

Phylogeography of *Reticulitermes* Termites From California

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Abstract

A molecular genetics study involving DNA sequencing of a portion of the mitochondrial DNA 16S gene was conducted to determine the extent of genetic variation within *Reticulitermes* from California. A total of 89 samples were analyzed. Twenty-five nucleotide sites were variable in *R. hesperus*, and 19 mtDNA haplotypes were observed in the 428 bp mtDNA sequence. Fourteen haplotypes (37%) occurred only once, while the most common haplotypes, HE4 and HE9, each occurred in 18% of the samples. While some haplotypes were found to have a broad geographical range across the state, some were restricted to the southern region. Samples identified as *R. tibialis* were more exclusive to the southern region of California. Twelve haplotypes of a newly described species, *R. okanaganensis* Szalanski & Austin, were found. The first positive identification of *R. flavipes* from California is reported.

Introduction

There is a general consensus that the genus *Reticulitermes* is in desperate need of revision (Weesner 1970, Nutting 1990, Scheffrahn & Su 1994). This is an especially difficult task due to the problematic nature of this genus, namely the lack of discrete morphological characters, which accurately identify specimens within the genus. Recent studies of subterranean termites in the United States have implicated the identity of undescribed species of *Reticulitermes* (Isoptera: Rhinotermitidae) in California applying mitochondrial DNA (mtDNA) markers (Austin *et al.* 2002) and cuticular hydrocarbons (Haverty & Nelson 1997, Haverty *et al.* 1999, Nelson *et al.* 2001). Existing taxonomic studies of *Reticulitermes* from California provide information on two described species: *R. hesperus* Banks and *R. tibialis* Banks (Weesner 1970, Nutting 1970). The likelihood of undescribed species of *Reticulitermes* in California has been established with cuticular hydrocarbon data (Haverty *et al.* 1999ab, Delphia *et al.* 2003). In this study, a phylogenetic analysis of *Reticulitermes* applying the 16S mtDNA gene was conducted. In addition, the geographic distributions of *Reticulitermes* species and species' haplotypes throughout the region are discussed.

Fig. 1. Map of *Reticulitermes* haplotype distribution in California.



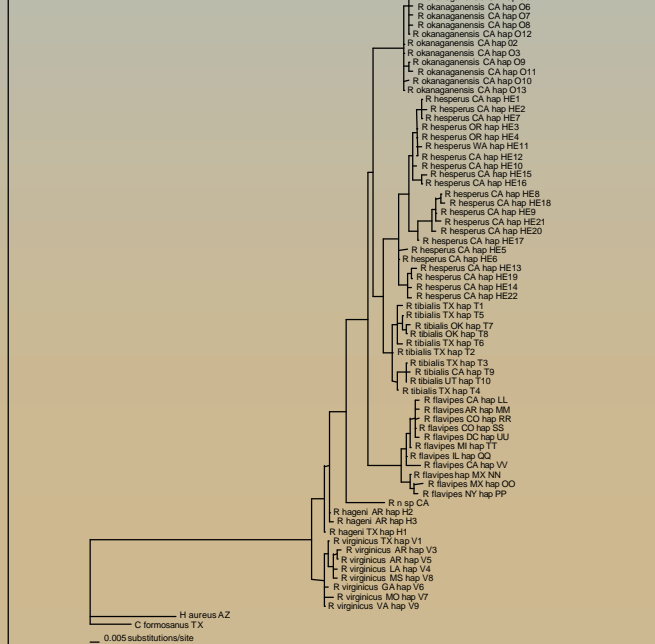
Materials and Methods

Termites were collected from various locations in California, both from our own collecting efforts and from the 2002 National Termite Survey (Fig. 1). DNA extraction, PCR, and DNA sequencing was conducted per Szalanski *et al.* (2004 Ann. Entomol. Soc. Amer. 97: 556-566). Mitochondrial DNA haplotypes were aligned using MacClade v4 (Sinauer Associates, Sunderland, MA). DNA sequences were aligned using CLUSTAL W (Thompson *et al.* 1994). Mitochondrial 16S sequences from additional *R. hesperus*, *R. tibialis*, *R. hageni* Banks, *R. okanaganensis*, *R. flavipes*, and *R. virginicus* (Banks) (Szalanski *et al.* 2003, submitted; Austin *et al.* 2004abc) were added to the California dataset for comparison along with DNA sequences from the Formosan termite, *Coptotermes formosanus* Shiraki (GenBank AY558910), and *Heterotermes aureus* (Snyder) (GenBank AY280399), which were added to act as outgroup taxa. Maximum likelihood and unweighted parsimony analysis on the alignments was conducted using PAUP* 4.0b10 (Swofford 2001) per Austin *et al.* (2004a) with the exception of the transition/transversion ratio, which was set to 2.541707.

Table 1. Collection data and haplotypes for *Reticulitermes*.

Species	City	County	Hap	N	Species	City	County	Hap	N
<i>R. flavipes</i>	Sacramento	Sacramento	F LL	1	<i>R. okanaganensis</i>	Chino	San Bernardino	O1	1
	El Cajon	San Diego	F VV	1		Grass Valley	Nevada	O1	1
<i>R. hesperus</i>	Novato	Marin	HE 1	1		Irvine	Orange	O1	1
	Riverside	Riverside	HE 1	1		Napa	Napa	O1	1
	San Luis Obispo	San Luis Obispo	HE 1	2		Placerville	El Dorado	O2	1
	San Leandro	Alameda	HE 2	1		Auburn	Placer	O3	2
	Alabama Hills	Calaveras	HE 4	1		Bakersfield	Kern	O3	1
	Napa	Napa	HE 4	2		Davis	Yolo	O3	1
	San Francisco	San Francisco	HE 4	1		Dimuba	Tulare	O3	1
	San Mateo	San Mateo	HE 4	1		Lafayette	Contra Costa	O3	1
	Strathmore	Tulare	HE 4	1		Lake Arrowhead	San Bernardino	O3	1
	Visalia	Tulare	HE 4	1		Napa	Napa	O3	1
	Groveland	Tuolumne	HE 5	1		Strathmore	Tulare	O3	1
	Palm Springs	Riverside	HE 6	1		Walnut Creek	Contra Costa	O3	1
	Riverside	Riverside	HE 6	2		Sebastopol	Sonoma	O5	1
	Westminster	Orange	HE 6	1		Walnut Creek	Contra Costa	O5	1
	Brea	Orange	HE 7	1		Burbank	Los Angeles	O6	1
	Napa	Napa	HE 7	1		Santa Maria	Santa Barbara	O6	1
	Hanford	Kings	HE 8	1		Mission Viejo	Orange	O7	1
	Long Beach	Los Angeles	HE 9	2		Riverside	Riverside	O7	1
	Monarch Beach	Orange	HE 9	1		Walnut	Los Angeles	O7	2
	Norco	Riverside	HE 9	1		Pauma Valley	San Diego	O8	1
	Santa Catalina Island	Los Angeles	HE 9	1		San Marcos	San Diego	O8	1
	Topanga Park	Los Angeles	HE 9	1		Davis	Yolo	O9	1
	Walnut	Los Angeles	HE 9	1		Napa	Napa	O9	2
	Hanford	Kings	HE 10	1		Oakland	Alameda	O9	1
	Stockton	San Joaquin	HE 13	1		Oakland	Alameda	O10	1
	Stockton	San Joaquin	HE 14	1		Rocklin	Placer	O10	1
	Porterville	Tulare	HE 15	1		Napa	Napa	O11	1
	Porterville	Tulare	HE 16	1		Corona Del Mar	Orange	O12	1
	Los Angeles	Los Angeles	HE 17	1		Covina	Los Angeles	O12	1
	Santa Maria	Santa Barbara	HE 18	1		Culver City	Los Angeles	O12	1
	Sacramento	Sacramento	HE 19	1		Laguna Beach	Orange	O12	1
	Glendale	Los Angeles	HE 20	1		Los Angeles	Los Angeles	O12	2
	Los Angeles	Los Angeles	HE 21	1		Pomona	Los Angeles	O12	1
	Penn Valley	Nevada	HE 22	1		San Juan Capistrano	Orange	O12	1
<i>R. tibialis</i>	-	San Bernardino	T 3	1		Upland	San Bernardino	O12	1
	Diamond Bar	Los Angeles	T 9	1		W. Covina	Los Angeles	O12	1
	San Bernardino	San Bernardino	T 9	1		Walnut	Los Angeles	O12	1
undescribed species									
<i>R. californicus</i>	Arrowbear	San Bernadino		1		Westminster	Orange	O12	1
<i>Coptotermes formosanus</i>	Baton Rouge, LA		outgroup			Stockton	San Joaquin	O13	1
<i>Heterotermes aureus</i>	Santa Rita, AZ		outgroup						

Fig. 2. Maximum likelihood cladogram.



Results and Discussion

DNA sequencing of the 16S rRNA amplicon revealed an average size of 428 bp. The average base frequencies were A = 0.41, C = 0.23, G = 0.13, and T = 0.23. Among the 89 *Reticulitermes* mtDNA 16S DNA sequences, a total of 21 nucleotide sites were variable. *Reticulitermes hesperus* was found in 17 counties of the 25 California counties sampled (Fig. 1). Eighteen distinct haplotypes (lineages) were observed (Table 1). Fourteen haplotypes occurred only once, while the most common haplotypes, HE4 and HE9, each accounted for 18% of the *R. hesperus* samples. Haplotype HE4 was found over the largest geographical area (Fig. 1).

Twelve haplotypes of the newly described species *R. okanaganensis* were found in 17 of the counties sampled. Haplotype O12 was the most common, occurring in 27% of the samples, and was only present in samples from the southwestern extreme of the state (Fig. 1). Haplotype O3 was also common, occurring in 22% of the samples and covered the broadest geographic area (Fig. 1). The identification of *R. okanaganensis* haplotypes O1 through O13 demonstrates considerable variation in this species given the number of samples (43) evaluated in the present study. A single sample of a genetically distinct species, *R. sp. nov. "californicus"*, was discovered in Arrowbear, CA in San Bernardino county.

Phylogenetic analysis of *Reticulitermes* from California reveals discrete clades, which support the monophyletic nature of *R. hesperus* and *R. tibialis* (Fig. 2). The identification of *R. flavipes*, the Eastern Subterranean termite, from Sacramento and El Cajon likely represents either extreme western distributions of the species or accidental introductions from anthropogenic sources. The latter seems more plausible given the omission of information about the species' presence in California as well as the geographic distance separating the two samples. Because *R. flavipes* is a considerable pest of structures in the United States and around the world, evaluation of the presence of this species in California should be carefully evaluated to see if its presence will compete with *R. hesperus* as a destructive pest in the future.

Acknowledgements

We thank M. Rust for critical review of this manuscript, P. Pachamuthu and S. Vega of Western Exterminating (Anaheim, CA), and numerous pest management professionals for providing samples. This research was supported in part by the University of Arkansas, Arkansas Agricultural Experiment Station.