

GENETIC VARIATION AND GEOGRAPHICAL DISTRIBUTION OF THE SUBTERRANEAN TERMITE GENUS *RETICULITERMES*¹ IN TEXASJames W. Austin², Allen L. Szalanski², Roger E. Gold³, and Bart T. Foster³

ABSTRACT

A molecular genetics study involving DNA sequencing of a portion of the mitochondrial DNA 16S gene was undertaken to determine the extent of genetic variation with *Reticulitermes* spp. and the distribution of *Reticulitermes* spp. subterranean termites in Texas. From 42 Texas counties a total of 68 *R. flavipes*, seven *R. hageni*, eight *R. virginicus*, and nine *R. tibialis* were identified. No genetic variation was observed in *R. virginicus* and *R. hageni*, while seven haplotypes were observed in *R. tibialis* and 13 for *R. flavipes*. Among the 13 *R. flavipes* haplotypes, 9 nucleotides were variable and genetic variation ranged from 0.2 to 1.6%. Phylogenetic analysis did not reveal any relationships among the *R. tibialis* and *R. flavipes* haplotypes, and there was no apparent geographical structure to the haplotypes. The high amount of genetic variation, but a lack of genetic structure in *R. flavipes* supports the hypothesis that this termite species has been distributed randomly by man due to its association with structures.

INTRODUCTION

The most abundant native termite in Texas is the subterranean genus *Reticulitermes* Holgren (Rhinotermitidae). Four species, the eastern subterranean *Reticulitermes flavipes* (Kollar), light southern *R. hageni* Banks, arid *R. tibialis* Banks, and dark southern *R. virginicus* (Banks), are known to occur in Texas (Howell et al. 1987). These species are among the most destructive and costly termites for homeowners and businesses alike, and are of considerable economic importance. Su (1993) estimated that over \$1.5 billion is spent annually for termite control in the U.S., of which 80% is spent to control subterranean termites. More recent estimates by the National Pest Management Association suggest the cost to exceed \$2.5 billion annually (Anonymous 2003). While there are no current estimates of the total economic impact of *Reticulitermes* in Texas, Howell et al. (1987) estimated that the costs for termite inspections, treatment of infestations, and repair of damage in Corpus Christi, Texas, alone was \$3.7 million annually and \$30 million annually for the greater Houston, Texas, area.

In 1979, an attempt to determine the geographical distribution of termites in the state of Texas was started by Howell et al. (1987), and the collection effort has continued to the present. This endeavor principally utilizes specimens provided from the professional

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pest management industry in Texas and specimens which are available in the insect museum at Texas A&M University. Correct identification is critical for pest insects, such as termites, which may require very different control methods depending on the target species. Identifying workers is nearly impossible and separating soldiers is especially difficult given that precise measurements are required and overlap may occur between species (Sheffrahn and Su 1994). Difficulties arise in species determination at individual collection sites since the majority of the termites encountered are workers. Finding an alate in a collection is seasonal and quite rare. Soldiers represent only 1-3% of *Reticulitermes* colonies and are morphologically variable; use of this caste alone can result in equivocal species determinations. Subtle clinal variations imposed by geographic boundaries can be misleading in correct species determination. Molecular genetic methods are able to differentiate species regardless of the caste encountered (Szalanski et al. 2003). Also, genetic information obtained from collections is an integral component to phylogenetic studies as a whole. Remarkable as it may seem, there are currently no known studies that have attempted to look at the extent of genetic variation and subsequent gene flow in *Reticulitermes* from Texas.

Information on how genetic variation is partitioned within populations and among termite species can be useful for determining the extent of gene flow and for developing molecular diagnostics for identifying species. Previous studies have focused on *Reticulitermes* spp. from the southeastern United States and Western Europe (Jenkins et al. 1998, 2001; Marini and Mantovani 2002). More recently, Austin et al. (2002) included locations within Texas and other areas, but additional populations are needed to establish their respective genetic parameters.

Both cytochrome oxidase II (COII) and 16S rRNA of the mitochondrial DNA (mtDNA) have proved useful for determining phylogenetic relationships of termites (Austin et al. 2002; Jenkins et al. 1999, 2001; Kambhampati and Smith 1995, Kambhampati et al. 1996; Lo et al. 2000; Miura et al. 1998). Using a new molecular diagnostic method for discriminating between closely related *Reticulitermes* spp. (Szalanski et al. 2003), we hope to not only confirm existing distributions but to expand their known occurrences. For example, within the insect collection at Texas A&M University, College Station, Texas (entowww.tamu.edu/new/research/systematics/collection.html), there are presently 227 *Reticulitermes* samples, of which only 96 have been classified to species (85% *R. flavipes*, 7% *R. virginicus*, 7% *R. hageni* and 1% *R. tibialis*). Fifty-eight percent, representing 131 vials, have not yet been identified to species. Identification of existing specimens, using molecular techniques as outlined in this study, from existing collections such as this, can add significant information on their distribution and gene flow. Additional information provided by observing the genetic variation and gene flow can elucidate existing patterns of migration, potential hybridization events and general speciation of *Reticulitermes* spp. in Texas.

We investigated the extent of genetic variation within and among Texas *Reticulitermes* termites, evaluated the utility of these genetic markers for identifying species, and updated the geographical distribution of these taxa.

MATERIALS AND METHODS

Termites were collected from various locations in Texas and preserved in 100% ethanol (Table 1). In addition to our own collecting efforts, we solicited the assistance of Pest Management Professionals (PMPs) throughout the state for the purpose of interpreting the predominant species recovered from infested structures. PMPs were provided with collection kits and 611 samples were collected throughout the state. A subsample, representative of various geographic zones throughout the state, was used for

molecular analysis. *Reticulitermes* were morphologically identified to species when either alates or soldiers were available using the keys of Krishna and Weesner (1969), Scheffrahn and Su (1994), Hostettler et al. (1995) and Donovan et al. (2000). For the remaining samples, species identification was conducted using DNA sequences (Szalanski et al. 2003). Two additional taxa (Table 1) were included as outgroup taxa to corroborate relationships within the genus for our phylogenetic analysis. Voucher specimens, preserved in 100% ethanol, are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

TABLE 1. Collection data, and haplotypes for Texas *Reticulitermes* and outgroup taxa.

Species	City	County	Haplotype	N
<i>R. flavipes</i>	Corpus Christi	Nueces	A	1
	Del Rio	Val Verde	B	1
	Carrollton	Dallas	C	1
	Houston	Harris	C	1
	Taylor	Williamson	C	1
	San Antonio	Bexar	D	1
	Waco	McLennan	D	1
	Lake Jackson	Brazoria	E	1
	Dallas	Dallas	E	1
	Friendswood	Galveston	E	1
	Granbury	Hood	E	1
	Beaumont	Jefferson	E	1
	Buffalo	Leon	E	1
	The Woodlands	Montgomery	E	1
	Austin	Travis	E	1
	Hempstead	Waller	E	1
	Lewisville	Denton	F	3
	Odessa	Ector	F	1
	Houston	Harris	F	1
	Houston	Harris	F	1
	Nederland	Jefferson	F	1
	Paris	Lamar	F	1
	Austin	Travis	F	1
	Beeville	Bee	G	1
	Pittsburg	Camp	G	1
	Plano	Collin	G	1
	Dallas	Dallas	G	1
	Rowlett	Dallas	G	1
	Stephenville	Erath	G	1
	Spring	Harris	G	1
	Quinlan	Hunt	G	1
	Beaumont	Jefferson	G	1
	Combine	Kaufman	G	2
	Mabank	Kaufman	G	1
	Jewett	Leon	G	1
	Midland	Midland	G	1
	Livingston	Polk	G	1
	Troup	Smith	G	1
	Arlington	Tarrant	G	1
	Del Rio	Val Verde	G	1

	Mabank	Kaufman	G	1
	Onalaska	Polk	H	1
	Blanco	Blanco	H	1
	Gun Barrel City	Henderson	H	1
	The Woodlands	Montgomery	H	1
	Amarillo	Potter	H	1
	Sugarland	Fort Bend	I	1
	Lubbock	Lubbock	I	1
	Baytown	Harris	J	1
	Magnolia	Montgomery	J	1
	The Woodlands	Montgomery	J	1
	Richardson	Dallas	J	1
	Red Water	Bowie	K	1
	Addison	Dallas	L	1
	Garland	Dallas	L	2
	Irving	Dallas	L	2
	Rowlett	Dallas	L	1
	Fritch	Hutchinson	L	1
	Kemp	Kaufman	L	2
	Midland	Midland	L	1
	Milano	Milam	M	1
	Dumas	Moore	M	1
<i>R. hageni</i>	College Station	Brazos	H1	5
	Lewisville	Denton	H1	1
	Athens	Henderson	H1	1
<i>R. virginicus</i>	College Station	Brazos	V1	5
	Bryan	Brazos	V1	1
	Athens	Henderson	V1	2
<i>R. tibialis</i>	Fort Worth	Tarrant	T1	1
		Collin	T1	1
	Brackettville	Kinney	T2	1
	El Paso	El Paso	T3	1
	New Braunfels	Comal	T4	1
	Happy	Swisher	T5	1
	De Soto	Dallas	T6	1
	Athens	Henderson	T7	2
<i>Coptotermes formosanus</i>	Galveston Is.	Galveston	outgroup	
<i>Heterotermes aureus</i>	Santa Rita, AZ		outgroup	

Alcohol-preserved specimens were allowed to dry on filter paper, and DNA was extracted according to Liu and Beckenbach (1992) and Jenkins et al. (1999) on individual whole worker termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50µl of Tris:EDTA and stored at -20°C. Polymerase chain reaction was conducted using the primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati and Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al., 1994). These PCR primers amplify an approximately 428 bp region of the mtDNA 16S rRNA gene. The PCR reactions were conducted with 1µl of the extracted DNA (Szalanski et al. 2000), having a profile consisting of 35 cycles of 94°C for 45s, 46°C for 45s and 72°C for 60s. Amplified DNA from individual termites was purified and concentrated with minicolumns (Wizard PCRpreps, Promega, Madison, WI) according to the manufacturer's instructions. Samples

were sent to The University of Arkansas DNA Sequencing Facility (Fayetteville, AR) for direct sequencing in both directions. DNA sequences from representatives of each haplotype were submitted to GenBank, accession numbers AY441975 to AY441992. DNA sequences were aligned using the PILEUP command of GCG (Accelrys, San Diego, CA). Mitochondrial DNA haplotypes were aligned using MacClade v4 (Sinauer Associates, Sunderland, MA).

The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Mitochondrial 16S sequences from the Formosan termite, *Coptotermes formosanus* Shiraki, and *Heterotermes aureus* (Snyder), GenBank AY380299, were added to the *Reticulitermes* DNA sequences to act as outgroup taxa. The DNA sequences were aligned by the PILEUP program in GCG (Genetics Computer Group, Madison, WI) and adjusted manually. Maximum parsimony analysis on the alignment was conducted with PAUP 4.0b10 (Swofford 2001). Gaps were treated as missing data. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings and used the Branch and Bound algorithm of PAUP.

RESULTS

DNA sequencing of the 16S rDNA amplicon revealed that it averaged 428 bp in size. The average base frequencies were A = 0.39, C = 0.23, G = 0.14, and T = 0.24. From the DNA sequence analysis of *Reticulitermes* from 42 Texas counties, a total of 68 *R. flavipes*, 7 *R. hageni*, 8 *R. virginicus*, and 9 *R. tibialis* were identified based on species diagnostic nucleotide sites from Szalanski et al. (2003) (Table 1, Fig. 1).

No genetic variation was observed in *R. virginicus* and *R. hageni*, while seven unique haplotypes were found in *R. tibialis* and 13 in *R. virginicus* (Table 1). Pairwise Tajima-Nei distances (Tajima and Nei 1984) among *Reticulitermes* taxa ranged from 5.7% between *R. flavipes* and *R. hageni*, to 8.3% between *R. flavipes* and *R. tibialis*. A total of nine nucleotide sites were variable among the 13 *R. flavipes* haplotypes (Table 2), and genetic variation among the *R. flavipes* haplotypes ranged from 0.2 to 1.6%. Within *R. tibialis* a total of six nucleotide sites were variable among the seven haplotypes, and variation among the *R. tibialis* haplotypes ranged from 0.2 to 1.2%.

TABLE 2. Haplotype variation at nine nucleotide sites among *R. flavipes* from Texas.

Haplotype	131	158	168	179	206	236	270	271	274
A	G	A	A	C	T	A	T	T	A
B	*	G	G	*	*	*	*	*	*
C	A	G	*	T	*	C	*	*	*
D	A	G	*	*	*	C	C	*	G
E	A	*	*	*	*	*	*	*	G
F	A	G	G	*	*	C	*	*	G
G	*	G	*	*	*	C	*	C	G
H	A	G	*	*	*	C	*	*	G
I	A	G	*	T	C	C	*	*	G
J	*	G	*	*	*	C	*	*	G
K	*	G	*	T	C	C	*	*	G
L	A	G	*	T	*	C	*	*	G
M	*	*	*	*	*	C	*	*	G

The aligned DNA data matrix, including the outgroup taxa resulted in a total of 436 characters. Of these characters, 86 (20%) were variable and 50 (11%) were

phylogenetically informative. Bootstrap analysis of the aligned *Reticulitermes* species and the outgroup taxa resulted in a consensus tree (Fig. 2), (length = 132, CI = 0.697, RI = 0.752), as documented using the Branch and Bound search algorithm of PAUP. The distinct clades from the maximum parsimony analysis were: *R. flavipes* forming poorly supported sister group with *R. tibialis*, *R. hageni* and *R. virginicus*. The distinctive relationship of these termite taxa has been observed repeatedly in other genetic studies (Austin et al. 2002; Jenkins et al. 1998, 1999). There was no haplotype structure observed among the *R. tibialis* and *R. flavipes* haplotypes in the present study.

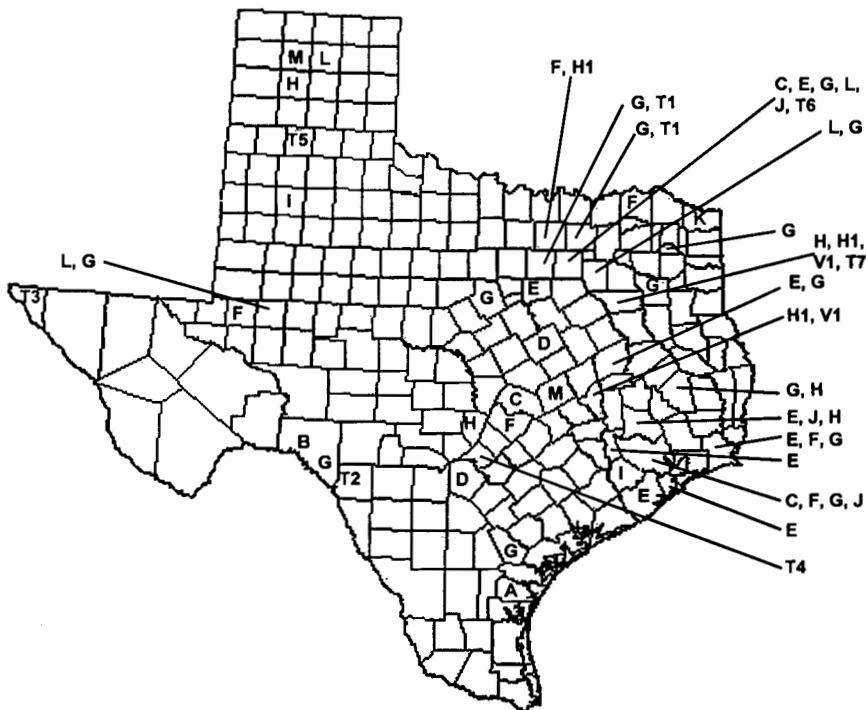


FIG. 1. Distribution of *Reticulitermes* species and haplotypes in Texas.

DISCUSSION

This study represents the first attempt in over 16 years to update the current geographic distribution and genetically categorize the genus *Reticulitermes* in Texas. In the present study, a clear bias associated with the frequency of occurrences from various *Reticulitermes* species which attack structures is reflected. The Eastern subterranean termite *R. flavipes* is the predominant species observed in Texas. This is not surprising as previous surveys in Texas and other Gulf Coast states similarly reflect this observation (Howell et al. 1983, Wang and Powell 2001, Messenger et al. 2002). However, with increased urban expansion into woodland habitats the occurrence of other species may be

more frequently realized. Because of the limited number of locations where samples of *R. hageni* and *R. virginicus* used in this study originated from, little variation within these two species was detected.

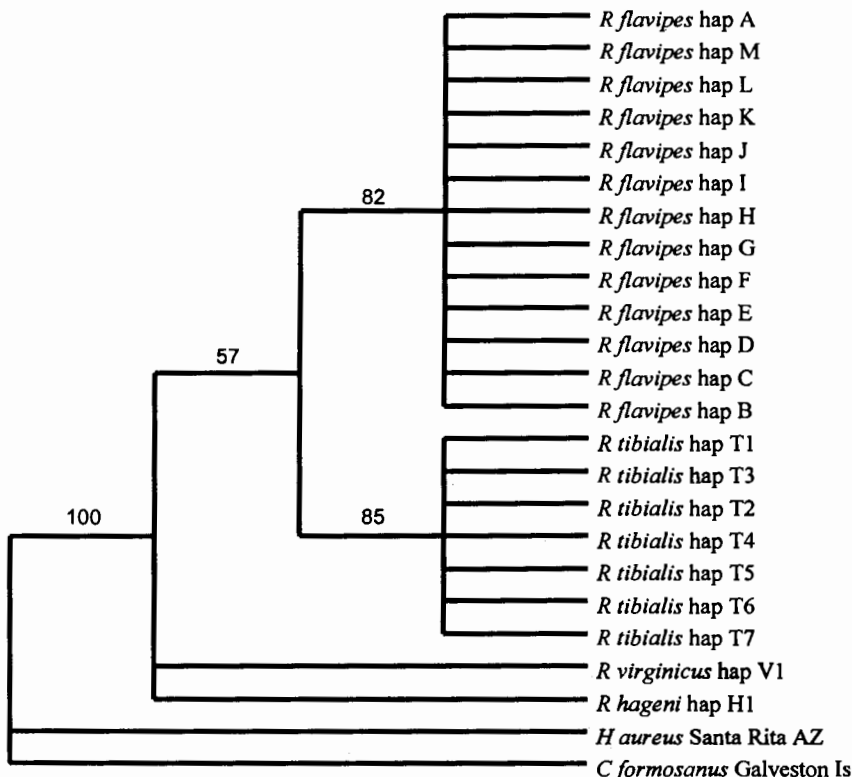


FIG. 2. 16S single most parsimonious tree during a branch and bound search using PAUP*. Bootstrap values for 1,000 replicates are listed above the branches supported at $\geq 50\%$

Using the 16S rRNA gene, we have found that genetic divergence can range from as much as 2.6% between *R. virginicus* and *R. hageni* to 9.2% between *R. virginicus* to *R. tibialis* (JWA, unpublished data). While less commonly encountered, *R. tibialis* was represented by seven distinct haplotypes in this study. The fact that 77% of the *R. tibialis* populations represent a unique haplotype is intriguing and merits further investigation.

The lack of geographic patterns based on haplotypes observed in *R. flavipes* is not surprising given the numerous opportunities for anthropogenic disruptions. Some *R. flavipes* haplotypes appear to be unique or possibly correlated with geography and warrant further investigation of *Reticulitermes* species in neighboring states. By investigating the genetic variation of *Reticulitermes* from larger geographic zones, the complex ecological demands of this genus can be better understood. Genetic variation is important because it impacts on a species ability to respond to natural selection: selection is inversely proportional to genetic variation (Fisher 1958). While an individual's fitness is determined by interactions between its phenotype and the environment, social organisms'

individual fitness is influenced by direct interactions between phenotypes (Hochberg et al. 2003). The numerous haplotypes, without genetic structure as often imposed by geographic isolation or distance, observed in the present study might suggest a degree of interaction that has been observed in “open” (when termites accept alien homospecific individuals) termite populations. To more accurately assess this, intensive collecting at various locations should be performed and more robust statistical procedures should be applied. This phenomenon is variable, but has been demonstrated in ants (Forel 1920, Soulié 1960, Scherba 1964, Passera 1963, Benois 1972, Provost 1979) and occasionally in termites where the degree of aggression between homospecific individuals may vary from one nest to another [*Nasutitermes corniger*, *Nasutitermes ephratae*, *Amitermes* and *Armitermes* (Thorne 1982), *Reticulitermes santonensis* and *Reticulitermes grassei* (Clément 1978)]. For this reason, it is becoming increasingly important to evaluate the genetic relationship between *Reticulitermes* so that a broader understanding of how sympatric homospecific populations interact. This is important because it allows us to better understand the dynamic nature of controlling termites with newer control strategies (e.g. termite baiting regimes).

It has been demonstrated that *Reticulitermes* colony structure and, therefore, gene flow can be more clearly understood in North America and Europe when using mtDNA data (Austin et al. 2002; Jenkins et al. 2001, 2002; Marini and Mantovani 2002). The Eastern subterranean termite *R. flavipes*, incorrectly identified as *R. santonensis*, has been moved about in Europe along trade corridors, expanding its known range throughout France and Europe (Lainé 2002). Likewise in Texas, movement of *R. flavipes* is greatly influenced by trade corridors and partially explains the lack of haplotype structure observed in this study based on geography. Colonies that merge shared physical space and resources may demonstrate no intercolony agonistic behavior (Houseman et al. 2001). This lack of agonistic behavior between disjunct *Reticulitermes* populations has been demonstrated extensively in Europe (Plateaux and Clement 1984, Clement 1986) and even in some instances hybridization may be achieved (Clement 1977, 1979). The discontinuity of *R. flavipes* in Texas suggests that fragmentation of *Reticulitermes* populations due to anthropogenic disturbances induces variations in their observed haplotypes.

Thirteen distinct haplotypes from 68 different populations suggests a considerable amount of genetic variation in this species, even without a geographic correlation as observed in other Rhinotermitidae (ALS unpublished data, Jenkins et al. 2002). More pronounced differences may yet be uncovered in other endemic *Reticulitermes* species in Texas as they are more frequently collected. Szalanski et al. (2003) have developed a quick and inexpensive molecular method which can easily identify *Reticulitermes* species. The lack of diagnostic castes for morphological species determination can be overcome employing molecular diagnostic methods. Molecular methods also allow the utilization of samples already in various collections can help facilitate more robust comparisons.

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