

Genetic Variation of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) in North America Applying the Mitochondrial rRNA 16S Gene

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Ann. Entomol. Soc. Am. 98(6): 980-988 (2005)

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 within *Reticulitermes flavipes* (Kollar) in North America. This study was done because differences in morphological variants (of *R. flavipes*) would presumably translate into genetic differences, and there are no prior studies that describe its genetic variation from the extent of its North American range. In total, 493 samples were analyzed from the United States, Canada, and Mexico. Nineteen nucleotide sites were variable in the 428-bp mitochondrial DNA (mtDNA) sequence, and 47 mtDNA haplotypes were observed. Nine haplotypes (19%) occurred only once, whereas the most common haplotype, F, occurred in 17% of the samples. Four haplotypes were found over a broad geographical range encompassing at least nine states each. The single haplotype found in Toronto, Canada, also occurs in Arkansas, whereas two of the three haplotypes found in Mexico are unique to that country. Based on this research, there seems to be numerous *R. flavipes* haplotypes that are widespread, perhaps due to human involvement, whereas other haplotypes may be more rare and could represent locally adapted populations.

KEY WORDS 16S, DNA sequence, genetic variation, *Reticulitermes*, termite

THE MAJORITY OF PESTIFEROUS subterranean termites in North America belong to the endemic genus *Reticulitermes* (Isoptera: Rhinotermitidae). *Reticulitermes* species are found in every state in the continental United States except Alaska, but they are most common in the warm and humid southeastern region (Su et al. 2001). The eastern subterranean termite, *Reticulitermes flavipes* (Kollar), is the most widely distributed *Reticulitermes*, and it is found in the entire eastern region of North America as far north as Ontario, Canada, and south to Florida. The known western distribution of the species extends through the central plains to the Rocky Mountains and down to Monterey, Mexico (Banks and Snyder 1920, Snyder 1954, Weesner 1965, Nutting 1990, Messenger 2003). The species was first described by Kollar (1837) in Austria from infested root stocks stored in pots arriving from the United States. More recent evidence has established *R. flavipes* as an exotic pest in Europe, South

America (Austin et al. 2005), and the Bahamas (Scheffrahn et al. 1999).

R. flavipes is unquestionably the most economically important termite in North America. Of the \$2.5 billion annually spent for termite control in the United States (NPMA 2003), subterranean termites account for 80% of the cost, and the majority of this is probably due to *R. flavipes* (Su et al. 1993).

In general, studies of variation within and among species are more easily interpreted when evaluated through a phylogenetic framework (Avice 1994), because most modern systematists infer phylogenetic relationships using quantitative methods (Felsenstein 2004). For this reason, application of the phylogenetic species concept (PSC) has been particularly useful when applied to this genus. In essence, PSC is a typological species concept that diagnoses species for their discrete, diagnosably distinct monophyletic clusters that share common ancestry (Coyne and Orr 2004). Differences among distinct geographic populations of the same species have been extensively studied, in part because they are critical for distinguishing species (Futuyma 1998). Populations differ in allele and genotype frequencies, often very considerably and in many different characteristics (Futuyma 1998). At least some of these differences seem to be adaptive consequences of occupying different environments (Futuyma 1998). Austin et al. (2004a, b, c) have demonstrated that although some genetic haplotypes of *R.*

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cally identified to species when alates were available using the keys of Krishna and Weesner (1969), Hostettler et al. 1995, and Donovan et al. (2000). For the remaining samples, species identification was conducted using mtDNA 16S sequences (Szalanski et al. 2003). Voucher specimens preserved in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

Alcohol-preserved specimens were dried on filter paper, and DNA was extracted according to Liu and Beckenbach (1992) on individual whole worker termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 μ l of Tris-EDTA and stored at -20°C . Polymerase chain reaction (PCR) was conducted using the primers LR-J-13007 (5'-TTACGCTGTTATC-CCTAA-3') (Kambhampati and Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al. 1994). These PCR primers amplify an \approx 428-bp region of the mtDNA 16S rRNA gene. The PCR reactions were conducted with 1 μ l of the extracted DNA (Szalanski et al. 2000), having a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s, and 72°C for 60 s. 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 Medical Center DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. Additional *R. flavipes* mtDNA 16S sequences were obtained from our previous studies of *Reticulitermes* from Texas, Oklahoma, Arkansas, and Louisiana (Austin et al. 2004a, b, c) and from Indiana (Ye et al. 2004). GenBank accession numbers were DQ001951 to DQ001973 for the new *R. flavipes* haplotypes found in this study and DQ004945 to DQ004948 for the four *Reticulitermes hesperus* Banks haplotypes that were used. Consensus sequences for each sample were obtained using Bioedit 5.09 (Hall 1999). Mitochondrial DNA haplotypes were aligned using MacClade version 4 (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 *R. hesperus*, *Reticulitermes tibialis* Banks, *Reticulitermes hageni* Banks, and *Reticulitermes virginicus* Banks (Szalanski et al. 2003; Austin et al. 2004a, b, c) were added to the *R. flavipes* data set 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. DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994). Maximum likelihood and unweighted parsimony analysis on the alignments was conducted using PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing characters for all analysis. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis in-

cluded 1000 resamplings by using the Branch and Bound algorithm of PAUP*.

Results

R. flavipes was found in 38 of the 42 U.S. states sampled, Washington, DC, Canada, and Mexico (Fig. 1). 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 493 *R. flavipes* mtDNA 16S DNA sequences, 19 nucleotide sites in total were variable (Table 1). Forty-seven distinct haplotypes (lineages) were observed (Table 1), and genetic divergence among these haplotypes ranged from 0.23 to 1.9%. Nine haplotypes occurred only once, whereas the most common haplotype, F, occurred in 17% of the samples (Table 2). The haplotypes found over the largest geographical area were haplotypes F, M, Z, and EE (Fig. 1). The single sample from Toronto, Canada, was haplotype V, that also occurs in Arkansas. Among the 11 Mexican samples, two were haplotype B, which is also found in Texas, whereas the remaining samples consisted of eight haplotype NN and one haplotype OO, which are unique to Mexico.

Some haplotypes seem to have some geographical isolation significance. For example, haplotype A was only recovered in the extreme south of Texas, whereas its congener haplotype B was found both in southern Texas and Mexico. In California, haplotype LL was found in Sacramento, and in Nevada, *R. flavipes* haplotype Z was found (Table 2). To our knowledge this is the first report of *R. flavipes* from California and Nevada. Similarly, on the eastern Atlantic sea board of the United States, it would seem that haplotype EE is only found east of the Mississippi River.

We conducted a phylogenetic analysis on all described *Reticulitermes* species from North America to clarify *R. flavipes*' phylogenetic relationship within the genus. Parsimony analysis of the aligned *Reticulitermes* spp. and the outgroup taxa used 442 characters, of which, 87 were variable (20%) and 59 (13%) were informative. This analysis had a single consensus tree with a length of 216 and a CI value of 0.486, verifying that all 47 *R. flavipes* haplotypes were monophyletic (Fig. 2). In general, there did not seem to be any genetic structure among the *R. flavipes* haplotypes; however, haplotypes X, Y, Z, EE, HH, NN, OO, and PP formed a distinct clade as did haplotypes R and S (Fig. 2).

Discussion

This is the first genetic analysis of *R. flavipes* from North America as a whole, and the phylogenetic relationships of this species provide potentially important information on the history of evolutionary changes in the species. We do not propose that the collection and use of sequence data alone are comprehensive enough to reveal all possible populations, but this study, coupled with more intensive efforts

Table 1. Variable nucleotide sites among 47 *R. flavipes* haplotypes

Hap	5	8	9	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	n	
	5	0	6	2	0	1	1	7	8	2	8	0	2	0	7	1	2	5	5		
A	G	C	T	A	A	G	G	A	A	G	A	-	A	C	T	T	T	A	G	2	
B	G	.	G	-	2
C	A	.	.	G	.	.	-	.	T	7
D	A	.	.	G	.	.	-	.	.	.	C	.	G	.	.	4
E	A	-	G	.	.	18
F	A	.	.	.	G	.	G	A	-	G	.	.	83
G	.	.	.	T	G	.	.	-	C	G	.	.	42
H	A	.	.	.	A	.	.	.	G	.	.	-	G	.	.	16
I	A	.	.	.	A	.	.	.	G	.	.	-	.	T	C	.	.	G	.	.	4
J	G	.	.	-	G	.	.	9
K	G	.	.	-	.	T	C	.	.	G	.	.	1
L	A	.	.	G	.	.	-	G	.	.	38
M	A	.	.	G	.	.	-	G	.	.	24
N	A	.	.	G	A	.	-	.	T	2
O	C	.	.	G	A	.	-	G	.	.	1
P	A	.	.	G	.	.	-	C	G	.	.	24
Q	A	.	.	G	.	G	-	.	T	.	.	.	G	.	.	10
R	A	.	.	G	.	.	-	G	A	.	10
S	A	.	.	G	A	.	-	G	A	.	5
T	A	.	.	G	.	.	-	.	.	C	C	.	G	.	.	14
U	A	.	.	G	.	G	-	.	.	C	.	.	G	.	.	16
V	A	.	.	G	A	G	A	-	G	.	.	8
W	A	A	.	.	G	.	.	-	.	.	C	C	.	G	.	.	2
X	.	T	C	G	.	.	-	.	A	.	.	C	G	.	.	10
Y	.	T	C	.	.	A	A	.	G	.	.	-	.	A	.	.	C	G	.	.	12
Z	.	T	C	.	.	A	.	.	G	.	.	-	.	A	.	.	C	G	.	.	44
AA	T	.	.	.	G	A	.	-	G	.	.	4
BB	A	A	.	G	.	.	-	.	T	.	.	C	G	.	.	3
CC	A	.	.	G	.	.	-	.	.	.	C	G	.	.	.	3
DD	A	A	.	G	.	.	-	.	T	.	.	.	G	.	.	1
EE	.	T	C	.	.	A	.	.	G	.	.	-	.	A	.	.	C	G	.	.	2
FF	A	A	.	G	.	.	-	.	.	.	C	G	.	.	.	22
GG	A	.	.	G	.	G	G	-	G	.	.	3
HH	.	T	C	.	T	.	.	.	G	.	G	-	.	A	.	.	C	G	.	.	5
II	A	.	.	G	.	G	G	-	T	.	.	.	G	.	.	1
JJ	A	.	.	G	.	G	G	-	11
KK	.	T	C	.	T	.	.	.	G	.	G	-	.	A	.	.	C	G	.	.	2
LL	.	A	.	.	.	A	.	.	G	.	G	G	-	G	.	.	1
MM	A	.	.	G	.	G	G	-	T	.	.	.	G	.	.	1
NN	.	T	.	.	.	A	.	.	G	.	.	-	.	A	.	.	C	G	.	.	8
OO	.	T	C	-	.	A	.	.	C	G	.	.	1
PP	.	T	C	.	.	A	.	.	G	A	.	-	C	G	.	.	3
QQ	A	.	.	G	.	.	-	G	.	.	7
RR	A	.	T	G	.	G	G	-	G	.	.	3
SS	A	.	.	G	.	G	G	-	G	.	.	4
TT	A	.	.	G	.	G	A	-	G	.	.	1
UU	A	.	.	G	.	G	G	-	.	C	.	.	G	.	.	1

(especially at local levels) may provide significant information for subsequent research.

Nucleotide variation was assessed from the mitochondrial 16S rRNA gene. The application of mitochondrial DNA to elucidate both the identity (Szalanski et al. 2003) and phylogeny (Austin et al. 2002, 2004a, b, c) of *Reticulitermes* termites, and *R. flavipes* (Austin et al. 2005) in particular, has been recently demonstrated. Mitochondrial DNA is a single circular molecule that is apparently inherited only through females and does not undergo recombination; it lacks complicating features such as introns and transposable elements; evolving as much as 10 to 20 times faster than most nuclear genes (Brown et al. 1979, Futuyma 1998), perhaps because of a higher mutation rate owing to less effective DNA repair (Futuyma 1998). Apart from its general versatility, mtDNA is a valuable marker

because it eliminates maternal gene flow through maternal lines (Avice 1991, 1994) and because mtDNA is more numerous than nuclear DNA (the ratio of mitochondria to nuclei being far greater per cell), it can be more easily detected in old samples (Loxdale and Lushai 1998). This is particularly important when dealing with samples that are recovered from older collections or in variable circumstances that preclude careful handling or preservation of samples.

Uva et al. (2004) demonstrated that nuclear markers such as the ribosomal internal transcribed spacer (ITS2) can be useful, but not without their own difficulties. For example, the application of this nuclear marker could not fully resolve the phyletic relationships between 10 *Reticulitermes* haplotypes in their study, but there was strong bootstrap support for branching patterns of haplotypes that corresponded to

Table 2. Collection data and haplotypes (frequency) for *R. flavipes* from the United States, Canada, and Mexico

Location (n)	County/parish, haplotype(s) (n)
United States	
Alabama (5)	Bullock (Z); Colbert (X); Dallas (Z); Henry (FF); Houston (FF)
Arkansas (93)	Arkansas (F-2); Boone (F, Q, U); Carroll (F); Clark (F); Clay (G, Q); Conway (G, J); Craighead (G, P, R, X, Y); Desha (F-2); Faulkner (S-3, Z); Franklin (BB); Garland (F-2, P, T); Grant (V); Greene (F); Howard (F-2); Independence (F); Jackson (F, G, Q-2, U); Jefferson (F, M); Lawrence (F-2, Q-2, U-2, W, V); Little River (M); Madison (F-3, R-2, QQ-3); Monroe (F); Newton (F); Phillips (F); Pike (G, Q); Polk (F-2); Pope (R); Pulaski (G-2, QQ-2, V-2, AA); Randolph (F, V-2); Sebastian (F, M, U); Sharp (F); Union (F); Washington (F-3, M, Q, T, R-2, U-2, V, Y, GG); White (R)
Arizona (1)	Cochise (M)
California (1)	Sacramento (LL)
Colorado (5)	Boulder (SS); El Paso (RR); Jefferson (L, QQ); Pueblo (RR)
Connecticut (5)	Hartford (PP, Y, Z); Tolland (FF); Windham (EE)
Washington, DC (1)	DC (UU)
Delaware (20)	New Castle (FF, KK, SS, TT, Z-6); Sussex (Z-10)
Florida (8)	Alachua (W, U-2); Broward (P, DD); Okaloosa (FF); Volusia (FF, M)
Georgia (3)	Colquitt (CC, KK); Tift (HH)
Iowa (15)	Johnson (GC, JJ-12, II); Monona (M)
Illinois (1)	Pike (QQ)
Indiana (5)	Grant (MM); Marion (FF); Porter (M-2); Tippecanoe (M)
Kansas (13)	Douglas (Q); Osage (F); Reno (M); Russell (H); Sedgwick (F, L); Shawnee (F-3, L, M, P, U)
Kentucky (2)	Christian (X); Floyd (M)
Louisiana (20)	Beauregard (GG); Caddo (G, M); Calcasieu (M, P); Craighead (G, P); E. Baton Rouge (F-2); Grant (QQ); Jackson (G); Lafourche (F, GG); Ouachita (J); Plaquemines (F); Richland (F); Terrebonne (F-2, S); Vernon (P)
Massachusetts (2)	Chicopee (Z); Hampshire (FF)
Maryland (6)	Cecil (FF-4, Y); Washington (Z)
Michigan (4)	Jackson (Z-2); Kent (Z); Muskegon (TT)
Minnesota (1)	Le Sueur (Z)
Missouri (4)	Clay (U); Howard (R); Jackson (U); Laclede (R)
Mississippi (23)	Hancock (AA, F-2, M-3, X-2, FF-2, HH-2); Harrison (F, M, AA, CC, FF-3); Lee (D); Oktibbeha (EE); Stone (CC); Washington (U)
North Carolina (4)	Brunswick (P, R); Guilford (FF); Wake (Z)
Nebraska (2)	Howard (F); Lancaster (P)
New Jersey (5)	Essex (PP, Z); Gloucester (HH); Middlesex (X); Ocean (Z)
New Mexico (2)	Dona Ana (BB); Harding (BB)
Nevada (1)	Churchill (Z)
New York (6)	Cattaraugus (Z); Kings (Z); Nassau (PP); Otsego (Y, FF); Westchester (Z)
Ohio (7)	Ashtabula (Z); Cuyahoga (Z); Hamilton (Z-2); Shelby (Z); Trumbull (Z-2)
Oklahoma (63)	Adair (P); Beaver (L); Carter (L); Creek (H, T); Delaware (F, P-2, T-2); Greer (N-2, P-2); Kay (L); Le Flore (F); Love (H); McCurtain (F, L); Oklahoma (G, H, L-6, O, P-3, S, T-2); Osage (H, J, L, T-4); Ottawa (Q, U); Payne (C, E-2, F, G, L); Pawnee (P); Tulsa (H, P-7, T-3); Wagoner (P, T)
Pennsylvania (3)	Allegheny (Z); Cumberland (Z); Philadelphia (Z)
Rhode Island (3)	Kent (FF); Providence (FF); Washington (FF)
South Carolina (7)	Aiken (Y); Pickens (F, Y-5)
Tennessee (8)	Haywood (X); Shelby (F-4, M, X, AA)
Texas (124)	Bee (G); Bexar (D); Blanco (H); Bowie (K); Brazoria (E); Brazos (L); Camp (G); Coleman (E); Collin (G); Dallas (C, E-2, F-3, G-4, J, L-6); Denton (C, F); Ector (F, L-6); Edwards (B); El Paso (H); Erath (F, G-2); Fort Bend (G, I); Galveston (E, I); Harris (C, E, F-2, J); Henderson (C, F-2, G, H, J); Hood (E, H); Hunt (E, G); Hutchinson (L, G); Jefferson (E, F-2, G, L); Kaufman (E, F, G, L); Lamar (F, G-4, L-2); Leon (F, G); Lubbock (E, F, I); McLennan (D, I); Midland (D, G, L); Milam (G, L, M); Montgomery (H, J, M); Moore (E, H, J-2, M); Nueces (A, M); Polk (A, G, H); Potter (G, H); Smith (H, G); Tarrant (C, G-2, L-2); Travis (E, F, G); Val Verde (E, F, G); Waller (B, E, G); Williamson (C, E, F)
Utah (1)	Unitah (L)
Virginia (2)	Suffolk (L, U)
Wisconsin (6)	Brown (X); La Crosse (RR), Milwaukee (H); Rock (SS-2); Trempealeau (RR)
West Virginia (2)	Fayette (X); Kanawha (Z)
Wyoming (1)	Goshen (U)
Canada	
Ontario (1)	Toronto (V)
Mexico	
Nuevo Leon (11)	Monterrey (B, NN); Garza—San Pedro (NN)
Jalisco (1)	Guadalajara (OO)

samples from isolated geographic designations. Furthermore, there was no significant difference ($df = 15$, $P = 0.30$) in the rate(s) of sequence evolution when

applying a molecular clock (Uva et al. 2004). Based on findings from our laboratory, the 16S rRNA mtDNA marker has proven to be more consistent and reliable

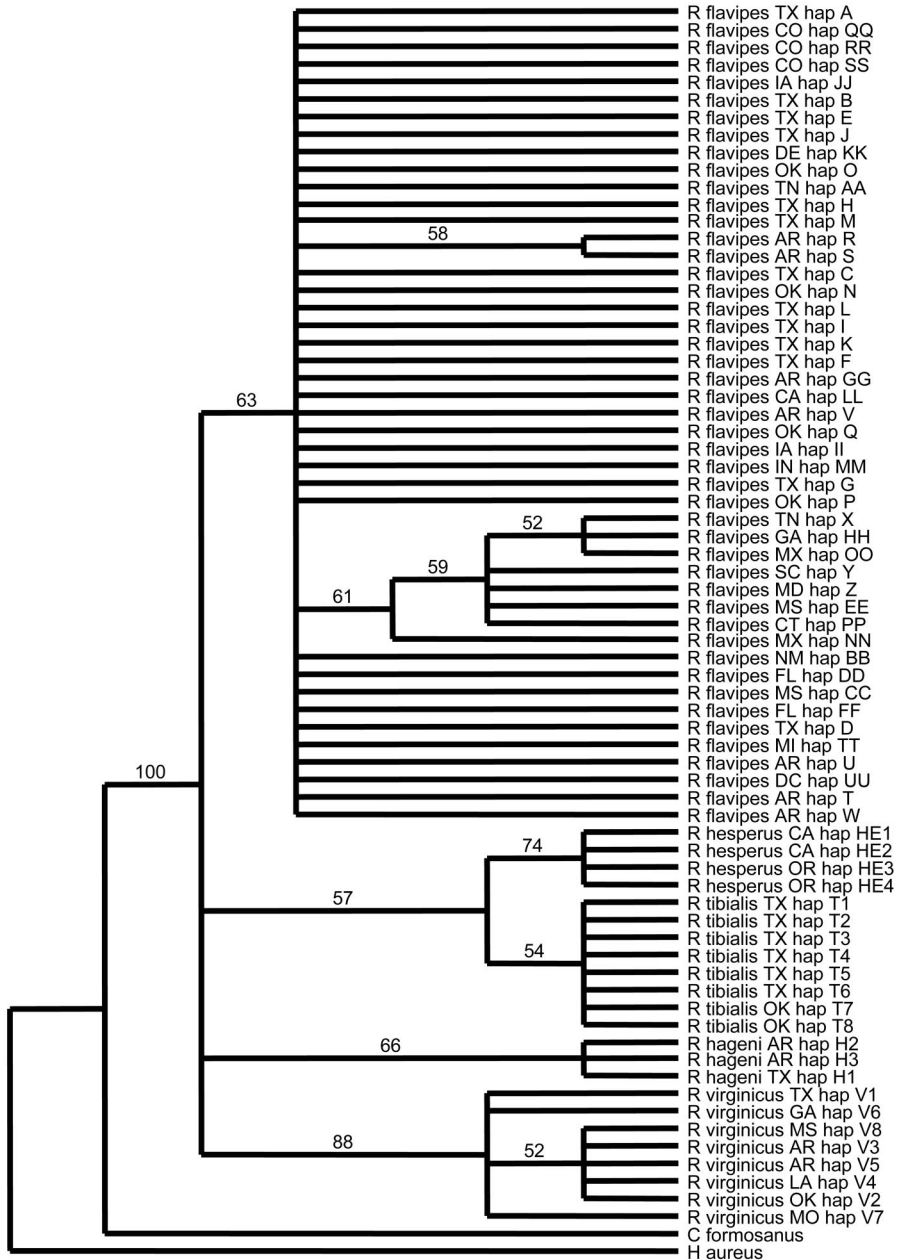


Fig. 2. Single most parsimonious tree during a branch and bound search using PAUP*. Bootstrap values for 1000 replicates are listed above the branches supported at $\geq 50\%$.

than other mtDNA markers or nuclear markers when applied to specimens from the genus *Reticulitermes*.

Genetic analysis revealed a large amount of genetic variation within *R. flavipes*, more than any other *Reticulitermes* species from North America (J.W.A., unpublished). Variation in genes as well as in environmental conditions is translated by processes of development into variation in phenotypic characters, such as morphological features (Futuyma 1998), which may provide some ideas for the differences (in

haplotypes), we observe in the current study. An extensive evaluation of *R. flavipes* has revealed that some recognized species share common haplotypes with the sequence data presented herein. For example, *R. arenicola* Goellner (Goellner 1931) taken from the sand dunes of Lake Michigan in Indiana share haplotype M, a common haplotype found in numerous states. Because *R. arenicola* has already been demonstrated to be phylogenetically most similar to *R. flavipes* (Ye et al. 2004), there is little surprise that

the extensive nature of this study has revealed that *R. arenicola* is most probably a morphological variant of *R. flavipes*. It could be argued that a nutrient poor environment, such as the sand dunes of Lake Michigan, provide just such an environment that may contribute to isolation of this morphologically variable *R. flavipes* (= *R. arenicola*) sample.

R. flavipes from Canada haplotype V was also found in 9.0% of the samples from Arkansas. First noticed in the Toronto waterfront area in 1938, they have since spread to ≈ 1000 city blocks, imposing a potential economic risk of \$12 billion to property with a \$120 million annual devaluation (Myles 2004). Morphologically variable samples from Monterrey, Mexico, also were identified as *R. flavipes* (Table 2). Although not the aim of this study, there were additional samples from Europe and South America that share common haplotypes with *R. flavipes* from the continental United States (Austin et al. 2005).

Although one goal of applying molecular markers such as 16S is to identify morphologically ambiguous samples (Szalanski et al. 2003), another might be to identify the source of introductions to nonnative areas (Szalanski et al. 2004a). Recently, Austin et al. (2005) have documented the presence of *R. flavipes* in South America and that introductions there share identical genetic haplotypes from both the United States and Europe (France and Germany). Exotic introductions of *R. flavipes* should be viewed with caution in South America, where Constantino (2002) suggested the likely importation to neighboring Brazil, Paraguay, and Argentina. Misidentified as *R. lucifugus* (Aber and Fontes 1993, Aber 1998), populations of *R. flavipes* were found in Santiago, Chile, and from Montevideo, Uruguay. More intensive collections of *Reticulitermes* from Mexico would be highly desirable to determine whether the relative proximity of Mexico and/or possibly trade between Mexico and South America may suggest an introduction of *R. flavipes* (to South America) other than from the United States or Europe.

We found a single *R. flavipes* haplotype in California, haplotype LL that has likely been introduced from eastern or neighboring states. However, the presence of undetected residing populations is equally plausible. We speculate that these allopatric populations are introduced (to California) because they are well beyond their described distribution range (Banks and Snyder 1920, Snyder 1954, Weesner 1965), and we would expect their destructive nature would have alerted their presence to researchers or pest management professionals. In addition, we propose that rapid transit opportunities and human aided disturbances may contribute to the poor resolution of population structure (based on geographic correlations of mtDNA haplotypes) of *R. flavipes* in North America.

Because the dispersal capacity of *R. flavipes* varies, it is difficult to assign geographic limits to any given population. Therefore, a more plausible explanation (of population structure) would involve identification of unique population entities based on habitat preference. For example, Clément (1986) proposed that in moister habitats with abundant wood resources, alien

conspecific individuals may contribute to extrinsic sources of gene flow forming large complex groups. This means that they may contribute to variable haplotype patterns for any given population. Alternatively, termites that occupy drier habitats with more limited food resources may result with populations that are relatively small and far apart.

In an extensive population genetic analysis of *R. flavipes* from North Carolina, Vargo (2003) suggests that there is extensive gene flow in *R. flavipes*, even at large spatial scales, and this is likely due to populations genetically adapted to local ecological conditions. The lack of population structure based on the relationship between mtDNA haplotypes and geography observed in our study has also been supported in previous studies from Georgia (Jenkins et al. 1999), Texas, Oklahoma, Arkansas, and Louisiana (Austin et al. 2004a, b, c). Applying allozymes, Reilly (1986) found some structure across a relatively larger range, whereas Bulmer et al. (2001) was unable to detect significant differences over a smaller spatial scale. In fact, in many real populations, there may not be any obvious individual populations or substructure at all, and the populations are said to be continuous. This scenario is unlikely given the large geographic distances between populations in this study. Even in effectively continuous populations, different areas can have different gene frequencies, because the whole metapopulation (a "population of populations") is not panmictic. For example, the *R. arenicola* samples we evaluated in the current study were morphologically consistent with the descriptions for this species by Goellner (1931), but they were genetically consistent with *R. flavipes* haplotype M, a common haplotype found throughout the United States. Likewise, it cannot be stated that our genetic marker lacks sufficient variability among termite groups to effectively differentiate them. The application of the 16S rRNA mtDNA marker has proven successful in phylogenetic studies of *Nasutitermes* (Scheffrahn et al. 2005a, b), *Heterotermes* (Szalanski et al. 2004a), *Coptotermes* (Scheffrahn et al. 2004), *Anacanthotermes*, *Kalotermes*, *Cryptotermes/Procratotermes* (J.W.A., unpublished), other *Reticulitermes* (Austin et al. 2004a, b, c, 2005) groups, and in molecular diagnostics of *Coptotermes* (Szalanski et al. 2004b). Molecular evidence suggests that some species of termites have been misidentified due to variable or indiscernible morphological characters. For example, *Coptotermes gestroi* (Wasmann) (Kirton and Brown 2003), *Nasutitermes corniger* (Motschulsky) (Scheffrahn et al. 2005b), and *Reticulitermes santonensis* (Feytaud) (Austin et al. 2005) have all been synonymized with other species that have equivocal morphological identities that have only recently been clarified, some with the application of molecular markers such as the 16S rRNA gene. Many specimens (e.g., collections without diagnostic termite castes) are still waiting to be identified by more comprehensive techniques like DNA sequencing (Austin et al. 2004b) or by molecular diagnostics (Szalanski et al. 2003).

Because some researchers historically interpreted *Reticulitermes* variants at more local levels (Goellner

1931), some identities remain elusive. For example, *R. flavipes* found in Arizona, California, and Nevada were presumably introduced from eastern states, but they have gone largely unnoticed or ignored because our biases associated with what we think we know about their historical distributions. Similarly, new genetic evidence has resurrected the identity of some cryptic termite species in the eastern United States and has targeted potentially new species that warrant further investigation (J.W.A., unpublished).

This study demonstrates that *R. flavipes* has the largest distribution of any *Reticulitermes* in North America, with more western distributions than previously reported (Fig. 1). This is not surprising given its establishment ability around the world (Austin et al. 2005). Furthermore, *R. flavipes* possesses significant genetic variation while maintaining its monophyly (Fig. 2) with all described sympatric congeners from North America.

Acknowledgments

We thank R. Gold, B. Foster, M. Hafley, B. Kard, R. Houseman, D. Miller, M. Fisher, G. Henderson, J. Hyde, T. Myles, R. Saran, P. Pachamuthu, S.-W. King, and numerous pest management professionals for providing samples. This research was supported in part by the University of Arkansas, Arkansas Agricultural Experiment Station.

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Received 12 November 2004; accepted 30 June 2005.