CORRELATION OF MITOCHONDRIAL HAPLOTYPES WITH CUTICULAR HYDROCARBON PHENOTYPES OF SYMPATRIC *Reticulitermes* SPECIES FROM THE SOUTHEASTERN UNITED STATES

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Abstract—Three sympatric *Reticulitermes* species have been identified in Georgia, USA, based on morphological characters from alates and soldiers: *R. flavipes*, *R. virginicus*, and *R. hageni*, but species identification at individual collection sites is often difficult because alate production is seasonal and soldiers comprise 1–3% of the colony. We therefore set up an experiment to determine if chemical phenotypes and mtDNA haplotypes can be used together to separate species of subterranean termites. Subterranean termites of the order *Reticulitermes* (Isoptera: Rhinotermitidae) were collected from 20 inspection ports across four soil provinces in Georgia. Each collection was identified to species using dichotomous keys. Two collections, HH11 and BH25, however, could not be unequivocally keyed to species and were classified as unknown. The mitochondrial cytochrome oxidase II (COII) gene was sequenced from individual members of each collection and the variation in cuticular hydrocarbon phenotypes from these same collections was characterized. The cuticular hydrocarbon and mtDNA phylogenetic analyses show agreement with both unknown collections falling out in a separate clade.

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Specimens from HH11 nad BH25 are different morphologically, chemically, and genetically from the three known sympatric species in Georgia. Our results suggest that these two collections may represent at least one new taxon in *Reticulitermes*. Furthermore, the association of cuticular hydrocarbon phenotypes and mtDNA haplotypes demonstrates that, when combined with morphological characters, they are useful in separating known species, determining new species, and understanding termite evolution.

Key Words—*Reticulitermes*, MtDNA, haplotype, cuticular hydrocarbon, phenotype.

INTRODUCTION

Three species of *Reticulitermes* from the southeastern United States have been described: *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks), and *R. hageni* Banks (Nutting, 1990; Weesner 1965, 1970). These three species are sympatric throughout Georgia, the largest state in the region. Georgia covers 15.1 million hectares and provides a variety of subterranean termite habitats (Figure 1): steeply sloped mountains in the Blue Ridge (BR) in the north, Southern Piedmont (SP) foothills underlain with metamorphic rock; gently sloping terrain covering marine sands, loam, and/or clary in the Southern Coastal Plain (SCP); and barrier islands in the Atlantic Coastal Flatwoods (ACF) along the Atlantic Coast in the southeast (Perkins and Shaffer, 1977). Although additional *Reticulitermes* species have been suggested in Georgia on the basis of soldier secretions (Clément et al., 1985, 1986), chemical analysis of cuticular hydrocarbons (Haverty et al., 1996a, 1999a), and phylogenetic analysis of mtDNA sequence data (Jenkins et al., 1999a), no new species have been confirmed or assigned.

Dichotomous keys have been published for identifying *Reticulitermes* species based on characters of alates and/or soldiers (Snyder, 1954; Weesner, 1965; Nutting, 1990; Scheffrahn and Su, 1994), relying on synonymies published in a catalog of the Isoptera (Snyder, 1949) and on material published nearly 80 years ago (Banks and Snyder, 1920). Difficulties arise in species determination at individual collection sites because the worker caste is the most abundant within a subterranean termite colony. Finding an imago (or 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 often results in equivocal species determinations (Weesner, 1965). To make matters even more confusing, we have collected termites from a single piece of wood and keyed the soldiers to *R. hageni* and the alates to *R. virginicus* (Haverty et al., 1996a). Recent work using chemotaxonomic, genetic, and behavioral characters (Haverty and Nelson, 1997; Haverty et al., 1996a, 1999a,b; Polizzi and Forschler, 1998; Jenkins et al., 1999a) reiterate the need to revise the taxonomy of the genus *Reticulitermes*, as has been suggested frequently (Weesner, 1970; Nutting, 1990; Scheffrahn and Su, 1994).

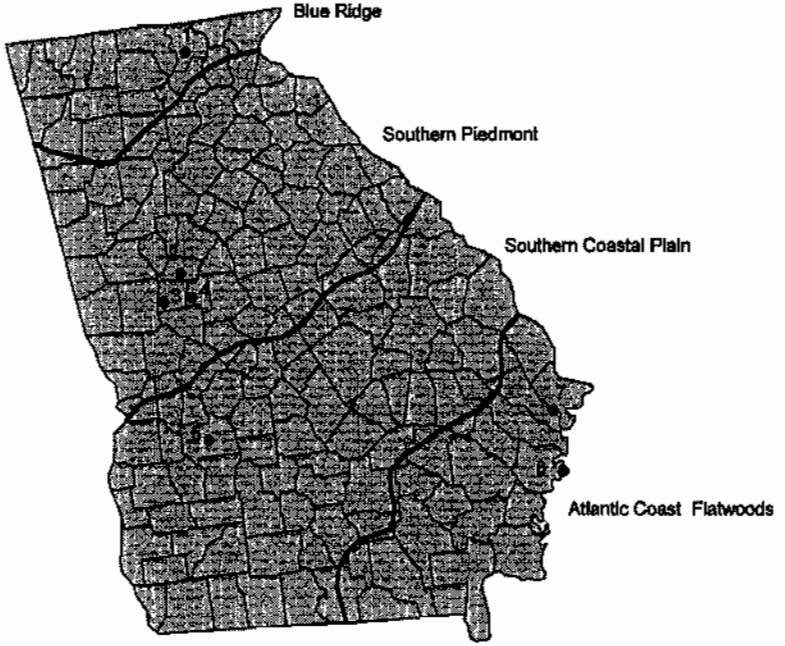

FIG. 1. Sampling sites and soil provinces in the state of Georgia, U.S.A. Numbers refer to sampling sites listed in Table 1 and soil provinces are designated at the right of the map.

Hydrocarbon phenotypes and mitochondrial DNA (mtDNA) haplotypes have been independently examined to assess the validity of described species of *Reticulitermes* from Georgia. First, there is mounting evidence that hydrocarbon mixtures are species-specific in termites (Brown et al., 1990, 1996; Watson et al., 1989; Bagnères et al., 1990; Haverty et al., 1988, 1990, 1991, 1992; Haverty and Nelson 1997; Kaib et al., 1991; Howard and Blomquist, 1982; Howard et al., 1988). Cuticular hydrocarbons were extracted from 91 different groups of workers sampled from colonies across four soil provinces in Georgia. Chemical analyses showed 15 unique hydrocarbon phenotypes among those *Reticulitermes* collections (Haverty et al., 1996a, 1999a). Based on keys to the alates and soldiers, three of the cuticular hydrocarbon phenotypes correspond with *R. flavipes* and one with *R. virginicus*. The remainder all key to *R. hageni*, based on soldiers, and either *R. virginicus* or *R. hageni* based on alates.

Second, assuming that a single pair of adults initiates a subterranean termite colony, each colony should have a unique and possibly species-specific mitochondrial DNA (mtDNA) genotype, because mtDNA is a haploid, matrilineally inherited molecule. We also recently extracted mtDNA from *R. flavipes* and *R. virginicus* individuals sampled from across four soil provinces in Georgia (Jenkins et al., 1999a). A 400-bp fragment from their A+T-rich region was sequenced and phylogenetically evaluated indicating population and species differences (Jenkins et al., 1999a). Subterranean termite colony associations are presently based on traditional field protocols that not only can provide ambiguous results (Forschler and Robinson, 1999), but cannot unequivocally delineate a colony, or its "movement and resource utilization patterns" (Jenkins et al., 1999b). We use the word population to refer to collections from inspection ports (Table 1), none of which are known to be connected by mark–release–recapture (MRR) (Forschler and Jenkins 2000). MRR is a technique in which termites are taken from an inspection port, marked with a dye, and released back into the inspection port from whence originally collected. After a specified time all the inspection

TABLE 1. GEORGIA TERMITE COLLECTION DESIGNATIONS AND GENERAL INFORMATION FOR EACH COLLECTION SITE

Species[a]	Collections	Sample size	Map No.[b]	Soil province[c]	CHP[d]
R. flavipes	C1	4	1	BR	A
	PCH2	4	1	BR	A
	BB	2	3	SP	A
	BWS	6	3	SP	A
	Tyler3	2	4	SP	A
	P9	3	5	SCP	A
	N2	3	6	ACF	AB
	BH17	4	6	ACF	AB
	BH2	3	6	ACF	AB
	Conf2	3	7	ACF	AB
R. virginicus	93	4	2	SP	C
	94	7	2	SP	C
	95	2	2	SP	C
	N4	3	6	ACF	C
R. hageni	Andy1	3	4	SP	D
	Dukes	3	4	SP	D
	H19-2	3	4	SP	D
	Tyler6	3	4	SP	D
Unknown	HH11	3	5	SCP	I
	BH25	3	7	ACF	L

[a] Species determined from alate and soldier morphology. When alate and soldier morphology do not agree or when difficult to identify from morphological characters, we consider the population to be unknown.
[b] Refer to Figure 1.
[c] Soil provinces: BR = Blue Ridge, SP = Southern Piedmont, SCP = Southern Coastal Piedmont, and ACF = Atlantic Coastal Flatwoods.
[d] Cuticular hydrocarbon phenotype.

ports near where the marked termites were released are sampled. Inspection ports with marked termites are assumed to be connected and part of a colony unit.

Here we report the results of a study in which cuticular hydrocarbons (CH) and mtDNA sequence data were used to evaluate the accepted taxonomy of *Reticulitermes* in Georgia. Our primary goal was to determine if chemical phenotypes and mtDNA haplotypes could be used together to separate species. These data may then be used, along with morphological and soldier defense secretion characters, in future taxonomic and phylogenetic studies.

METHODS AND MATERIALS

Specimens. Termites were collected from established inspection ports located in four soil provinces in Georgia or by directed collections from infested wood (Forschler and Townsend, 1996; Haverty et al., 1996a, 1999a, Jenkins et al., 1999a). Species were identified as *R. flavipes*, *R. virginicus*, or *R. hageni* using published dichotomous keys (Weesner, 1965; Nutting, 1990), or as unknown based on lack of congruence with these published keys (Haverty et al., 1996a). Localities and specific details of each inspection port used in this study are presented in Figure 1 and Table 1. Sample collection methods and cuticular hydrocarbon analysis were detailed in Haverty et al. (1996a, 1999a). Voucher specimens for each collection were placed in 70% ethanol and are maintained by BTF at the University of Georgia. *Coptotermes formosanus* Shiraki, collected in New Orleans, was used as the outgroup to root both the mtDNA and CH cladograms. Each collection contained enough individuals for cuticular hydrocarbon characterization (200 workers), DNA sequencing (2–7 workers), and voucher specimens (≥5 soldiers, workers, and/or alates).

Characterization of Cuticular Hydrocarbon Phenotypes. Cuticular hydrocarbons were characterized and cuticular hydrocarbon phenotype separations were made according to procedures in Haverty et al. (1996a, 1999a), without prior knowledge of the species determinations. Cuticular hydrocarbons were identified by gas chromatography–mass spectrometry (GC-MS). The 142 cuticular hydrocarbons identified from *Reticulitermes* phenotypes GA-A, GA-AB, GA-C, GA-D, GA-I, and GA-L were used as phenotypic characters (Haverty et al., 1996a,b, 1999a). In the text and figures, we use shorthand nomenclature to identify hydrocarbons. This shorthand uses a descriptor for the location of methyl groups (X-me), the total number of carbons (CXX) in the molecule exclusive of methyl branches, and the number of double bonds follows a colon (CXX:Y). Thus *n*-pentacosane becomes *n*-C25; 13-methylpentacosane becomes 13-meC25; 11,15-dimethylpentatriacontane becomes 11,15-dimeC35; and pentacosatriene becomes C25 : 3. In no case were the locations of the double bonds determined. None of the individual *Reticulitermes* taxa or *C. formosanus* contained all 142 hydrocarbons.

Phylogeny Analyses Based on Cuticular Hydrocarbon Phenotypes. Parsimony analyses were performed using PAUP [Mac version 3.1.1 of Swofford (1993)] to compare degrees of relatedness among *Reticulitermes* based on chemical phenotypes and mtDNA analyses. Each discrete hydrocarbon character state was scored as a fraction of the total cuticular hydrocarbon: 0 = absent or not detected, 1 ≤0.3%, 2 = 0.3–1.0%, 3 = 1.0–3.0%, 4 = 3.0–6.0%, 5 = 6.0–10.0%, 6 = 10.0–20.0 5, and 7 ≥20%. With the small data set of seven taxa and 142 hydrocarbon characters, the exhaustive search algorithm of PAUP was used to find the most parsimonious trees. Support for relationships among phenotypes was assessed by bootstrap analysis using 1000 replications.

DNA Extraction, Amplification, Sequencing. Genomic DNA was extracted from individual whole termites (Jenkins et al., 1999a). Primers TM-N-193 (TGGGGTATGAACCAGTAGC) and SR-J-14613 (TAGGGTATCTAATCCTAGTT) (Taylor et al., 1993) were used to amplify the entire A+T-rich region. Primer AT-J-T1 (CACTAAGGATAATCAATTATACGTC) was designed from *R. flavipes*, *R. virginicus*, and *R. hageni* original sequence data (Jenkins et al., 1999a) and was used as a reverse primer to amplify and sequence a 400-bp fragment.

Polymerase chain reaction (PCR) amplification was done according to Jenkins et al. (1999a) in a Perkin-Elmer Gene Amp PCR System 9600. Electrophoresis of amplification products and size standards, as well as fragment size estimation, was accomplished according to Jenkins et al. (1999a,b). Primers AT-J-T1 and TM-N-193 were then used to sequence complementary strands of a 400-bp fragment from 67 individual samples representing 20 collection sites or inspection ports (Jenkins et al., 1999a) in Georgia. (Table 1). Insect mtDNA was verified as in Jenkins et al. (1999a).

Standardization. Insect mtDNA has both a gene order and transcriptional orientation unique to its class. Therefore, we used the standardized format for insect mtDNA primer names and strand identification (J for majority strand and N for minority strand) established by Simon et al. (1994). Also as per Simon et al. (1994), the primers that were designed from our unpublished sequence data do not have numerical locations. Sequence data have been submitted to GenBank.

Statistical Analysis. Sequencer individual chromatograms were edited; contigs made, and alignments done for 312 segregating sites beginning at $tRNA^{gln}$ and ending 150 bp into the A+T-rich region. Consensus sequences were then assigned, multiple consensus sequences of all samples aligned with Malign (Hein, 1989), and aligned sequences reformatted for PHYLIP (Felsenstein, 1993) and bootstrapped 1000 times (Jenkins et al., 1999a). The same bootstrapped data were then used with DNAPARS and CONSENSE to produce the parsimony tree (P) (Felsenstein, 1993). The parsimony tree was drawn with TREEVIEW PPC (Page, 1999). A second neighbor-joining (NJ) tree was also constructed (not shown) as in Jenkins et al. 1999a,b).

The Hudson, Boos, and Kaplan (HBK) (Hudson et al., 1992) statistic was used to test the null hypothesis of no genetic differentiation between populations as outlined in Jenkins et al. (1996).

The Kluge and Farris (1969) consistency index (CI) that measures the level of support for each tree and the Farris (1969) retention index (RI) that measures the congruency of characters to the cladogram and to each other were calculated for the PAUP parsimony analysis of cuticular hydrocarbon phenotypes (Figure 7 below). The CI will equal 1 when the data set explains the tree as well as possible, and the RI will equal 1 when the characters in a data set are totally congruent with each other and the tree.

RESULTS

All but two of the collections were positively identified using published morphometric keys. Specimens taken from inspection ports HH11 and BH25 could not be assigned to species unequivocally. The alates from HH11 keyed to *R. virginicus* and the soldiers keyed to *R. hageni*. We collected no alates from BH25, but soldiers collected keyed to *R. hageni*. Cuticular hydrocarbon phenotypes for these two equivocal samples were each unique and were identified as phenotype I (Figure 2) and phenotype L (Figure 3) for HH11 and BH25, respectively (Haverty et al., 1996a, 1999a). In addition, the cuticular hydrocarbon and mtDNA cladograms show HH11 (I) and BH25 (L) in separate clades (Figures 4 and 5), as do the neighbor-joining (NJ) phenograms for both (not shown). Of the remaining collections, *R. flavipes* specimens were collected from all soil provinces, *R. virginicus* specimens from both the SP and ACF soil provinces. All of the *R. hageni* specimens, verified by both soldier and alate morphology, were collected from the SP soil province (Figure 1, Table 1). Specimens collected from BH2 and BH17 inspection ports were determined to be part of the same colony (Forschler, unpublished MRR data). Cuticular hydrocarbon phenotypes, like the mtDNA haplotypes, corresponded well with the morphometric species determinations, with two exceptions. The chemical phenotypes are identified as A and AB for *R. flavipes* (Figure 6), C for *R. virginicus* (Figure 7), and D for *R. hageni* (Figure 8).

Phylogenetic analyses utilizing cuticular hydrocarbon characters indicate that phenotypes AB and C are closely related and different from the closely related phenotypes I and L. The phenotypes of the colonies studied here fall into two clades or lineages: phenotypes A, AB, and C belong to a lineage with a predominance of internally branched mono- and dimethyl alkanes, and phenotypes D, I, and L belong to a lineage with a preponderance of unsaturated hydrocarbons (Figure 4). Phenotypes A and AB correlate with morphological determinations for *R. flavipes:* AB phenotype from *R. flavipes* collected from

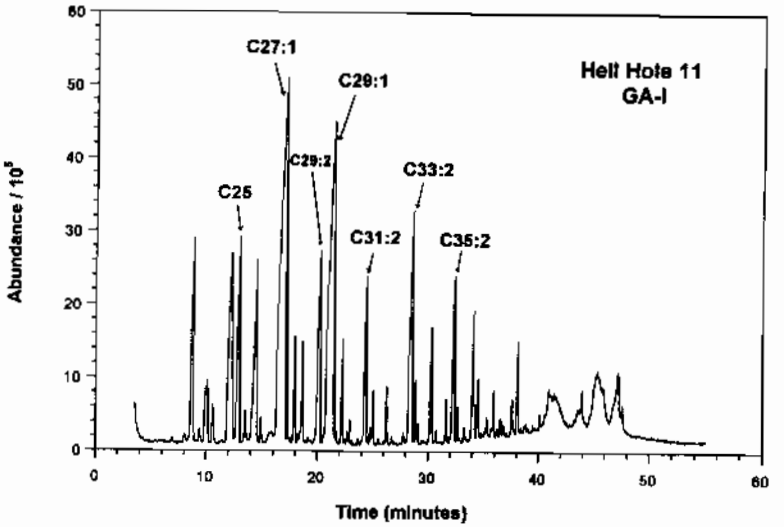

FIG. 2. Total ion chromatogram of cuticular hydrocarbons from phenotype I specimens [Hill Hole (HH11)] from Plains, Sumter County, Georgia, and identified as *Reticulitermes hageni* from soldiers and *Reticulitermes virginicus* from alates.

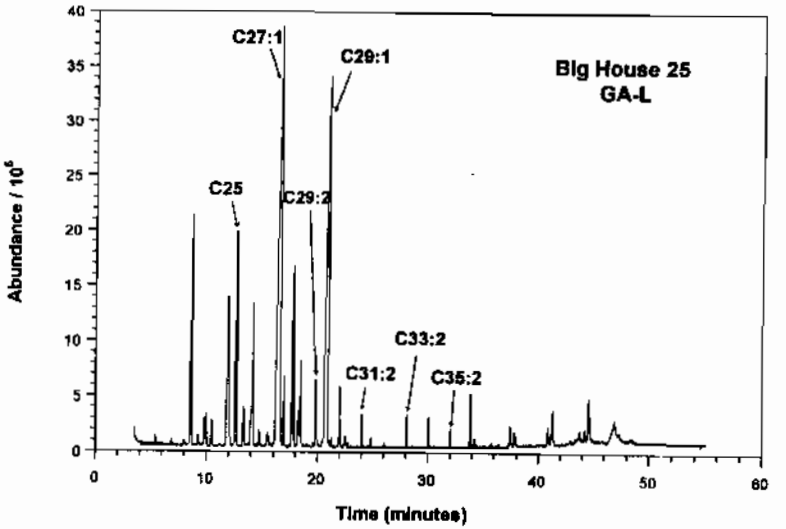

FIG. 3. Total ion chromatogram of cuticular hydrocarbons from phenotype L specimens [Big House (BH25)] from Saplo Island, McIntosh County, Georgia, and identified as *Reticulitermes hageni* from soldiers and *Reticulitermes virginicus* from alates.

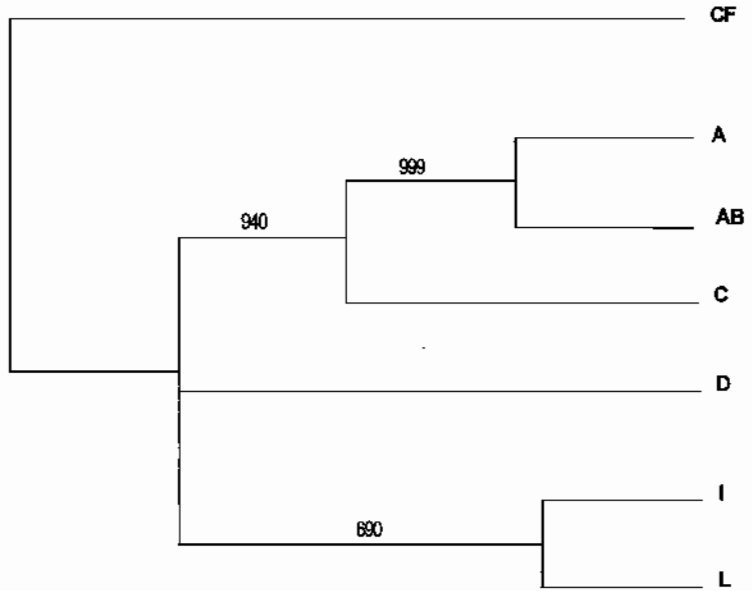

FIG. 4. A strict consensus tree of two trees of equal parsimony for *Reticulitermes* chemical phenotypes from phylogenetic analysis of cuticular hydrocarbon characters with *Coptotermes formosanus* as the outgroup. The tree length is 422 steps; consistency index of Kluge and Farris (1969) is 0.791; retention index of Farris (1969) is 0.573. The numbers above branches are bootstrap values for 1000 replications.

inspection ports on Sapelo Island and A phenotype from *R. flavipes* collected from all other inspection ports. These phenotypes differ primarily by the large quantities of C25 : 3 in phenotype A and the near absence of C25 : 3 and C33 methylalkanes in phenotype AB (Haverty et al., 1996a). This difference is similar to the difference between *Reticulitermes* phenotypes CA-A and CA-A′ from California: phenotypes CA-A and CA-A′ likely represent variants of the same species (Haverty and Nelson, 1997). Phenotypes A and AB have apparent disjunct geographic distributions. Thus far, phenotype AB has only been collected from the coast of Georgia near Savannah and on nearby Sapelo Island. Phenotype C correlates with morphological determinations for *R. virginicus*. This is the only phenotype of *Reticulitermes* to have significant quantities (>3% of the total hydrocarbon component) of 11-meC31 and 11,15-dimeC31 (Haverty and Nelson, 1997; Haverty et al., 1996a, 1999a). Phenotype C is consistent over a wide geographic range, from Louisiana to southern Florida, through Georgia to Maryland and Virginia (Haverty et al., unpublished observations). Phenotype D, I, and L all key to *R. hageni* based on soldier morphology: the width of the

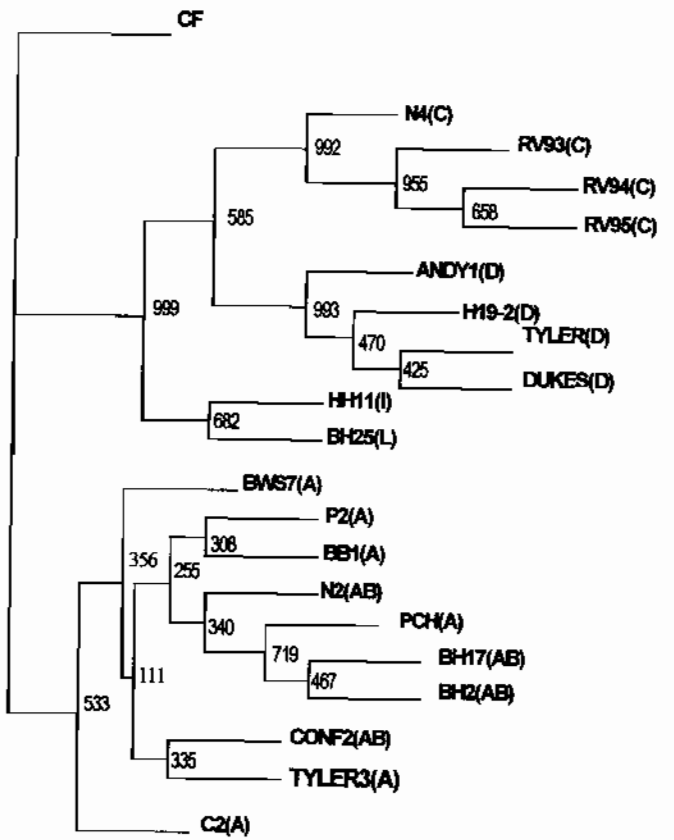

FIG 5. Rooted maximum parsimony (MP) cladogram (Felsenstein, 1993) comparing consensus sequences, fragment of 312 segregating sites (Jenkins et al., 1999a). Numbers at the nodes were generated with SEQBOOT (Felsenstein, 1993) and represent the number of times out of 1000 the node occurred. Capital letters in parentheses represent the cuticular hydrocarbon phenotypes for the specific inspection port.

pronotum is less than 0.7 mm (Scheffrahn and Su, 1994). We have alates from HH11 of phenotype I that appear to be *R. virginicus:* the color of the body varies from dark brown to black and the distance of the ocellus from the compound eye is less than the diameter of the ocellus (Scheffrahn and Su, 1994). Phenotype D differs from phenotypes I and L because D contains a large amount (>20%) of C27 : 2, which is absent in I and L. Phenotypes I and L contain abundant quantities (>10%) of C29 : 1, which is absent in D. Phenotype I differs from L in the abundance and diversity of later eluting components, particularly the

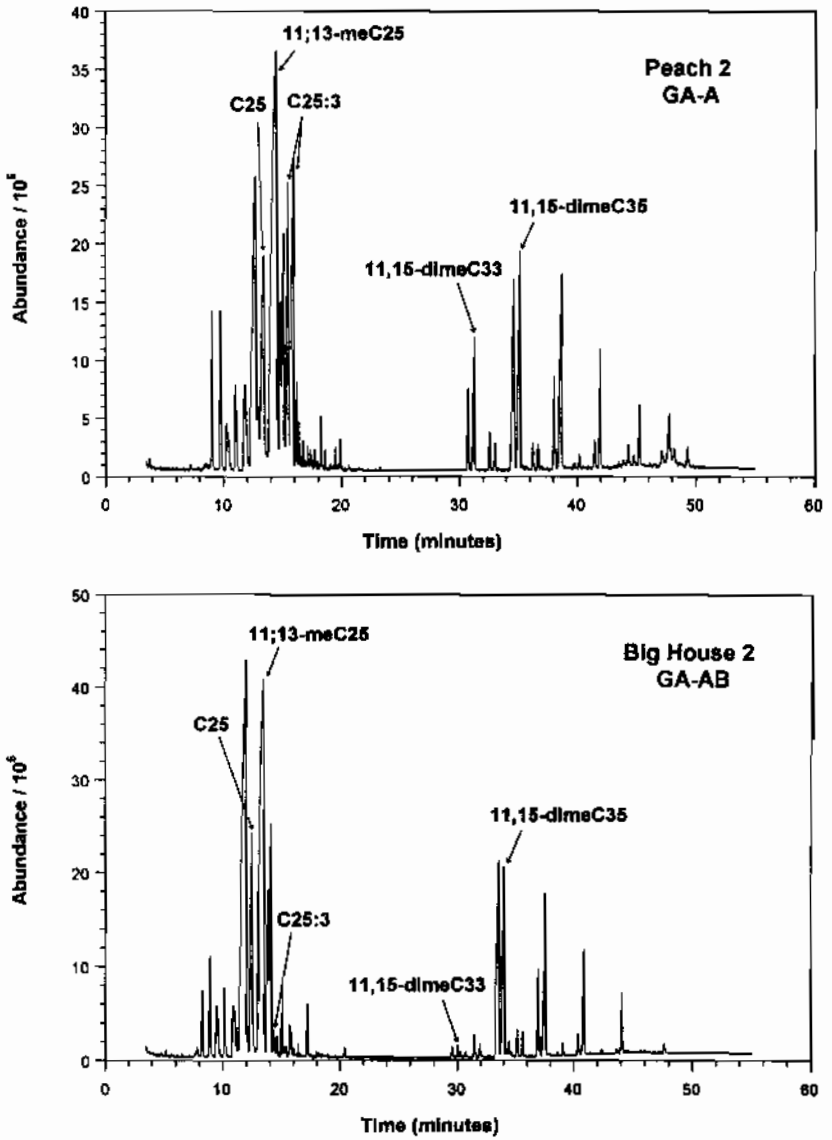

FIG. 6. Total ion chromatogram of cuticular hydrocarbons from phenotype A specimens (Peach colony) from Blairsville, Union County, Georgia, and phenotype AB specimens [Big House (BH 2) from Sapelo Island, McIntosh County, Georgia, and identified as *Reticulitermes flavipes* from soldiers and alates.

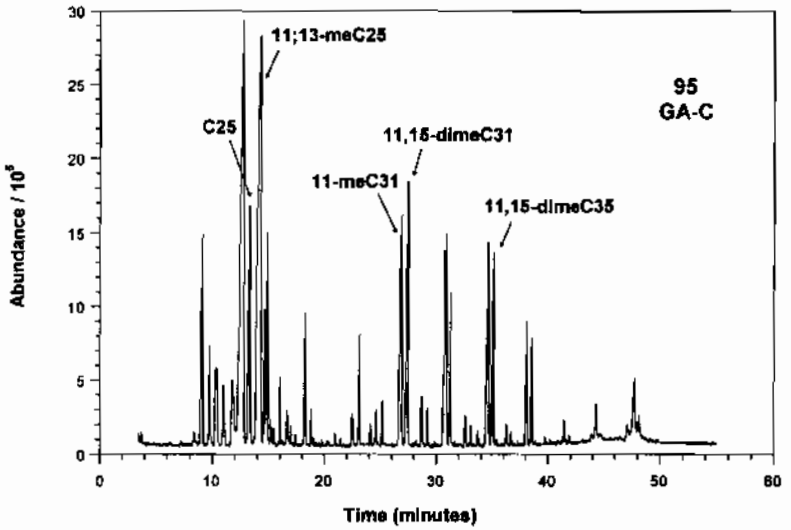

FIG. 7. Total ion chromatogram of cuticular hydrocarbons from phenotype C specimens (95) from Westbrook Farm, Spalding County, Georgia, and identified as *Reticulitermes virginicus* from soldiers and alates.

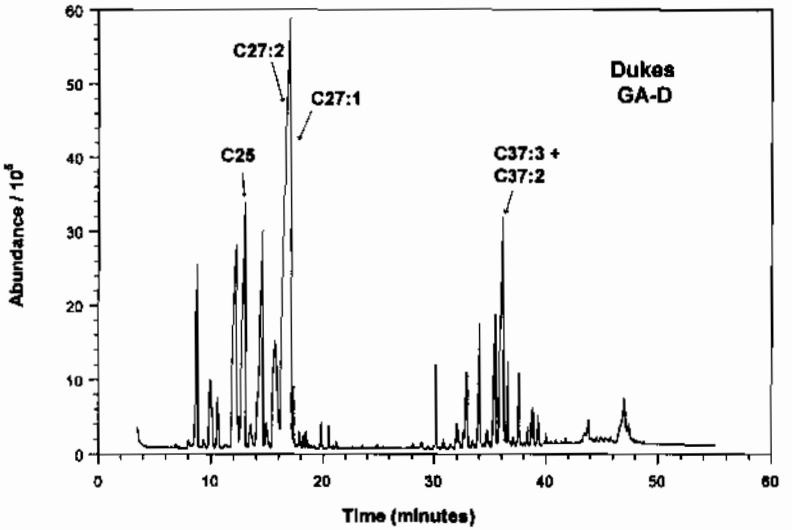

FIG. 8. Total ion chromatogram of cuticular hydrocarbons from phenotype D specimens (Dukes) from Barnesville, Lamar County, Georgia, and identified as *Reticulitermes hageni* from soldiers and alates.

dienes (Haverty et al., 1996a, 1999a). These relationships are corroborated in the mtDNA analyses.

Cladistic analyses of both cuticular hydrocarbon and mtDNA data sets generally agree (Figures 4 and 5). *Reticulitermes flavipes* (hydrocarbon phenotypes A and AB) forms a very supported (999) chemical clade (Figure 4) and a supported (533) mtDNA clade (Figure 5). Figure 4 shows a supported clade [to which both *R. virginicus* (Nina 4, 93, 94, 95), phenotype C and *R. hageni* (Andy 1, Tyler 6, Dukes, House 19-2), phenotype D belong]. This major clade also includes collections from HH11 and BH25 (Figure 4). These individuals that could not be identified unequivocally using morphological keys have unique cuticular hydrocarbon phenotypes and mtDNA haplotypes. They also form a supported clade (682) separate from the three sympatric species. Although the mitochondrial COII gene marker has bootstrap support for several clades including *R. flavipes*, *R. virginicus*, *R. hageni*, and others (Jenkins et al., 1999b) within Georgia, there is little nodal support within the *R. flavipes* using this A+T-rich mtDNA marker (Figure 4).

The HBK statistic was calculated to determine genetic partitioning among species groups and clades identified in the cladogram (Figure 4). Partitioning among *R. flavipes*, *R. virginicus*, and *R. hageni* (Ks^* = 2.2480–2.2580, Zs^* = 3.1587–3.2981) was significant ($P < 0.05$), indicating genetic differentiation. The Ks^* and Zs^* values for comparison of HHII/BH25 clade and *R. hageni*, D clade (Ks^* = 1.6695, Zs^* = 1.0965) and for *R. virginicus*, C clade and HHII/BH25 clade (Ks^* = 1.9103, Zs^* = 1.0965) were not significant ($P > 0.05$). HBK statistic for *R. flavipes*, A and AB clade and HH11/BH25 clade (Ks^* = 2.4660, Zs^* = 3.0672) was significant ($P < 0.05$). Thus, the HBK statistic genetically partitioned the HH11/BH25 clade from *R. flavipes* clades A and AB, but not *R. virginicus* clade C and *R. hageni* clade D.

DISCUSSION

Mitochondrial DNA has been used to demonstrate intraspecific geographical localization from which phylogeography has been deduced (Juan et al., 1996) and colonization patterns elucidated (Jenkins et al., 1996a). Inspection of the rooted phylogenetic patterns in Figure 5 reveals low bootstrap values for *R. flavipes* that likely reflect a limited number of substitutions, a situation that we know improves when more sequence data are used (Jenkins et al., 1999b). The cladogram topology shows no geographic localization (Figures 1 and 5, Table 1). Gene flow, therefore, appears to be unrestricted, especially in the *R. flavipes* collections. Figure 5 demonstrates that the clade containing *R. virginicus*, *R. hageni*, HH11 (phenotype I), and BH25 (phenotype L) is separated from the *R. flavipes* clade by supported nodes. The *R. flavipes* clade, however, demonstrates

strikingly low levels of molecular divergence with unsupported nodes (Figure 5). Thus, it is difficult to resolve *R. flavipes* lineages using phylogenetic analyses with this mtDNA marker. Since individual internal nodes are not supported, they should be collapsed into a single node. This means that *R. flavipes* collections do not show geographical localization with this marker, whereas there is geographic separation, as mentioned previously, of the two hydrocarbon phenotypes of *R. flavipes*, A and AB. The phylogenetic analysis indicates that gene flow is unhindered by either geographic or physical barriers (Figure 5).

Cuticular hydrocarbon analysis was done on collections from inspection port BH2. MRR results (Forschler, unpublished data) indicated that BH2 and BH17 were connected. Thus, it was expected that mtDNA sequences from BH2 and BH17 would be the same. They were not (Figure 5). Although the bootstrap results were <50% (Figure 5), the mtDNA sequence between BH2 and BH17 was different (Figure 5). The molecular data for these two inspection ports, as in recent studies (Jenkins et al., 1999b), appear to differ from field data and expectations for colony structure. Furthermore, because the matrilineal line collected from BH2 was not the same as that collected from BH17, the cuticular hydrocarbon phenotype assigned to BH2 could not be designated, as expected, for BH17 (Figure 5).

Collections from inspection ports HH11 and BH25 both had soldiers that keyed to *R. hageni*. The HBK statistics of the mtDNA sequence data revealed that, while the three sympatric species in Georgia were significantly different genetically ($P < 0.05$), neither *R. virginicus* nor *R. hageni* was significantly different from the HH11/BH25 clade ($P > 0.05$). The *R. flavipes* or A and AB clade, however, was significantly different from HH11/BH25 ($P < 0.05$). the statistical analyses appear to indicate that HH11 and BH25 are more closely related to either *R. virginicus* or *R. hageni* than to *R. flavipes*; the hydrocarbon data suggest a much closer affinity to *R. hageni* or phenotype D.

Although HH11 and BH25 were sampled from two disparate soil provinces, SP and ACF, respectively (Figure 1, Table 1) they are more related phylogenetically to each other than to either *R. virginicus* or *R. hageni* (Figures 4 and 5). All external nodes for *R. virginicus*, *R. hageni*, and HH11 and BH25 show that they form unique clades. The internal nodes in Figure 5 indicate the strength of support. HH11 and BH25 have the same ancestor as *R. virginicus* and *R. hageni*, but have a supported node (683) that indicates that they are different from either *R. virginicus* or *R. hageni*. Thus, the HH11 and BH25 inspection ports could represent a saltational speciation event (Mayr, 1954; Carson, 1968; Templeton, 1980), which could be explained by either molecular genetics or population genetics phenomena.

First, HH11 and BH25 could be the result of a molecular genetics phenomenon. A hybridization event could have caused a molecular reorganization that resulted in changes in gene expression or regulation. This change in molec-

ular genetics may have so influenced organismal evolution as to result in a rapid speciation event (Avise, 1994, p. 257). The genetic change, having an adaptive advantage, was established and perpetuated by the colony's social structure.

A population genetics argument for the genotypic and phenotypic uniqueness of HH11 and BH25 may have an explanation in a founder event (Mayr, 1954). If geographically isolated and relatively small, a founder event might have resulted in a "genetic revolution" (Mayr, 1954) that, coupled with a "founder-flush" or rapid expansion (Carson, 1968), produced novel recombinants that were adaptive and, therefore, perpetuated. This could then have caused a shift to a new adaptive peak (Wright, 1951) due to a rapid and temporary accumulation of inbreeding that did not deplete genetic variability (Templeton, 1980).

The agreement between the chemical phenotypes and the mtDNA haplotypes adds robustness to the phylogenetic analyses as it calls attention to the two variant taxa, HH11 and BH25. The use of multiple characters also provides more insight into the speciation process because the collective uniqueness of each assists in understanding organismal evolution generally. Collections from HH11 and BH25 inspection ports form a supported clade (682) (Figure 5) that differs from *R. flavipes*, *R. virginicus*, and *R. hageni* clades genetically, chemically, and morphologically. We suggest, therefore, that these two collections may represent at least one new taxon in *Reticulitermes*. The association of cuticular hydrocarbon phenotypes with mtDNA haplotypes not only provides further support for the species specific nature of cuticular hydrocarbon phenotypes first seriously proposed by Howard and Blomquist (1982), but for using multiple data sets to evaluate taxa. Furthermore, the phenotypic plasticity and genotypic similarity of colonies of *R. flavipes* provide some caution for overinterpreting slight differences in cuticular hydrocarbon mixtures.

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