Cuticular Hydrocarbons for Species Determination of Tropical Termites[1]

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Abstract: Cuticular hydrocarbons can be used to discriminate species in *Coptotermes* and *Nasutitermes*, here discussed for selected species from locations in the Pacific Rim and several Caribbean islands. We recently reexamined the cuticular hydrocarbons of *Coptotermes formosanus* and identified several dimethylalkanes that may be unique to this species. Cuticular hydrocarbons of *C. curvignathus* are similar to those of *C. formosanus*; the primary difference is the absence of 9,13-DimeC_{27} and 13,15-DimeC_{29} in *C. curvignathus*. Hydrocarbons of *C. acinaciformis* include abundant quantities of $C_{27:1}$, which does not occur in any other species of *Coptotermes* examined thus far. *C. lacteus* makes two unusual dimethylalkanes: 15,17-DimeC_{37} and 15,17-DimeC_{39}. *C. testaceus* is notably different from the other *Coptotermes* species described here. Nearly all of the hydrocarbon components are monomethylalkanes. No *n*-alkanes or alkenes were identified. The predominant hydrocarbons in *Nasutitermes costalis* are mono- and dimethylalkanes: 13,17-DimeC_{31} accounts for nearly half of the total hydrocarbon. The most abundant hydrocarbons in *N. ephratae* are also mono-, di- and trimethylalkanes. Two dimethylalkanes (15,17-DimeC_{37} and 15,17-DimeC_{39}) separate *N. ephratae* from *N. costalis*. The hydrocarbon mixtures of *N. gaigei* and *N. guayanae* contain only late-eluting alkenes ($C_{37:1}$ through $C_{47:1}$). The major difference between these two species is the degree of unsaturation. The hydrocarbon profile of *N. acajutlae* appears to be bimodal: early-eluting hydrocarbons consist of *n*-alkanes, alkenes, and monomethylalkanes, while late-eluting compounds consist entirely of alkenes. *N. intermedius* predominantly makes long-chain alkenes with one or two double bonds ($C_{37:2}$ through $C_{41:1}$), including a homologous series of alkenes with an even number of carbons in the chain ($C_{36:2}$ through $C_{40:1}$).

The outer layer of the cuticle of all terrestrial insects consists of a thin layer of wax (Hadley 1985). This wax plays a key role in survival of the insect by providing protection from desiccation (Hadley 1980; Lockey 1988), as well as serving as a barrier to abrasion, microorganisms, and chemicals (Blomquist and Dillwith 1985). Hydrocarbons are ubiquitous components in insect cuticular lipids and can comprise up to 90 percent of the material (Blomquist and Dillwith 1985; Hadley 1985; Lockey 1988). They have been shown to be important semiochemicals, and have been postulated as species and caste recognition cues in termites (Howard and Blomquist 1982).

Alkanes occur in all insect surface lipids reported thus far in the literature (Blomquist and others 1987). *n*-Alkanes generally range from 21 to 31 carbon atoms; molecules with an odd number of carbons predominate. Terminally branched and internally branched monomethylalkanes are also prevalent in insect surface lipids and range from simple compositions, in which only one compound is present, to complex mixtures. In most monomethylalkanes, the methyl branch is located on an odd-numbered carbon atom between carbons 3 and 17. As careful analyses of mono-, di- and trimethylalkanes are made on more organisms, it appears that the methyl groups can be positioned almost anywhere on the chain.

n-Alkenes, with one, two, three, or four double bonds, have been characterized in about one-half of the insect species examined to date (Lockey 1988). The chain length of cuticular *n*-alkenes ranges from 23 to 47 carbon atoms, with odd-numbered chain lengths predominating. The position of the double bond can be almost anywhere in the chain, but is common at carbon 9 (Blomquist and Dillwith 1985).

Moore (1969) was the first to report the composition of cuticular hydrocarbons in a termite, *Nasutitermes exitiosus* (Hill). He found the majority of the hydrocarbons to be unsaturated with the degree of unsaturation ranging from four to eight double bonds; the major component was nonatriacontatetraene ($C_{39:4}$). Also present were paraffins from C_{24} to C_{47}; compounds with an odd number of carbons in the parent chain predominated. Blomquist and others (1979) and Howard and others (1978, 1980, 1982a,b) were next to completely characterize the cuticular hydrocarbons of three termite species. They found that *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks), and *Zootermopsis nevadensis* (Hagen) possess drastically different hydrocarbon profiles, and all three of these profiles differ markedly from those reported earlier for *N. exitiosus*. On the basis of these early results, Howard and Blomquist (1982) hypothesized that each insect species had a mixture of cuticular hydrocarbons that was peculiar to that species and potentially useful as taxonomic characters.

Insects synthesize most if not all of their complement of cuticular hydrocarbons *de novo* (Blomquist and Dillwith 1985). Insect species generally have from 10 to 40 major components in their hydrocarbon mixtures. The relatively large number of possible hydrocarbon components found in the cuticle of insects, ease of chemical analysis and identification of hydrocarbons, and apparent species-specific compositions for many insects make hydrocarbons attractive characters for use in chemotaxonomy.

We have been examining cuticular hydrocarbons as taxonomic characters with termites as well as other groups of economically important forest insects (Haverty and others 1988, 1989, 1990a,b, 1991; Page and others 1990a,b). We have found that characterization of cuticular hydrocarbons often leads to subsequent morphological, biological, or chemical studies which clarify taxonomic questions (Haverty and Thorne 1989; Thorne and Haverty 1989). However, by comparing our taxonomic separations on the basis of cuticular hydrocarbons with existing taxonomic divisions based on morphology, behavior, etc., we are broadening the data base of cuticular hydrocarbons as taxo-

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nomic characters of termites as suggested by Prestwich (1983). The groups of termites we have recently chosen to investigate are the economically and ecologically important, tropicopolitan genera: *Coptotermes* and *Nasutitermes.*

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is considered one of the most voracious subterranean termites and a serious threat to wood in structures throughout its range. In addition to buildings, *C. formosanus* attacks live trees, creosoted transmission poles, structural pilings, and even underground utility cables. *C. formosanus* was introduced into Japan from mainland China before 1600 and subsequently into Hawaii before 1907. Since its introduction into Hawaii, *C. formosanus* has been imported to Guam, Midway, Sri Lanka, Taiwan, South Africa, and the mainland United States (Su and Tamashiro 1987). Although many of the infestations in the continental United States were first noticed after 1965, *C. formosanus* colonies were most likely established by movement of military goods after World War II (Beal 1987). Since 1980, established infestations in inland areas indicate that this species may now be transported in infested wood products via domestic surface commerce (Chambers and others 1988; La Fage 1987; Sponsler and others 1988).

Knowledge of how introductions of *C. formosanus* have been spread will help regulatory agencies formulate and reexamine quarantine policies and procedures. Has *C. formosanus* been introduced numerous times from Asia or Hawaii, or once introduced, has it spread from port to port via domestic, maritime, or surface commerce? Are all populations of *Coptotermes* in the mainland United States *C. formosanus,* or is it possible that introductions of other species of *Coptotermes*, such as *C. havilandi* (Holmgren) or *C. testaceus* (L.), have been introduced and simply misidentified? We hope that cuticular hydrocarbons will help to elucidate this problem.

Nasutitermes Dudley is the largest genus in the Isoptera, containing approximately 212 species worldwide (based on compiled lists in Snyder 1949; Roonwal 1962; Araujo 1977). Taxonomic discrimination is based on morphological characters. These characters are often subtle and variable, or most prominent in imagoes which are rarely collected. For example, soldiers of *N. corniger* and *N. costalis* are currently impossible to discriminate without locality information. Nest architecture is also indistinguishable. When collections are made outside of the confirmed *N. corniger* range (Central America, see Thorne 1980) or away from the recognized *N. costalis* distribution (Caribbean islands: Snyder 1949; Araujo 1977), species determination is equivocal. There is currently confusion over the extent of the distribution of each of these species in northern South America, with overlapping reports of each, and identifications of each from as far as Bolivia and Brazil (Roonwal and Rathore 1976, reviewed in Thorne 1980).

As part of a broad examination of the taxonomy of Neotropical *Nasutitermes,* other termites of the Caribbean Basin, and the pantropical *Coptotermes,* we decided to examine cuticular hydrocarbons as an adjunct to the study of morphology and behavior for taxonomic distinction of species. This paperbehavior for taxonomic distinction of species. This paper reports supplemental data on surface hydrocarbons of *Nasutitermes* (Howard and others 1988; Haverty and others 1990b) and *Coptotermes* (Brown and others 1990; Haverty and others 1990a; McDaniel 1990), and discusses whether this information helps us resolve diagnostic problems within the genus (Howard and Blomquist 1982).

Materials and Methods

Detailed descriptions of collection and extraction procedures for specific termite species have been published elsewhere (Blomquist and others 1979; Howard and others 1978, 1982b, 1988; Haverty and others 1988, 1990a,b). In this study, cuticular lipids were extracted by immersing 15 to 500 termites (depending on size and total quantity of cuticular hydrocarbon), as a group, in 2 to 10 ml of hexane for 10 min. After extraction, hydrocarbons were separated from other components by pipetting the extract and an additional 2 to 8 ml of hexane through 3 cm of activated BioSil-A in Pasteur pipette mini-columns. Hydrocarbon extracts were evaporated to dryness under a stream of nitrogen and redissolved in 30 to 100 µl of hexane for gas chromatography-mass spectrometry (GC-MS) analyses.

All termite specimens except *C. curvignathus* Holmgres were dried at the field location. Dried termites were then shipped to the laboratory in Berkeley, California, for hydrocarbon extraction and chromatographic analysis. Extracted termites, or additional termites from the same colony, were stored in 70 percent ethanol for later identification by morphological features and to serve as voucher specimens.

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 5970B Mass Selective Detector interfaced with Hewlett Packard Chemstation computer. The GC-MS instrument was equipped with a fused silica capillary column (30 m x 0.2 mm ID, HP-1) and operated in split mode (with a split ratio of 20:1). Each mixture was analyzed by a temperature program from 200°C to 320°C at 3°C/min with a final hold of 10 to 20 minutes. Electron impact (EI) mass spectra were obtained at 70 eV. *n*-Alkanes were identified by comparing their retention times and mass spectra with external standards (n-C$_{22}$, n-C$_{24}$, n-C$_{28}$ and n-C$_{32}$). Alkenes and methyl-branched alkanes were tentatively identified by calculating their equivalent chain lengths; mass spectra of methylalkanes were interpreted as described by Blomquist and others (1987).

Six colonies of *C. formosanus* were collected from the University of Hawaii campus; another came from Kaneoe on the island of Oahu. *C. formosanus* samples were separated from wood debris and carton material and subsequently dried in a desiccator. They were held dry at room temperature until hydrocarbons were extracted. Samples of *C. curvignathus* from Malaysia and Thailand were extracted live in hexane in the field. Samples of *C. acinaciformis* (Froggatt) from Darwin, Australia, *C. lacteus* from Erica Forest near Melbourne, Australia, and *C. testaceus* (L.) from Trinidad were dried in Australia or Trinidad and extracted in our Berkeley laboratory.

Samples from colonies of various *Nasutitermes* species were collected as follows: *N. costalis* (Holmgren) from Trinidad, Grenada, and Montserrat; *N. ephratae* (Holmgren) from Trinidad, Tobago, and Montserrat; *N. gaigei* Emerson from Trinidad and

Tobago; *N. guayanae* (Holmgren) from Trinidad and Tobago; *N. intermedius* Banks from Trinidad; and *N. acajutlae* (Holmgren) from Great Camino, Guana Island and Scrub Island in the British Virgin Islands, and Vieques Island (off Puerto Rico). Entire arboreal nests, or large sections of nests, were collected in the field and confined in plastic bags. Within 24 hours, live insects were removed from the carton matrix by briskly tapping a portion of nest material over sorting trays. Workers and soldiers were selected with forceps for extraction of hydrocarbons. Species were determined on the basis of nest architecture and soldier morphology (Emerson 1925; Snyder 1956; Thorne 1980; Thorne and Levings 1989).

Results and Discussion

Coptotermes

Recently we characterized the cuticular hydrocarbons from colonies of *C. formosanus* from four different geographic locations in the United States (Haverty and others 1990a). Concurrently, McDaniel (1990) characterized the hydrocarbons from nine colonies of *C. formosanus* collected from Lake Charles, Louisiana, and one from Honshu, Japan. McDaniel (1990, *table 1)* identified numerous mono- and dimethylalkanes, which occurred in trace amounts, that were not detected by Haverty and others (1990a). McDaniel (1990) also identified a unique trimethylalkane, 13,15,17-TrimeC$_{29}$, in trace amounts, which was not detected by Haverty and others (1990a). However, the major constituents reported by McDaniel (1990) and Haverty and others (1990a) agree with a few exceptions.

In this most recent re-examination of the cuticular hydrocarbons of *C. formosanus* from Hawaii, we identified fourteen hydrocarbons in small amounts that were not detected by McDaniel (1990) nor by Haverty and others (1990a) (9,13-DimeC$_{27}$; n-C$_{28}$; 3,17- and 3,15-DimeC$_{27}$; 2-MeC$_{28}$; 13,15-DimeC$_{29}$; 3-MeC$_{29}$; 3,15- and 3,17-DimeC$_{29}$; 3,7-DimeC$_{29}$; 13,15-DimeC$_{30}$; 13,15-DimeC$_{31}$; 15,17-DimeC$_{39}$; 16-MeC$_{40}$; 14,18-DimeC$_{40}$; and 15,17-DimeC$_{41}$—see *table 1).* We also detected 13,15,17-TrimeC$_{29}$, as well as some later-eluting monomethyl- and dimethylalkanes (see *table 1*) reported by McDaniel (1990), but not seen in our previous analysis (Haverty and others 1990a). Of the five species of *Coptotermes* we have investigated thus far, several unusual dimethylalkanes with one methylene group between the methyl groups (such as 13,15-DimeC$_{29}$) appear to be unique to *C. formosanus*, and could be considered diagnostic for this species. Analogous, but different, dimethylalkanes have also been identified in *Coptotermes* from Australia (Brown and others 1990).

A preliminary survey of the hydrocarbon composition of four additional species of *Coptotermes* demonstrates clear qualitative differences from *C. formosanus (table 1). C. curvignathus* Holmgren from Thailand is the most similar of these four species to *C. formosanus*. Nearly all of the major components in the hydrocarbon profiles of these two species are shared *(table 1).* Two significant components, 3-MeC$_{25}$ and 11,13,15,16-MeC$_{39}$, were not detected or occur in trivial amounts in *C. curvignathus*. The primary difference between *C. formosanus* and *C. curvignathus*, as mentioned above, is the absence of 9,13-DimeC$_{27}$ and 13,15-DimeC$_{29}$ in *C. curvignathus*.

In our characterization of the hydrocarbons of *C. acinaciformis* (Froggatt) from Darwin, Australia, we identified several abundant, unique hydrocarbons. Our hydrocarbon profile for *C. acinaciformis* from Darwin is nearly identical to the hydrocarbons of *C. acinaciformis* from Humpty Doo, Australia, as reported by Brown and others (1990), except that we did not identify any hydrocarbons that eluted after 13-MeC$_{29}$. The most dramatic difference that we, and Brown and others (1990), found in *C. acinaciformis* is the huge C$_{27:1}$ peak (ca. 52 percent of the total cuticular hydrocarbon composition). This hydrocarbon does not occur in any of the other *Coptotermes* species examined thus far.

We also characterized the hydrocarbons from workers of *C. lacteus* (Froggatt) from Erica Forest near Melbourne, Australia. Our results closely agree with hydrocarbon profiles of *C. lacteus* from Batemans Bay and Canberra, Australia (Brown and others 1990). Several minor components (2-MeC$_{28}$, *n*-C$_{29}$, 2-MeC$_{29}$ and 3-MeC$_{29}$) were not detected from our workers nor were they found by Brown and others (1990) in soldiers or nymphs of the colonies they examined. One intriguing difference is our identification of two dimethylalkanes (15,17-DimeC$_{37}$ and 15,17-DimeC$_{39}$) that coelute with their corresponding internally branched monomethylalkanes (X-MeC$_{37}$ and X-MeC$_{39}$). Hydrocarbons with the methyl branches separated by a single methylene group are not common in insects (Blomquist and others 1987). However, this type of compound has now been identified in two species of *Coptotermes* (Haverty and others 1990a, McDaniel 1990).

Finally, we report the hydrocarbon profile from *C. testaceus* (L.) from Trinidad. *C. testaceus* is notably different from *C. formosanus* and the *Coptotermes* species from Southeast Asia and Australia. Nearly all of the hydrocarbon components are terminally branched monomethylalkanes (such as 2-MeC$_{27}$ and 2-MeC$_{29}$) or internally branched monomethylalkanes. Small amounts of dimethylalkanes were found (11,15- and 13,17-DimeC$_{29}$; 13,17-DimeC$_{30}$; 13,17-DimeC$_{31}$). No *n*-alkanes, alkenes, 3-; 5-; or 7-methylalkanes were identified *(table 1).* Therefore, we can conclude with reasonable certainty that none of the four *Coptotermes* populations we have studied in the United States was misidentified *C. testaceus*. Next we need to convince ourselves that *C. havilandi* Holmgren, now established in the Caribbean (Scheffrahn and others 1990), has not been introduced into the mainland United States.

Nasutitermes

We have begun to expand our data base on the cuticular hydrocarbons of *Nasutitermes* from the Caribbean islands. Previously Haverty and others (1990b) characterized the cuticular hydrocarbons of *N. costalis* and *N. ephratae* from Trinidad. They found that these species have hydrocarbon mixtures that are qualitatively and quantitatively distinct from one another, with sixteen major hydrocarbon components. No unsaturated components were found.

In this study we included samples of these two species from Trinidad, as well as samples from other Caribbean islands.

Table 1—*Composition of hydrocarbon mixtures from workers of five species of* Coptotermes[1]

	Coptotermes species[2]				
Hydrocarbon[3]	form	curv	acin	lact	test
n-C_{23}	o	o	o	tr	o
9-;11-MeC_{23}[4]	o	o	o	tr	o
2-MeC_{23}	o	o	o	+	o
n-C_{24}	o	o	o	+	o
9-;10-;11-; 12-;13-MeC_{24}[4]	o	o	o	+	o
2-MeC_{24}	o	o	+	++	o
$C_{25:1}$	o	o	tr	o	o
n-C_{25}	+	tr	++	++	o
9-;11-;13-MeC_{25}[4]	+	+++	++	+++	o
2-MeC_{25}	+++	+++	+++	+++	o
3-MeC_{25}	++	o	++	+++	o
n-C_{26}	+	+	++	+	o
9-;10-;11;12-;13-MeC_{26}[4]	++	++	tr	++	o
2-MeC_{26}	++	++	o[5]	+	o
3-MeC_{26}	tr	o	o	tr	o
$C_{27:1}$	o	o	+++[5]	o	o
n-C_{27}	++	++	++	tr	o
9-;11-;13-;15-MeC_{27}[4]	+++	+++	+++	+	+
7-MeC_{27}	o	o	++	o	o
2-MeC_{27} + 9,13-DimeC_{27}[6]	+++	+++[7]	++[7]	tr[7]	++[7]
3-MeC_{27}	+++	++	++	tr	+
$C_{28:1}$	o	o	+	o	o
n-C_{28}	+	++	o	o	o
3,17-;3,15-DimeC_{27}[4]	+	o	o	o	o
3,7-DimeC_{27}	o	o	tr	o	o
9-;11-;12-;12-; 13-;14-;15-MeC_{28}[4]	++	++	tr	o	+
2-MeC_{28}	+	o	+	o	+
3-MeC_{28}	tr	o	o	o	o
$C_{29:1}$	o	o	++	o	o
n-C_{29}	o	+	tr	o	o
9-;11-;13-;15-MeC_{29}[4] + 13,15-DimeC_{29}[6]	+++	++[7]	+[7]	o	+++[7]
9,13-DimeC_{29}	+	o	o	o	o
11,15-;13,17-DimeC_{29}[4] + 2-MeC_{29}[5]	tr[7]	o	o	o	+++
3-MeC_{29}	+	o	o	o	+
13,15,17-TrimeC_{29}	+	o	o	o	tr
3,15-;3,17-DimeC_{29}[4]	+	o	o	o	o
3,7-DimeC_{29}	+	o	o	o	tr
10-;11-;12-;13-MeC_{30}[4]	o	o	o	o	++
13,15-DimeC_{30}	+	o	o	o	o
13,17-DimeC_{30} + 2-MeC_{30}[6]	o	o	o	o	tr
11-;13-;15-MeC_{31}[4]	o	o	o	o	+++
13,15-DimeC_{31}	+	o	o	o	o
13,17-DimeC_{31}	o	o	o	tr	+
3,7-DimeC_{31}	tr	o	o	o	tr
10-;12-;16-MeC_{32}[4]	o	o	o	o	+
11-;13-;15-;17-MeC_{33}[4]	+	o	o	+	++
13,17-;15,19-DimeC_{33}[4]	o	o	o	+	o
10-;11-;12-;14-MeC_{34}[4]	o	o	o	tr	tr
12,16-DimeC_{34}	o	o	o	tr	o
11-;13-;15-;17-MeC_{35}[4]	+	o	o	++	++
11,15-;13,21-DimeC_{35}[4]	o	o	o	++	o
10-;11-;12-;13-;14-; 15-;16-;17-MeC_{36}[4]	o	o	o	+	tr
12,16-DimeC_{36}	o	o	o	+	o
11-;13-;15-;17-;19-	+	o	o	o	+
MeC37[4] + 15,17-DimeC_{37}	o	o	o	+[8]	o
11,15-;13,17-; 15,19-DimeC_{37}[4]	+	o	o	+++	o
12-;14-;15-;16-; 17-;18-MeC_{38}[4]	tr	o	o	+	o
12,16-DimeC_{38}	o	o	o	+	o
11-;13-;15-;16-MeC_{39}[4]	++	o	o	o	tr[7]
+ 15,17-DimeC_{39}[6]	+	o	o	++[8]	o
11,15-;13,17-; 15,19-DimeC_{39}[4]	++	o	o	++	o
16-;15-;14-;13-MeC_{40}[4]	+	o	o	o	o
14,18-DimeC_{40}	+	o	o	o	o
13-;15-MeC_{41}[4] +15,17-DimeC_{41}[6]	++	o	o	o	o
13,17-;15,19-DimeC_{41}[4]	++	o	o	o	o
13-;15-MeC_{43}[4]	+	o	o	o	o
13,17-;15,19-DimeC_{43}[4]	+	o	o	o	o

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[1] Hydrocarbons are quantified as follows: +++ indicates ≥ 5.0 percent of the total, ++ from 1.0 to 5.0 percent of the total, and + from 0.5 to 1.0 percent of the total hydrocarbon component. Some trace (tr) components appear infrequently or consistently in very small quantities (< 0.5 percent of the total). A zero indicates the hydrocarbon has not been identified for the species.

[2] form = *Coptotermes formosanus* from Honolulu, Hawaii, curv = *C. curvignathus* from near Bangkok, Thailand, acin = *C. acinaciformis* from Darwin, Australia, lact = *C. lacteus* from Erica Forest near Melbourne, Australia, and test = *C. testaceus* from Trinidad.

[3] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC_{25} = 3-methylpentacosane.

[4] An isomeric mixture. These components co-elute in this peak.

[5] 2-MeC_{26} co-elutes with $C_{27:1}$.

[6] Because of incomplete separation of these hydrocarbons both peaks are included as one value.

[7] Only the monomethylalkane occurs in this species.

[8] Only the dimethylalkane occurs in this species.

Since the earlier study by Haverty and others (1990b) and the report by Howard and others (1988) on *N. ephratae* and *N. corniger* (Motschulsky), we have discontinued the use of a flame ionization detector (FID) on our capillary gas chromatograph and now use a mass selective detector (MSD). We have also changed our methods for collecting, shipping, and extracting specimens. We now dry specimens at the field location and extract dry specimens in the laboratory in Berkeley, rather than extracting fresh specimens in the field and shipping liquid or dried extracts to the laboratory. These changes have resulted in much superior separation, identification and quantification of the hydrocarbon components, especially the later-eluting components.

The predominant classes of cuticular hydrocarbons in *N. costalis* are internally branched, mono- and dimethylalkanes *(table 2)*. Normal alkanes and an alkene ($C_{27:1}$) occur, but only in relatively small quantities (≤ 1.0 percent). No terminally branched, monomethylalkanes were found. The preponderance of one component (13,17-DimeC_{31}) accounts for nearly half of the total hydrocarbon of *N. costalis*.

The hydrocarbon profile of *N. ephratae* is much more complex than previously described (Haverty and others 1990b; Howard and others 1988). These earlier reports failed to identify the compounds that elute after 13,17-DimeC_{33}. In our samples from Trinidad and Tobago we consistently identified 24 hydrocarbons in significant (≥ 1.0 percent of the total hydrocarbon) quantities *(table 2)*. As with *N. costalis*, the predominant classes of hydrocarbons are the internally branched, mono-, di- and trimethylalkanes. Most of these compounds have isoprenoid spacing between the methyl branches. We also found trimethylalkanes (13,15,17-TrimeC_{31}; 13,15,17-TrimeC_{37}; and 13,15,17-TrimeC_{39}) with only a methylene group between the methyl branches. These rare trimethylalkanes elute very soon after the analogous dimethylalkanes (15,17-DimeC_{31}; 15,17-DimeC_{37}; and 15,17-DimeC_{39}). *N. ephratae* does make significant quantities of *n*-alkanes (n-C_{23} and n-C_{27}) and alkenes ($C_{23:1}$, $C_{25:1}$, $C_{27:1}$, and $C_{29:1}$). We found no terminally branched, monomethylalkanes.

Although we found no qualitative differences in hydrocarbon mixtures between workers and soldiers of the same colony, we think it is important to point out significant differences between the hydrocarbon profiles of *N. ephratae* from Trinidad versus those from Tobago *(table 2)*. Our samples from Trinidad contain two isomers of $C_{29:1}$, whereas those from Tobago apparently do not make these compounds. Instead, the Tobago specimens produce a 12,16-DimeC_{28} which elutes at about the same time as $C_{29:1}$. In addition, our samples from Tobago include 11,15- and 13,17-DimeC_{39}; 13,17-DimeC_{41}; 11,15,19-TrimeC_{39}; and 13,17,21-TrimeC_{41}. These hydrocarbons were not found in our sample from Trinidad. These differences will require further elucidation.

N. gaigei and *N. guayanae* make a very simple, yet unique, hydrocarbon mixture. The only hydrocarbons we recovered from these species are late-eluting alkenes ($C_{37:1}$ through $C_{47:1}$) *(table 2)*. We found only trivial amounts of an occasional *n*-alkane or methylalkane. The major difference between these two species is the degree of unsaturation. Hydrocarbons of *N. gaigei* have either three or four double bonds; those of *N. guayanae*

Table 2—*Mean percent composition of hydrocarbons from workers of six species of* Nasutitermes[1]

	Nasutitermes species[2]					
Hydrocarbon[3]	cost	ephr	gaig	guay	acaj	inte
$C_{23:1}$	o	+	o	o	tr	o
n-C_{23}	tr	++	o	o	+	o
n-C_{24}	tr	o	o	o	tr	o
2- or 4-MeC_{24}	o	o	o	o	+	o
$C_{25:1}$	tr	++	o	o	+++	o
n-C_{25}	+	+	o	o	+++	o
11-;13-MeC_{25}[4]	tr	+	o	o	+	o
9,13-DimeC_{25}	o	+	o	o	o	o
2- or 4-MeC_{25}	o	o	o	o	++[5]	o
3-MeC_{25}	o	o	o	o	++[5]	o
$C_{26:1}$	o	o	o	o	tr	o
n-C_{26}	o	o	o	o	+	o
11-;12-;13-MeC_{26}[4]	o	+	o	o	o	o
2- or 4-MeC_{26}	tr	o	o	o	+	o
$C_{27:1}$	+	+++	o	o	+	o
n-C_{27}	+	++	o	o	+++	+
9-;11-;13-MeC_{27}[4]	tr	+++	o	o	tr	tr
11,13-DimeC_{27}	o	++	o	o	o	o
11,15-DimeC_{27}	+[6]	++[6]	o	o	o	+[6]
9,13-DimeC_{27}	o	++[6]	o	o	o	o
2- or 4-MeC_{27}	+[6]	o	o	o	tr	+[6]
3-MeC_{27}	tr	o	o	o	tr	o
9,13,17-TrimeC_{27}	o	+	o	o	o	o
n-C_{28}	tr	tr	o	o	tr	o
12-;13-;14-MeC_{28}[4]	tr	+	o	o	o	o
11,15-;12,16-DimeC_{28}	tr	++[7]	o	o	o	o
2- or 4-MeC_{28}	+	o	o	o	tr	o
$C_{29:1}$	tr	++[7]	o	o	o	o
9,13,17-TrimeC_{28}	o	+	o	o	o	o
n-C_{29}	+	+	o	o	+	+
9-;11-;13-;15-MeC_{29}[4] + 13,15-DimeC_{29}	++[6]	+++[6]	o	o	tr	+
11,15-;13,17-DimeC_{29}	++	+++	o	o	o	o
9,19-DimeC_{29}	o	o	o	o	tr	o
9,17-DimeC_{29}	o	+++[6]	o	o	o	o
9,13-DimeC_{29}	o	+++[6]	o	o	o	o
11,15,19-; 9,13,17-TrimeC_{29}[4]	tr	++[8]	o	o	o	o
3-MeC_{29}	tr	o	o	o	o	o
12-;13-;14-; 15-;16-MeC_{30}[4]	tr	+	o	o	o	o
12,16-DimeC_{30}	++	++	o	o	o	o
$C_{31:1}$	tr	tr	o	o	tr	+
11,15,19-TrimeC_{30}	o	tr	o	o	o	o
n-C_{31}	tr	o	o	o	o	++

Table 2—*Mean percent composition of hydrocarbons from workers of six species of* Nasutitermes,[1] continued

Hydrocarbon[3]	cost	ephr	gaig	guay	acaj	inte
	Nasutitermes species[2]					
11-;13-;15-MeC$_{31}$[4]	++[6]	++[6]	o	o	o	+[6]
11,13-DimeC$_{31}$	++[6]	o	o	o	o	o
13,15-DimeC$_{31}$	++[6]	o	o	o	o	+
15,17-DimeC$_{31}$	o	++[6]	o	o	o	o
13,15,17-TrimeC$_{31}$	o	+	o	o	o	o
13,17-DimeC$_{31}$	+++	+++	o	o	o	o
11,15-DimeC$_{31}$	o	+	o	o	o	o
11,15,19-TrimeC$_{31}$	o	++	o	o	o	o
12-;14-MeC$_{32}$	+	o	o	o	o	o
14,18-DimeC$_{32}$	+	o	o	o	o	o
C$_{33:1}$	o	o	tr	o	o	+
n-C$_{33}$	o	o	tr	o	o	+
13-;15-;17-MeC$_{33}$	+[6]	+	o	o	o	o
+ 13,15-DimeC$_{33}$[4]	+[6]	o	o	o	o	o
15,19-DimeC$_{33}$	+	+[6]	o	o	o	o
13,17-DimeC$_{33}$	o	+[c]	o	o	o	o
11,15-DimeC$_{33}$	+	o	o	o	o	o
16-MeC$_{34}$	tr	o	o	o	o	o
C$_{35:2}$	o	o	o	o	o	+
C$_{35:1}$	o	o	o	o	o	+
17-MeC$_{35}$	tr	o	o	o	o	o
15,17-DimeC$_{35}$	o	tr	o	o	o	o
11,21-DimeC$_{35}$	tr	o	o	o	o	o
11,15-DimeC$_{35}$	tr	o	o	o	o	o
C$_{36:2}$	o	o	o	o	o	++
C$_{36:1}$	o	o	o	o	o	+
C$_{37:4}$	o	o	+++	o	o	o
C$_{37:3}$	o	o	+++	o	o	o
C$_{37:2}$	o	o	o	o	o	+++
C$_{37:1}$	o	o	o	++	tr	+++
15,17-DimeC$_{37}$	o	+	o	o	o	o
13,15,17-TrimeC$_{37}$	o	++	o	o	o	o
C$_{38:4}$	o	o	+	o	o	o
C$_{38:3}$	o	o	tr	o	o	o
C$_{38:2}$	o	o	o	o	o	++
C$_{38:1}$	o	o	o	+	tr	++
Unknown	o	o	o	o	+	o
C$_{39:4}$	o	o	+++	o	o	o
C$_{39:3}$	o	o	++	o	o	+
C$_{39:2}$	o	o	o	tr	tr	+++
C$_{39:1}$	o	o	o	+++	+++	+++
n-C$_{39}$	o	o	o	tr	o	o
11-;13-;15-MeC$_{39}$[4]	o	o	o	+	o	o
15,17-DimeC$_{39}$	o	++	o	o	o	o

Table 2—*Mean percent composition of hydrocarbons from workers of six species of* Nasutitermes,[1] continued

Hydrocarbon[3]	cost	ephr	gaig	guay	acaj	inte
	Nasutitermes species[2]					
13,15,17-TrimeC$_{39}$	o	++	o	o	o	o
13,17-;11,15-DimeC$_{39}$	o	++[9]	o	o	o	o
11,15,19-TrimeC$_{39}$	o	+[9]	o	o	o	o
C$_{40:4}$	o	o	tr	o	o	o
C$_{40:2}$	o	o	o	o	o	+
C$_{40:1}$	o	o	o	+	+	+
Unknown	o	o	o	o	++	o
n-C$_{40}$	o	o	o	tr	o	o
C$_{41:4}$	o	o	++	o	o	o
C$_{41:3}$	o	o	+	o	tr	o
C$_{41:2}$	o	o	o	+	+	+
C$_{41:1}$	o	o	o	+++	+++	++
13-MeC$_{41}$	o	o	o	+	o	o
15,17-DimeC$_{41}$	o	+	o	o	o	o
13,17-DimeC$_{41}$	o	+[9]	o	o	o	o
13,15,17-TrimeC$_{41}$	o	tr	o	o	o	o
13,17,21-TrimeC$_{41}$	o	+[9]	o	o	o	o
C$_{42:1}$	o	o	o	+	tr	o
Unknown	o	o	o	o	+	o
C$_{43:2}$	o	o	o	+	++	tr
C$_{43:1}$	o	o	o	++	++	+
13-MeC$_{43}$	o	o	o	tr	o	o
C$_{44:1}$	o	o	o	tr	tr	o
C$_{45:2}$	o	o	o	tr	+	o
C$_{45:1}$	o	o	o	++	++	o
C$_{47:1}$	o	o	o	+	++	o

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[1] Hydrocarbons are quantified as follows: +++ indicates ≥ 5.0 percent of the total, ++ from 1.0 to 5.0 percent of the total, and + from 0.5 to 1.0 percent of the total hydrocarbon component. Some trace (tr) components appear infrequently or consistently in very small quantities (< 0.5 percent of the total). A zero indicates the hydrocarbon has not been identified for the species.

[2] cost = *Nasutitermes costalis* from Trinidad and Grenada, ephr = *N. ephratae* from Trinidad and Tobago, gaig = *N. gaigei* from Trinidad and Tobago, guay = *N. guayanae* from Trinidad and Tobago, acaj = *N. acajutlae* from Great Camino, Guana Island, Scrub Island (British Virgin Islands) and Vieques, and inte = *N. intermedius* from Trinidad.

[3] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC$_{25}$ = 3-methylpentacosane.

[4] An isomeric mixture. These components co-elute in this peak.

[5] These isomers occur inconsistently; when present they are fairly abundant.

[6] Because of incomplete separation of these hydrocarbons both peaks are included as one value for *N. costalis, N. ephratae,* and *N. intermedius.*

[7] Two isomers of C$_{29:1}$ occur in *N. ephratae* from Trinidad and not in the sample from Tobago; 12,16-DimeC$_{28}$ occurs in the *N. ephratae* from Tobago but not in the sample from Trinidad.

[8] Only the 9,13,17-TrimeC$_{29}$ isomer was found.

[9] 13,17-DimeC$_{39}$; 13,17-DimeC$_{41}$; 11,15,19-TrimeC$_{39}$; and 13,17,21-TrimeC$_{41}$ have been found only in samples of *N. ephratae* from Tobago.

have one or two double bonds. The predominant hydrocarbon in *N. gaigei* has 37 carbons, whereas the major component of *N. guayanae* has 39 carbons.

The hydrocarbons of *N. acajutlae* differ from the four previously described species in that the chromatograms appear to be bimodal. The early-eluting hydrocarbons consist of *n*-alkanes (*n*-C_{23} through *n*-C_{29}), alkenes ($C_{25:1}$ and $C_{27:1}$), and terminally branched monomethylalkanes *(table 2)*. The late-eluting compounds consist entirely of alkenes with one, two or three double bonds *(table 2)*. Only trivial amounts of internally branched, monomethylalkanes were found. No di- or trimethylalkanes were found.

We hesitate to report upon the single colony of *N. intermedius* collected from Trinidad. However, given the consistency of hydrocarbon profiles among samples of other species of *Nasutitermes*, the uniqueness of this observation is worthy of mention. This species makes predominantly long-chain alkenes with one or two double bonds ($C_{37:2}$ or 1 through $C_{41:2}$ or 1) *(table 2)*. This species even produces a homologous series of alkenes with an even number of carbons in the chain ($C_{36:2}$ or 1 through $C_{40:2 \text{ or} 1}$). We have not seen this with the other species of *Nasutitermes*. In addition, this sample yielded no hydrocarbons before *n*-C_{27}, no terminally branched monomethylalkanes, and only trivial amounts of internally branched, mono- and dimethylalkanes.

The taxonomy of the Caribbean *Nasutitermes* is in need of revision and is a topic of our work in progress. Keys that distinguish these species on the basis of morphological characters (Banks 1919; Emerson 1925; Snyder 1956, 1959) often lead to equivocal determinations. Diagnostic keys and synonomies are being re-evaluated on the basis of comparisons with type specimens and studies of additional collections.[3] We are certain enough about morphological groupings, however, to be confident of species determinations made on material used in this study.

A brief discussion of diagnostic morphological characters among *Nasutitermes* involved in this project follows, with comments on inferred phylogenetic relationships among the species. *N. costalis* and *N. ephratae* overlap geographically on Trinidad and Tobago, and in northern South America based on known ranges (Araujo 1977; Emerson 1925). Morphologically, these two species are close, but imagoes are distinguished by position of ocelli (Emerson 1925; Snyder 1956) and soldiers may be separated on the basis of the pattern of setae on the tergal sclerites (Emerson 1925; Snyder 1956; Thorne 1980). Nests of these species are easily identified on the basis of both exterior and interior architectures (Darlington In press; Haverty and others 1990b; Thorne 1980). *N. costalis, N. ephratae*, and the Central/South American species *N. corniger* (Motschulsky) are all considered to be close relatives based on morphological similarities, but a comprehensive phylogenetic analysis involving these groups has not yet been undertaken.

In this paper we designate the specimens from the British Virgin Islands and Vieques Island as *N. acajutlae*, resurrecting a name previously synonomized as *N. nigriceps* by Snyder (1949). The "*N. nigriceps* complex" is a broadly dispersed and morphologically variable group, ranging throughout Central America and the Caribbean, with isolated reports from a number of locations in South America (Araujo 1977, American Museum of Natural History collection). In work still in progress, Thorne has examined material throughout the *N. "nigriceps"* range and isolated three morphological characters which consistently differentiate soldiers of *N. nigriceps sensu strictu* from soldiers found in the American and British Virgin Islands and Puerto Rico. The latter groups compare favorably to soldiers of the COTYPE specimens of *"Eutermes acajutlae"* (collected from St. Thomas, Virgin Islands, determined by N. Holmgren, American Museum of Natural History collection). The three distinguishing characters are: (1) density of hairs on the head capsule of *N. acajutlae* soldiers greatly exceeds that of *N. nigriceps* soldiers; (2) small hairs are found on the ventral surface of the soldier head on either side of the postmentum in *N. acajutlae* (absent on specimens of *N. nigriceps)*; and (3) the anterior margin of the soldier pronotum is distinctly emarginate in *N. acajutlae*, flatter in *N. nigriceps*. On the basis of these characters, specimens from Jamaica sort as *N. nigriceps*; samples from localities in the Lesser Antilles examined to date separate as *N. acajutlae*. A formal treatment of the taxonomy of the *N. nigriceps* complex is in progress, comparing morphological data with hydrocarbon analyses.[4]

Nasutitermes gaigei, N. guyanae and *N. intermedius* are each distinct morphospecies, identified in these samples on the basis of characters of the soldier caste and comparison to determined specimens. Diagnostic suites of characters and geographical distributions of each species are being revised.[5]

As we continue to survey *Nasutitermes* hydrocarbon profiles throughout the Caribbean, we anticipate that correlations between hydrocarbon profiles and morphology will assist us in further resolution of the taxonomy of this genus.

Our study represents only the fourth report of the cuticular hydrocarbons of species of *Nasutitermes*, one of the most ubiquitous and abundant pantropical groups of termites. Moore (1969) provided the first description of the cuticular lipids of the Australian termite, *N. exitiosus*. His report that the long-chain, unsaturated component $C_{39:4}$ was the predominant hydrocarbon is consistent with our characterization of four of the six species we have investigated.

The consistent similarities among the hydrocarbon mixtures of the species of *Nasutitermes* examined thus far (Moore 1969; Howard and others 1988; and Haverty and others 1990b) indicate that cuticular hydrocarbons can be valuable chemotaxonomic characters for all *Nasutitermes* species. As has been found with other taxonomic character systems (Gush and others 1985), hydrocarbon profiles may not show exact qualitative or quantitative species-specificity, especially across a broad geographic range. Further sampling within *Nasutitermes* and in other groups should be encouraged to determine the prevalence of such variability, and to ascertain its biological and taxonomic relevance.

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[3] Thorne, Collins and Darlington, unpublished data.

[4] Thorne, Haverty and Collins, unpublished data.

[5] Darlington, unpublished data.

Conclusions

There are still some unresolved questions to be addressed to optimize the use of cuticular hydrocarbons for taxonomic studies of termites. They are: (1) What is the influence of genetics and the environment (e.g., temperature, relative humidity, and diet) on hydrocarbon composition? Does drying the samples before extraction quantitatively or qualitatively change the hydrocarbon mixture? (2) Are cuticular hydrocarbons, or the other chemicals present in the wax layer, responsible for species or caste recognition? (3) Are hydrocarbon profiles within "good species" qualitatively identical or are there some exceptions to the rules? Can different biological species have identical hydrocarbon profiles? (4) What is the rate of change in hydrocarbon profiles as species evolve? Do slight differences in profiles mean that the species are closely related? and (5) Can hydrocarbons be used to determine phyletic relationships among groups within a genus? Resolution of these questions will greatly advance the precision of cuticular hydrocarbons as chemotaxonomic characters in all insects.

Even though there are numerous unresolved questions concerning the utility of hydrocarbons as taxonomic characters, an increasingly convincing body of knowledge is accumulating which indicates that hydrocarbon profiles are species-specific in termites (Brown and others 1990, Clément and others 1985, 1986, Haverty and others 1988, 1990b, Howard and others 1978, 1980, 1982a,b, 1988; Watson and others 1989). Our experience in applying knowledge of cuticular hydrocarbon composition to the identification of termite species has been encouraging. We have used hydrocarbons to initially sort specimens (Haverty and others 1988) to identify a new diagnostic morphological character for *Zootermopsis* species (Thorne and Haverty 1989). Geographical races of *Coptotermes formosanus* can be distinguished on the basis of the concentrations of individual hydrocarbon components (Haverty and others 1990a). And most importantly, we now realize that cuticular hydrocarbons are extremely useful taxonomic characters for separating species of termites where morphology has proven inadequate in the past (Brown and others 1990, Watson and others 1989).

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