isoptera (Liu and Beckenbach, 1992). The third, *Reticulitermes speratus* (Accession No. AB005584) was used to align sequences beginning with base 1 (Miura *et al.*, 1998).

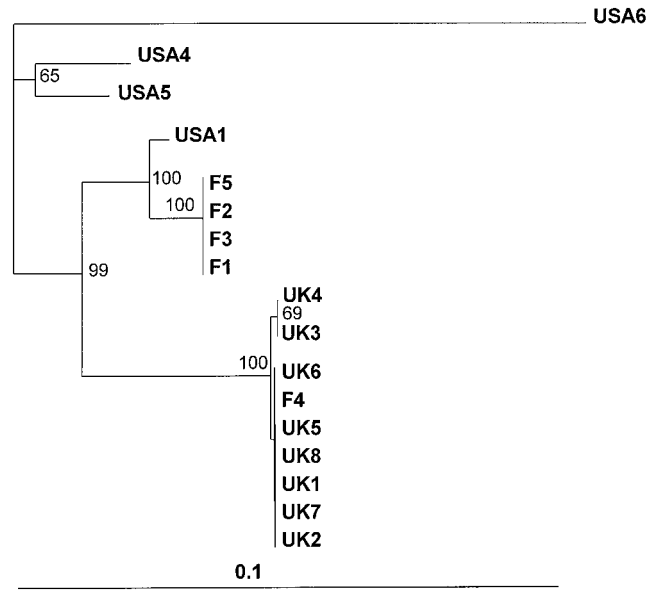

FIG. 1. COI neighbor-joining tree (Saitou and Nei, 1987) was created with PHYLIP v. 3.5c (Felsenstein, 1993) and rooted with *Coptotermes formosanus*. Bootstrap values (1000 pseudoreplicates) are written at each node. All branches ≤50 were collapsed.

Phylogenetic assumptions were tested according to Avise (1994, p. 124). CLUSTAL W 1.74 (Thompson *et al.*, 1994; Higgins *et al.*, 1996) was used to align sequences. The data were imported into PHYLIP (Felsenstein, 1993) version 3.5c and analyzed to generate distance, neighbor-joining (NJ) (Saitou and Nei, 1987) (Figs. 1–3), and parsimony (not shown) bootstrapped trees (1000 pseudoreplicates). Rooted (Figs. 1–3) and unrooted (not shown) trees were generated with TREEVIEW (Page, 1996). Additional parsimony analyses (not shown) was accomplished for all three data sets with the heuristic search option with stepwise addition in the Beta Version of PAUP* 4.0 (v. 4.0b4a) by David Swafford (distributed by Sinauer Associates, Inc., Fitchburg, MA).

An additional PHYLIP 3.5c (Felsenstein, 1993) COII analysis (not shown) was done with four *R. flavipes* samples, three from Georgia (strains France, P2, RF53) and one from Toronto, Canada (strain TO9), and one *R. speratus* sample from Japan downloaded from GenBank (Miura *et al.*, 1998). This additional phyloge-

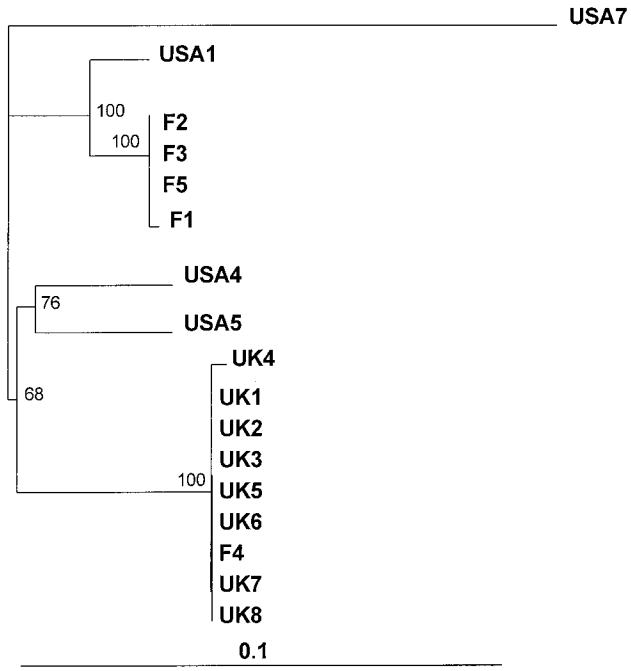

FIG. 2. COII neighbor-joining tree (Saitou and Nei, 1987) was created with PHYLIP v. 3.5c (Felsenstein, 1993) and rooted with *Coptotermes formosanus*. Bootstrap values (1000 pseudoreplicates) are written at each node ≥50.

netic analysis was done to determine whether the addition of multiple *R. flavipes* had an effect on extant groupings. *R. speratus* was added for species comparison.

Node support was assessed by 1000 nonparametric bootstrap pseudoreplicates (Hillis *et al.*, 1996, p. 523) for the neighbor-joining (Figs. 1–3) (Saitou and Nei, 1987) and parsimony (not shown) analyses. Also, 100 heuristic nonparametric bootstrap pseudoreplicates (Hillis *et al.*, 1996, p. 523) for the PAUP* parsimony analyses (not shown) were completed. Bootstrap node support ≥70% is referred to as strong (Hillis and Bull, 1993) (Figs. 1–3).

All GenBank accession numbers are in Table 2.

RESULTS

Neighbor-joining dendrograms (Saitou and Nei, 1987) were generated to examine relationships among extant *Reticulitermes* species (Figs. 1–3). All branches ≤50 were collapsed. Maximum-parsimony (MP) analyses were done (not shown) to determine tree topologies of character-state distributions across taxa, confirm clade designations, and identify possible problems in the tree topologies. Furthermore, NJ topologies were developed for both rooted (Figs. 1–3) and unrooted (not shown) data sets to determine whether clade designation varied with and without character-state polarities (Avise, 1994, p. 125), which it did not.

Phylogenetic analysis of partial COI sequence (Fig. 1) demonstrates that genetic partitioning is strong for all *Reticulitermes* species groups (Table 1), e.g., *R. virginicus* (USA5), *R. hageni* (USA3), *R. flavipes* (USA1, F1, F2, F3, F5), and *R. lucifugus grassei* (UK1–UK8, F4). Unrooted NJ and rooted and unrooted MP dendrograms (not shown) also confirmed this partitioning. The Rlg clade was composed of nine *R. lucifugus grassei* populations, eight from the UK (UK1–UK8) and one collected in France (F4) (Table 1), that were partitioned into three maternal types. The Rf clade genetically partitioned two haplotypes (Fig. 1). The sequence variation was between *R. flavipes* (USA1) and *R santonensis* (F1, F2, F3, F5). There was no differentiation among F1, F2, and F3 from southwest France and F5 from Île d' Olérons, France. PAUP* parsimony analyses (not shown) presented the same strongly supported Rlg and Rf clades as did the NJ tree.

The NJ and MP (not shown) dendrograms for the COII gene sequences presented a topology similar to that resulting from the COI phylogenetic analyses (Figs. 1 and 2). Genetic partitioning along species lines was strongly supported (Fig. 2). The monophyletic Rf clade (Fig. 2) demonstrates no genetic partitioning among *R. santonensis* samples. Two COII haplotypes were partitioned for Rlg populations, which formed a monophyletic clade with two *Reticulitermes* species, *R. virginicus* (USA5) and *R. hageni* (USA4). Parsimony analyses accomplished with PAUP* strongly supported both Rlg and Rf clades (100%) in both rooted and unrooted trees (not shown) with nodal support ≥72%. *R.*

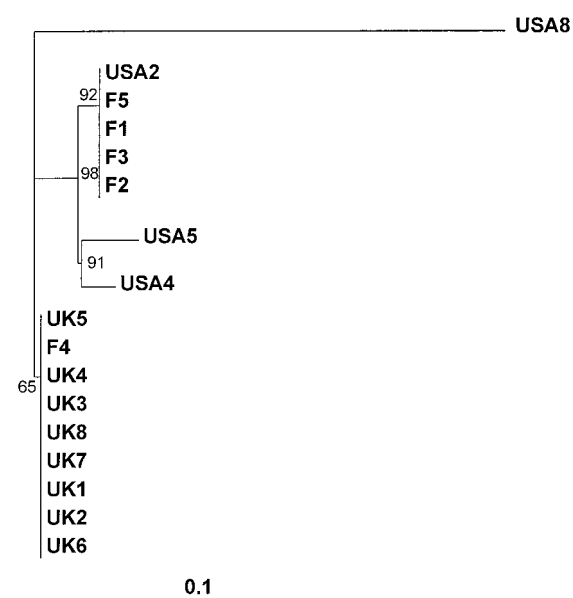

FIG. 3. ITS2 neighbor-joining tree (Saitou and Nei, 1987) was created with PHYLIP v. 3.5c (Felsenstein, 1993) and rooted with *Coptotermes formosanus*. Bootstrap values (1000 pseudoreplicates) are indicated at the nodes. All nodes are ≥50%.

TABLE 2

GenBank Accession Numbers

Sample code[a]	Species[b]	GenBank Accession Nos.		
		COI	COII	ITS2
UK1	Rlg	AY027456	AY027488	AY027454
UK2		AY027457	AY027484	AY027450
UK3		AY027460	AY027483	AY027449
UK4		AY027461	AY027482	AY027448
UK5		AY027462	AY027480	AY027446
UK6		AY027463	AY027481	AY027447
UK7		AY027459	AY027486	AY027451
UK8		AY027458	AY027485	AY027452
F4		AY027464	AY027487	AY027453
F1	Rs	AY027467	AY027473	AY027441
F2		AY027466	AY027474	AY027442
F3		AY027468	AY027475	AY027443
F5		AY027465	AY027476	AY027440
USA1	Rf	AY027469	AY027477	—
USA2		—	—	AY027450
USA3	Rh	—	AY027478	AY027444
USA4		AY027470	—	—
USA5	Rv	AY027471	AY027480	AY027445
USA6	Cf	AY027472	—	—
USA7		—	AY027489	—
USA8		—	—	AY027455

[a] Refer to Table 1 footnote *a*.
[b] Refer to Table 1 footnote *b*.

speratus COII sequence was downloaded from GenBank (Miura *et al.*, 1998) for comparison with other *Reticulitermes* spp. COII sequence data. The resulting rooted (Fig. 2) and unrooted (not shown) NJ dendrograms still showed two clades, except that *R. speratus* separated with the extant Rlg populations (Table 1) 61% of the time in the rooted NJ tree and 100% of the time in an unrooted NJ tree (not shown).

The NJ phylogeny in Fig. 3 demonstrated little sequence variability or few polymorphic sites for the ITS2 region compared to either the partial COI or the COII gene sequences. Interestingly, all *R. santonensis* and *R. flavipes* populations had the same consensus sequence for the ITS2 region (Fig. 3) and formed a distinct genetic group with strong support (92%). Figure 3 shows four genetic groups with no substructure. Three of these groups are strongly supported, Rf (92%) and Rv and Rh (91%), and one, Rlg, is moderately supported (65). Results were the same for rooted and unrooted (not shown) NJ and MP trees. Analyses of ITS2 sequences with PAUP* (not shown) showed 61% nodal support for the Rlg clade (UK2–8, F4) and 93% nodal support for the Rf clade (USA2, F1, F2, F3, F5).

Four additional *R. flavipes* samples three from Georgia and one from Toronto, Canada, and one *R. speratus* sample from Japan downloaded from GenBank (Miura *et al.*, 1998), were added to the COII sequence data set to determine the stability of gene tree topology. Neighbor-joining phylogenetic analyses showed, as in Figs. 1 and 2, that *R. flavipes* and *R. santonensis* samples still formed a strongly supported clade (100%) (not shown). *R. speratus*, however, fell out in the strongly supported Rlg clade (72%) (not shown).

DISCUSSION

Gene Flow and Populations

R. lucifugus grassei colony structure was discovered to be opened or closed, depending on geography and seasonal variations (Clemènt, 1986). A closed colony centers on a single reproductive pair and is characterized by a single mtDNA haplotype, but an open or a meta-colony promotes resource sharing by intraspecific groups and likely produces several mtDNA haplotypes. The pruning of intraspecific maternal haplotypes by stochastic lineage sorting (Avise, 1994, p. 126) likely resulted in the lack of genetic subdivision observed in the Rlg clade (Figs. 1–3). Interestingly, the Rlg sample from Royan, France (F4) had sequence identical to five COI sequences, all COII sequences, and all ITS2 sequences (Figs. 1–3). The majority of collections, therefore, came from the same maternal line as did the F4 sample from the Bordeaux region of France (Table 1). The observation that *R. speratus* from Japan was part of the Rlg clade warrants further phylogenetic study of individuals from more populations to verify the genetic partitioning.

The lack of genetic subdivision observed for the Rlg clade could be the result of an open colony with several closely related maternal lines pruned over time due to differential reproduction. However, whether opened or closed, the lack of genetic variation reflected a close evolutionary relationship and a lack of restrictive gene flow.

The Rf clade, which forms a species group distinct phylogenetically from the Rlg clade and other species used for comparison, demonstrated a close genetic relationship between *R. flavipes* from the United States and *R. santonensis* from France (Figs. 1–3). The genetic subdivision within the Rf clade reflects the environmental and temporal patterns inherent in variability and suggested a history of fragmented gene flow exacerbated by genetic drift and founder effect.

R. flavipes was introduced to northern Europe several times, e.g., Vienna, Hallein (Austria), and Hamburg (Becker, 1970, 1981). However, expansion has not occurred in these areas to any great extent as it has in the Bordeaux region of France despite the fact that importation of *R. flavipes* into Vienna occurred prior to 1837 (Kollar, 1837) when it was described. The likely explanation for this phenomenon is the relatively unfavorable environmental conditions that probably prevented extensive subterranean termite population growth in these areas.

Phylogenetic Patterns and Taxonomy

Confusion over the systematics of *R. flavipes* and *R. santonensis* existed prior to (Grassies, 1855) and since Feytaud's (1924) characterization (Grassè, 1954). The phylogenetic evidence herein supports the hypothesis that *R. santonensis* described by Feytaud (1924) was most likely *R. flavipes*, as it confirmed the taxonomy of *R. lucifugus grassei*.

Phylogenetic analyses showed that *R. flavipes* from North America and *R. santonensis* from France were always members of the same clade (Figs. 1–3). When COII sequence data sets were increased to include multiple *R. flavipes* samples from the United States and Canada, all *R. flavipes* and all *R. santonensis* formed the single Rf clade, as in Figs. 1–3.

The intraspecific homogenization and interspecific variation of ITS2 sequences argues for a homogenization mechanism that facilitated concerted evolution (Solignac *et al.*, 1986; Dover, 1982; Zimmer *et al.*, 1980). McLain and Collins (1982) made a similar observation for an intergenic spacer in the *Anophales gambiae* complex. The ITS2 sequences within *Reticulitermes* species clades, therefore, appear to have evolved together as they have diverged between species taxa (Figs. 1–3). The fixation of one ITS2 type within each species was likely a plerologic phenomenon. This suggests that the European *R. santonensis* and the North American *R. flavipes* belong to the same taxonomic group in which allopatry was a relatively recent event.

When Feytaud (1924) first described *R. santonensis*, he reported that *R. flavipes* and *R. santonensis* were morphologically indistinguishable. Chemical phenotypes for *R. santonensis* and *R. flavipes* were similar (Bagnères *et al.*, 1990) and now phylogenetic analyses of DNA sequences show them to be members of a monophyletic clade.

Dynamics of Exotic Introductions

The Devon *R. lucifugus grassei* introduction appears to have originated in the area around southwestern France, western Portugal, and northern Spain, where it is indigenous (Clèment, 1981). Phylogenetic analyses of mtDNA sequences revealed that at least three *R. lucifugus grassei* haplotypes comprise the United Kingdom's only known in-ground termite infestation (R. H. J. Verkerk, personal communication). The data, therefore, support two introduction scenarios, depending on whether the introduction consisted of an opened or a closed colony structure.

First, an opened, meta-colony structure composed of several populations (subcolonies) of kin-biased maternal groups would circumvent the fecundity issue of strict polygyny. Polygyny via neotenics was found to be potentially problematic in terms of reproductive strategies for termites as social insects because of the possible reduced fecundity of individual queens (Crozier and Pamilo, 1996, p. 123), which is well known in this species (Buchli, 1958). Alternatively, three or more populations, each headed by a single queen (monogyny), could have been introduced together or separately.

The infestation in Devon, United Kingdom was traced to two possible sources, wine crates from Bordeaux and/or plants from northern Spain. Fragmentation of a meta-colony in which cogenerational individuals were divided into maternal kin groups would result in populations with unique mtDNA haplotypes. These haplotypes would also be subjected to stochastic lineage sorting, which would be consistent with our phylogenetic data. A less parsimonious explanation, however, is that several unique maternal lines representing "closed," unique colonies were introduced in a single shipment of wine in which bottles were stored in multiple crates or in a single shipment of plants potted in separate containers. All but a few matrilineal lines were then lost over time due to sampling. This is more difficult to believe because it would mean that crates and pots were taken from disparate locations.

The lack of variability among *R. lucifugus grassei* collection sites was typical of a genetic bottleneck (Figs. 1–3), probably at the time of introduction, which could have exacerbated intraspecific resource sharing in an open social structure. A breakdown in colony boundaries would have further obscured colony distinctions (Queller, 2000; Suarez *et al.*, 1999) as the lack

of genetic variability encouraged social cooperation among individuals recognized as kin. Thus, a single fluid colony or supercolony structure composed of individuals with no kin boundaries (Queller, 2000) could have successfully evolved. As with other exotic social insect introductions (Queller, 2000; Suarez *et al.*, 1999; Holway, 1999, 1995), such success has significant economic and environmental import.

The area around Bordeaux may be the point of a *R. flavipes* introduction that was spread throughout France and Europe (Verkerk and Lainè, 2000). Extensive trade routes were in the region, notably canals and railroads. These provided ready transfer pathways for *R. flavipes* misidentified as *R. santonensis*, which, like *R. flavipes* in North America, has increased its range through the northern United States into Canada (Weesner, 1970). Records indicate that *R. santonensis* expanded its range during the 20th Century along these trade corridors (Verkerk and Lainè, 2000). Thus *R. flavipes* (*R. santonensis*) was efficiently introduced throughout France and Europe through trade as it was likely introduced into Europe from the United States during the 19th Century through trade.

CONCLUSION AND FUTURE DIRECTIONS

Reticulitermes colony structure and, therefore, gene flow was not clearly understood for either the European or the North American species and the taxonomy of the genus was often equivocal (Forschler and Jenkins, 2000). This study demonstrated the value of the use of phylogenetic analyses of multiple unique sequences to clarify gene flow and taxonomy and provided evidence for the dynamics of exotic introductions. The work has recently been expanded to include phylogenetic and gene flow analyses of multilocus DNA fingerprints and multiple mtDNA gene sequence analyses (per this study) of incipient and wild *Reticulitermes* spp. populations. These new results will have implications for the Devon introduction and the spread of *R. flavipes* throughout the world.

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