Preliminary Investigations of the Cuticular Hydrocarbons from North American Reticulitermes and Tropical and Subtropical Coptotermes (Isoptera: Rhinotermitidae) for Chemotaxonomic Studies.

by

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ABSTRACT

We collected *Reticulitermes* from Oregon, California, and Arizona, areas suspected to have only *R. hesperus* or *R. tibialis* and from areas where these two species are thought to be sympatric. We characterized four distinct cuticular hydrocarbon phenotypes. The two phenotypes from California are more similar to one another than to the two types found in Arizona. Samples of "*R. tibialis*" from distant locations in Arizona have profiles which are extremely different from "*R. hesperus*" and "*R. tibialis*" from California; one phenotype was found from Oregon to southeastern Arizona. These results imply that there are at least 3 species of *Reticulitermes* in western North America when only two are currently recognized. The Formosan subterranean termite, *Coptotermes formosanus*, in the United States shows no qualitative differences among geographically distant populations; quantitative differences in hydrocarbon components separate them into different relative concentration profiles. Our results suggest that *C. formosanus* colonies from Florida and Louisiana are not closely related to those from Hawaii. We strongly suspect that *C. formosanus* was introduced into Louisiana at least twice. Hydrocarbon profiles of *C. formosanus* from North America and Hawaii are different from samples of other *Coptotermes* species collected from northern Australia, Southeast Asia and the Caribbean. Hydrocarbon profiles in *Coptotermes* appear to be species-

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specific. These preliminary data suggest that cuticular hydrocarbons would be extremely useful for initially sorting specimens to search for diagnostic morphological characters within *Reticulitermes* and *Coptotermes*.

INTRODUCTION

The outermost layer of the cuticle of all terrestrial insects consists of a thin layer of lipids (Hadley 1985). This wax plays a key role in survival of insects 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 surface lipids (Blomquist and Dillwith 1985, Hadley 1985, Lockey 1988). They have been shown to be important semiochemicals, such as sex and trail pheromones, and have been postulated as species- and caste- recognition cues in termites (Howard and Blomquist 1982).

Several chemical classes of insect cuticular hydrocarbons have been identified. Alkanes occur in nearly all insect surface lipids investigated so far (Lockey 1988). *n*-Alkanes generally range from 21 to 36 carbon atoms, and those 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 positional isomer is present, to complex isomeric mixtures (Blomquist and Dillwith 1985, Lockey 1988). 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 more 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 usually ranges from 20 to 37 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) (Nasutitermitinae). He found the majority of the hydrocarbons to be a complex mixture of unsaturated components with the degree of unsaturation ranging from four to eight double bonds. All of the normal paraffins, from C24 to C47, were present: compounds with an odd number of carbons in the parent chain predominated. Blomquist *et al.* (1979) and Howard *et al.* (1978, 1980, 1982a,b) were next to completely characterize the cuticular hydrocarbons of three termite species. They found that *Zootermopsis angusticollis* (Hagen) (Termopsidae), *Reticulitermes flavipes* (Kollar), and *R. virginicus* (Banks) (Rhinotermitidae), possess drastically different hydrocarbon profiles, and all three of these profiles differ markedly from those reported earlier for *N. exitiosus*.

Howard and Blomquist and their colleagues postulated that hydrocarbons might serve as semiochemical cues in termites for recognition of different castes and species. On the basis of these early results, Howard and Blomquist (1982) hypothesized that each insect species has a mixture of cuticular hydrocarbons that is peculiar to that species and is potentially useful for taxonomic purposes. In his review of the chemical systematics of termite exocrine secretions, Prestwich (1983) also suspected that cuticular hydrocarbons have potential as taxonomic characters, but were understudied at the time of his review.

Insects synthesize most if not all of their complement of cuticular hydrocarbons *de novo* (Blomquist and Dillwith 1985). We assume this synthesis is genetically controlled and is affected only slightly by environmental parameters. 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 useful characters for use in chemotaxonomy (see references in Haverty *et al.* 1989; Page *et al.* 1990a). Hydrocarbons, that are reliable as taxonomic characters, should be abundant components (at least 1 percent, but preferably 5 percent of the total hydrocarbon mixture). They should also be unique or present in only a few of the species, or conversely,. they should be common in most of the species yet completely· absent or of insignificant quantities in one or a few. Furthermore, if one's laboratory does not have access to

a gas chromatograph/mass spectrometer, hydrocarbon peaks should have a unique elution times so that they do not co-elute with another hydrocarbon in the same species, nor should they elute at a time similar to that of a different hydrocarbon in a different species (Haverty *et al.* 1989). However, it is wise to have all components verified by mass spectrometry.

We have been studying existing taxonomies that are based on morphological, genetic and/or behavioral characteristics to evaluate the utility of cuticular hydrocarbons as taxonomic characters (Haverty *et al.* 1988, 1990b, Page *et al.* 1990a,b). Ideally, we should use these chemical characters much as classical taxonomists use morphology, behavior or genetics, i.e., to sort the groups of insects on the basis of chemical characters first, rather than after groups have already been sorted on the basis of existing (nonchemical) character criteria. 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 taxonomic characters of termites (Prestwich 1983).

We initially started our studies on the cuticular hydrocarbons of termites while trying to understand a synonymy of two species of scolytid cone beetles, *Conophthorus ponderosae* Hopkins and *C. lambertianae* Hopkins. Since these beetles have few useful diagnostic morphological characters, we decided to examine cuticular hydrocarbons as taxonomic characters for these beetles. To test our methodology we examined the hydrocarbons of the dampwood termite, *Z. angusticollis*, which had been characterized in the literature (Blomquist *et al.* 1979). We found our results to be qualitatively similar, yet quantitatively very different from those reported by Blomquist *et al.* (1979). Furthermore, on the basis of morphological characters our termite specimens were identified as *Z. nevadensis* (Hagen), not *Z. angusticollis*. Additional collections and analyses led us to identify an "extra" hydrocarbon phenotype of *Zootermopsis* (Haverty *et al.* 1988), and to find a morphological character for unequivocal identification of the three described species of *Zootermopsis* (Thorne and Haverty 1989).

These serendipitous discoveries encouraged us to expand our work to termites as well as other groups of economically important forest insects. We have found that characterization of cuticular

hydrocarbons often leads to subsequent biological, behavioral, or other chemical studies which clarify taxonomic questions (Haverty and Thorne 1989). The groups of termites we have chosen to investigate are the North American species of the wood-destroying genus *Reticulitermes* and the ecologically and economically important tropicopolitan genus *Coptotermes*.

Reticulitermes is, without a doubt, the most economically important genus of termites in the United States and Europe (Su and Scheffrahn 1990). It is widely acknowledged that this Holarctic genus is in need of revision. The morphology of the subterranean termites in the family Rhinotermitidae is quite variable among colonies within the same species. This holds true for *Reticulitermes* as well. Weesner (1970) states "The definition of the various species of *Reticulitermes* and, therefore, the limits of their distributions, is difficult. Certainly this genus is woefully in need of a critical taxonomic study...."

Currently the most common preventative and remedial control tactics used against these insects rely on the application of enormous quantities of toxic chemicals to the soil to form an impenetrable barrier between the soil-dwelling termites and the wooden structure at risk (Beal *et al.* 1989). This practice is beginning to fall into disfavor with the general public and environmental regulatory agencies. New, environmentally acceptable techniques will need to be developed. These new methods will require knowledge of the behavior of the termites, including knowledge of the semiochemicals on which they rely (Su and Scheffrahn 1990). It is our premise, therefore, that we will need to have a much better understanding of the taxonomy of this group before we can adequately study the biology and behavior of *Reticulitermes*. We hope that characterization of the cuticular hydrocarbons of *Reticulitermes* will help to resolve some taxonomic problems.

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is considered to be one of the most voracious subterranean termites and a serious threat to wood in structures throughout its range (Su and Scheffrahn 1990). In addition to buildings, *C. formosanus* attacks live trees, creosoted transmission poles, structural pilings, and even underground utility cables (Tamashiro *et al.* 1987). Even though the common name implies that this species is from Formosa (Taiwan), *C. formosanus* is considered

to be indigenous to mainland China (Kistner 1985). It was introduced into Japan prior to 1600 and subsequently into Hawaii before 1907 (Swezey 1914). 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 following World War II. Active movement of military goods from the Pacific Theater to storage facilities in port cities of the United States probably resulted in the establishment of *C. formosanus* in North America (Beal 1987). There has been a significant "spread" of *C. formosanus* to other southern port cities in the southern United States (La Fage 1987). Since 1980, indications of established infestations in inland areas suggest this species may now be transported in infested wood products via domestic, surface commerce (Chambers *et al.* 1988; La Fage 1987; Sponsler *et al.* 1988). Despite the overall success of *C. formosanus* in infesting new areas, distribution is generally confined to locations ca. 36° north or south of the equator (Su and Tamashiro 1987; Beal 1987). There is potential for infestation of the highly populated, coastal metropolitan area of Los Angeles, California, north to the San Francisco Bay Area.

With this latent threat to the most populous state in the United States, in addition to new locations along the entire Gulf Coast and much of the southern Atlantic Coast, effective quarantine procedures are imperative. Knowledge of how introductions of *C. formosanus* have been spread will help regulatory agencies formulate and/or 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 actually *C. formosanus*, or is it possible that introductions of another species of *Coptotermes*, such as *C. havilandi* (Holmgren) or *C. testaceus* (L.), have been introduced and simply misidentified? We feel that cuticular hydrocarbons will be useful for understanding the taxonomy of this genus, as well as identification of the origin of established infestations in the United States and the rest of the world (Haverty *et al.* 1990a).

MATERIALS AND METHODS

Samples of *Reticulitermes* were opportunistically collected in conjunction with biogeographic studies of the dampwood termites, *Zootermopsis*, and pine cone beetles, *Conophthorus*. We have collected throughout most of California, western Oregon and southern Arizona (Haverty *et al.* 1988, Page *et al.* 1990a). Additional samples were collected by us or colleagues in 1989 and 1990 from areas in California and Arizona reported to harbor either *R. hesperus* Banks or *R. tibialis* Banks (Light and Pickens 1934, Pickens 1934a,b, Weesner 1970, Nutting 1990) or *R. tumiceps* Banks (Banks and Snyder 1920) [*R. tumiceps* was synonymized with *R. tibialis* by Snyder (1949)]. Samples of *R. virginicus* (Banks) were collected near Clinton, Louisiana, and colonies of *R. flavipes* (Kollar) were collected near Gulfport, Mississippi; Ft. Lauderdale, Florida; and Boston, Massachusetts. Groups of ca. 100 larvae, nymphs and/or pseudergates and one or more soldiers (if they were present), were returned to the laboratory alive, then extracted. In some instances fewer than 100 pseudergates and/or nymphs were collected. Most of these smaller samples still provided well-resolved hydrocarbon chromatograms and were used in this preliminary study. Specimens from Louisiana, Mississippi, Florida and Massachusetts were dried in laboratories in those states before they were mailed to Berkeley, California. Exact localities as well as number and caste of each sample are presented in Table 1.

Samples of *C. formosanus* were collected from four geographically separated locations: Hallandale, Florida; New Orleans, Lousiana; Lake Charles, Louisiana; and Honolulu, Hawaii (Haverty *et al.* 1990a). Samples of *C. formosanus* were separated from wood debris and carton material, frozen, and subsequently dried in a desiccator. They were held dry at room temperature until hydrocarbons were extracted. Samples of *C. curvignathus* Holmgren from Thailand and *C. testaceus* (L.) from Trinidad were extracted live in hexane in the field. Samples from three colonies of *C. acinaciformis* (Froggatt) from Darwin, Australia, and from four colonies *C. lacteus* (Froggatt) from Erica Forest near Melbourne, Australia, were dried in Australia and extracted in our Berkeley laboratory.

Table 1. Location and castes of hydrocarbon samples of *Reticulitermes* spp.

Sample	Content[a]	Location
683	25PS, 3SO, 10NY	At Mud Creek near McCloud, Calif.
685	21PS, 2SO	On Hwy 97 23 km NE Weed, Calif.
687	19PS, 9SO, 11NY	On Hwy 299, 34 km W Redding, Calif., in pine stump
688	37PS, 5SO, 1NY	On Hwy 299, 40 km E of Eureka toward Willow Cr.
744	20PS, 1150, 4HY	On Hwy 20, 7.5 km west of Willits, Calif.
745	20PS, 6SO, 6NY	On Hwy 20, 25 km west of Willits, Calif.
749	25PS, 5SO, 3NY	26 km W of Red Bluff, Calif.
750	25PS, 11SO	76 km W of Red Bluff, Calif., in pine stump
752	8PS, 3SO	16 km N of Whiskeytown, Calif., in pine stump
753	20PS, 3SO	On Hwy 96 along Klamath River nr. Scott River
754	20PS, 3SO, 8NY	On Hwy 96 at Horse Cr. P.O. and Gen. Store, Calif.
829	??PS, 4SO	Fairbank, Ariz., in post along San Pedro River
835	??	St. David, Ariz., along San Pedro River
859	35PS, 2SO	On Hwy 34 near Trenholm, Oregon
934	28PS, 1SO, 1NY	Cloverdale, Calif.
968	30PS, 5SO	Isomata School, San Jacinto Mts., Calif.
969	30PS, 8SO	Onyx Summit, San Bernardino Mts., Calif.
971	60PS, 4SO	Eagles Roost Picnic Ground, San Gabriel Mts., Calif.
972	60PS, 3SO	Saunder's Meadow, San Gabriel Mts., Calif.
974	45PS, 15SO	Albin Meadows, San Gabriel Mts., Calif.
1101	??	Senator Hwy near Prescott, Ariz.
LSU1	(100PS)x3[b]	Idlewild Expt. Sta., Clinton, Louisiana
LSU2	(100PS)x3[b]	Harrison Expt. Forest near Gulfport, kmssissippi
HAST	200PS	Hastings Preserve, Carmel Valley, Calif.
LAF	100PS	Lafayette, Calif.
NYS1	100PS	Ft. Lauderdale, Florida
NYS2	100PS	Secret Woods, Davie, Florida
NYS3	100PS	Fern Forest, Coconut Creek, Florida
BLT1	100PS	Harvard Univ., Cambridge, Mass.
BLT2	100NY	Concord Field Station, Concord, Mass.
BLT3	100PS	Bedford, Mass.
BLT4	100PS	Sudbury, Mass.
DD1	200PS	Devil Dog Rd., at I-40, 5 km W of Williams, Ariz.
DD2	200PS	Devil Dog Rd., at I-40, 5 km W of Williams, Ariz.
PSCT1	200PS	Senator Hwy, 3 km SE of Prescott, Arizona
PSCT2	200PS	Senator Hwy, 3 km SE of Prescott, Arizona
PSCT3	200PS	Senator Hwy, 3 km SE of Prescott, Arizona
PSCT4	200PS	Schoolhouse Gulch, near Prescott, Arizona
GRSC5	200PS	Groom Creek School near Prescott, Arizona
GRSC6	200PS	Groom Creek School near Prescott, Arizona
GRSC7	200PS	Groom Creek School near Prescott, Arizona
PAL1	200PS	Palisades Ranger Stn, Santa Catalina Mts, Ariz.
PAL2	200PS	Palisades Ranger Stn, Santa Catalina Mts, Ariz.
SCAZ	200PS	Soldier Camp Road, Santa Catalina Mts, Arizona

[a] PS = pseudergate, SO = soldier, and NY = nymph.
[b] A composite sample from 3 colonies.

Detailed descriptions of extraction procedures for specific termite species have been published elsewhere (Blomquist *et al.* 1979; Howard *et al.* 1978, 1982, 1988; Haverty *et al.* 1988, 1990a,b). Cuticular lipids of *Reticulitermes* or *Coptotermes* were

extracted by immersing 15 to 200 termites (depending on sample size), as a group, in 10ml of *n*-hexane for 10 min. After extraction, hydrocarbons were separated from other components by pipetting the extract and an additional 8ml of *n*-hexane through 3cm 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 100µl of *n*-hexane for GC-MS analyses. Extracted termites, or additional termites from the same colony, were stored in 70 percent ethanol 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 a Hewlett Packard Chemstation computer. The GC-MS was equipped with an HP-1, fused silica capillary column (30m x 0.2mm ID) and operated in split mode (with a split ratio of 8:1 to 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 70eV. *n*-Alkanes were identified by comparing their retention times and mass spectra with external standards (*n*-C22, *n*-C24, *n*-C28 and *n*-C32). Alkenes and methyl-branched alkanes were tentatively identified by calculating their equivalent chain lengths; mass spectra of methylalkanes were interpreted as described by Blomquist *et al.* (1987) to identify methyl branch location(s).

Integration of the total ion chromatogram is performed by the data editing software (HP59974J Rev. 3.1.2) in the Hewlett-Packard Chemstation. The report generator program gives relative proportions of each peak (or hydrocarbon component) as a percentage of the total area. These relative proportions were then averaged for each hydrocarbon phenotype or species.

RESULTS AND DISCUSSION

We have characterized the cuticular hydrocarbons of numerous colonies of *Reticulitermes* from California, Oregon, and Arizona (Table 1). We have separated these samples into four different phenotypes (Types I, II, III, and IV) on the basis of their cuticular hydrocarbon components (Tables 2-5). At this point we are not prepared to assign species designations to these hydrocarbon types because the genus is in need of revision (Weesner 1970)

Table 2. Percent hydrocarbon composition from colonies of *Reticulitermes* spp. Type I collected in California.

Hydrocarbon[a]	ECL[b]	*Reticulitermes* sample[c]															
		683	685	687	688	744	749	750	752	753	754	934	969	971	972	INOL	Mean
n-C23	23.00	13.0	9.9	11.1	9.2	5.6	11.8	8.0	9.8	11.0	7.3	6.9	18.9	23.5	14.3	14.3	11.6
11-C23	23.39	0	0	1.1	0	0	0	3.1	0	0	0	0	0	0	0	2.6	0.5
2-MeC23	23.65	0	0	1.5	2.4	0	0	1.6	0	0	0	0	2.1	2.8	4.1	1.6	1.1
n-C24	24.00	10.9	0	4.5	4.7	2.8	5.8	2.5	0	0	3.7	4.4	5.6	6.1	7.2	4.7	4.2
10-;11-;12-MeC24[d]	24.36	0	0	2.0	0	2.4	0	3.1	0	0	0	0	0	0	0	2.7	0.7
2- or 4-MeC24	24.63	9.3	6.7	8.1	11.1	6.2	13.2	8.6	8.1	8.3	6.9	9.2	5.0	13.4	9.3	8.9	8.8
n-C25	25.00	39.4	26.7	21.7	21.0	11.9	27.1	10.8	10.9	26.6	22.8	23.5	36.8	30.6	27.3	17.9	23.7
9-;11-;13-MeC25[d]	25.38	15.5	19.3	24.2	15.3	36.4	8.7	35.1	35.5	15.5	19.0	12.5	10.3	4.8	4.0	22.3	18.6
11,15-DimeC25 + 2- or 4-MeC25[e]	25.60	5.5	5.1	5.9	4.9	6.9	5.5	10.0	8.6	8.7	8.6	6.4	3.7	1.3	1.1	10.8	6.2
3-MeC25	25.73	6.3	7.3	6.1	7.0	5.5	9.8	4.2	5.6	7.1	6.8	13.6	4.8	6.8	7.1	7.7	7.0
11-;13-MeC35[d]	35.31	0	7.6	2.2	3.8	4.6	4.1	1.7	2.4	5.4	3.8	5.0	3.1	0.7	0	0	3.0
11,15-DimeC35	35.60	0	6.6	4.8	3.7	6.2	5.1	7.1	10.7	10.9	12.7	4.6	1.2	0.5	0	0	4.9
11-;13-MeC37[d]	37.30	0	2.9	2.5	8.3	4.1	5.8	1.5	3.1	1.7	2.5	6.9	5.5	4.8	9.0	4.3	4.2
11,15-DimeC37	37.59	0	8.2	4.4	6.6	5.8	3.1	3.0	5.6	4.7	5.8	7.2	3.4	4.7	7.7	2.7	4.9
11-;13-MeC39[d]	39.30	0	0	0	1.8	0	0	0	0	0	0	0	0	0	4.5	0	0.4
11,15-DimeC39	39.60	0	0	0	0	2.1	0	0	0	0	0	0	0	0	4.8	0	0.5

[a] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC25 = 3-methylpentacosane.
[b] ECL = equivalent chain length.
[c] Sample content and locality in Table 1. Values of 0 may mean that these minor components are absent in a particular sample or simply in quantities below our level of detectability.
[d] An isomeric mixture. The 11-methyl isomer is predominant.
[e] These components co-elute in this peak.

Table 3. Percent hydrocarbon composition from colonies of *Reticulitermes* spp. Type II collected in California.

		Reticulitermes sample[c]				
Hydrocarbon[a]	ECL[b]	968	974	HAST	LAF	Mean
n-C23	23.00	14.1	16.1	7.8	5.5	10.9
11-C23	23.39	5.5	5.4	2.2	2.4	3.9
2-MeC23	23.65	3.7	1.9	2.2	1.5	2.3
9,13-;9,15-DimeC23 + 3-MeC23	23.65	0	1.9[e]	0	0	0.5
n-C24	23.73	8.0	1.9[e]	1.5	0.6	3.0
10-;11-;12-MeC24[d]	24.00	5.4	3.2	4.7	2.7	4.0
2- or 4-MeC24	24.36	0	0	1.3	1.5	0.7
C25:1	24.63	15.1	5.6	8.7	3.9	8.3
n-C25	24.70	1.9	7.2	2.4	1.5	3.3
9-;11-;13-MeC25[d] + 7,9-C25:2[e]	25.00	24.6	27.3	17.3	13.3	20.6
11,15-DimeC25 + 2- or 4-MeC25[f]	25.38	11.1	7.7	25.7	43.2	21.9
3-MeC25	25.60	1.9	2.9	3.9	3.5	3.1
11-;13-MeC35[d]	25.73	9.0	2.9	5.6	4.5	5.5
11,15-DimeC35	35.31	0	1.3	4.5	1.2	1.8
11-;13-MeC37[d]	35.60	0	1.3	0	0.9	0.5
11,15-DimeC37	37.30	0	6.1	5.3	5.6	4.3
11-;13-MeC39[d]	37.59	0	3.7	3.3	3.5	2.6
11,15-DimeC39	39.30	0	2.0	2.0	2.3	1.6
	39.60	0	1.6	1.6	2.2	1.4

[a]Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC25 = 3-methylpentacosane.
[b]ECL = equivalent chain length.
[c]Sample content and locality in Table 1. Values of 0 may mean that these minor components are absent in a particular sample or simply in quantities below our level of detectability.
[d]An isomeric mixture. The 11-methyl isomer is predominant.
[e]This component was detected only in samples HAST and LAF.
[f]These components co-elute in this peak.

and will likely be revised soon on the basis of soldier morphology (Timothy G. Myles, personal communication).

Type I, by far the most common type, was found in the Coast Range in northern California, in the San Francisco Bay Area, the San Gabriel, San Bernadino and San Jacinto Mountains in southern California, and even in a desert wash near Owens Lake, California. We did not collect this phenotype in Oregon or Arizona. Type I *Reticulitermes* differs from Types II, III, and IV by not containing any alkenes, specifically pentacosene (C25:1) or nonacosene (C29:1). The Type II phenotype was also found only in California, whereas the Type III phenotype was found in sites from Oregon,

Table 4. Percent hydrocarbon composition from colonies of *Reticulitermes* spp. Type III collected from Oregon, California and Arizona.

		Reticulitermes sample[c]					
Hydrocarbon[a]	ECL[b]	745	829	835	859	PSCT3	Mean
n-C23	23.00	15.2	7.7	6.3	8.4	5.8	8.7
2-MeC23	23.65	0	0.6	0.4	0.5	0	0.3
n-C24	24.00	5.0	2.7	2.2	6.3	3.0	3.8
10-;11-;12-MeC24[d]	24.36	0	1.5	0.6	0	0	0.4
2- or 4-MeC24	24.63	4.3	11.6	6.7	9.0	8.6	8.0
C25:1	24.70	0	0	1.5	0	7.0	0.4
n-C25	25.00	34.1	21.2	22.9	35.4	21.3	27.0
9-;11-;13-MeC25[d]	25.38	12.9	25.7	17.8	8.9	39.3	20.9
5-MeC25	25.50	10.0	3.0	2.9	17.3	2.0	7.0
11,15-DimeC25 + 2- or 4-MeC25[e]	25.60	4.9	4.7	4.0	4.0	3.7	4.3
3-MeC25	25.73	4.1	8.1	7.4	6.3	5.3	6.2
5,17-DimeC25	25.90	3.9	1.7	2.4	4.4	0	2.5
11-;13-MeC37[d]	37.30	0	3.5	3.3	0	0	1.4
11,15- DimeC37	37.59	5.7	0	0	0	0	1.1
5,17-DimeC37	37.80	0	3.4	4.0	0	0	1.5
12-MeC38	38.35	0	0	0.5	0	0	0.1
11-;13-MeC39[d]	39.30	0	3.0	5.9	0	0	1.8
13,17-DimeC39	39.60	0	0	1.3	0	0	0.3
5,17-DimeC39	39.80	0	1.4	3.7	0	0	1.0
13,17-DimeC41	41.60	0	0	0.9	0	0	0.2
5,17-DimeC41	41.80	0	0	5.2	0	4.1	1.9

[a] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC25 = 3-methylpentacosane.
[b] ECL = equivalent chain length.
[c] Sample content and locality in Table 1. Values of 0 may mean that these minor components are absent in a particular sample or simply in quantities below our level of detectability.
[d] An isomeric mixture. The 11-methyl isomer is predominant.
[e] These components co-elute in this peak.

California, and Arizona. Type II (as well as Type I) differs from Type III by the lack of 5-methylpentacosane (5-MeC25), which is consistently abundant in Type III, and the lack of any 5,17-dimethylalkanes. One hydrocarbon, 7,9-pentacosadiene, appears in Type II and *R. flavipes* (Table 6); this compound has been reported to be abundant in *R. flavipes* (Howard *et al.* 1978, Bagnères *et al.* 1991), *R. virginicus* (Howard *et al.* 1980), and *R. santonensis* (Feytaud) (Bagnères *et al.* 1991).

Type IV, which we (MH and MP) serendipitously collected for the first time near Prescott, Arizona, in April, 1988, is radically

Table 5. Percent hydrocarbon composition from colonies of *Reticulitermes* spp. Type IV collected from Arizona.

Hydrocarbon[a]	ECL[b]	*Reticulitermes* sample[c]												
		1101	PSCT1	PSCT2	PSCT4	DD1	DD2	PAL1	PAL2	GRSC5	GRSC6	GRSC7	SCAZ	Mean
3-MeC25	25.73	0.7	0	0	0.5	0.4	0.4	2.2	2.1	0.8	0.7	0.7	1.7	0.9
n-C26	26.00	0	0	0	0	0.3	0.3	0	0	0	0	0.3	0.4	0.1
2- or 4-MeC26	26.63	2.5	3.6	3.3	2.8	4.6	4.4	8.8	7.9	2.3	3.0	2.2	9.9[d]	3.8
C27:1	26.70	10.5	3.5	4.2	2.8	2.0	2.4	13.4	17.6	3.9	3.8	4.0	14.8[d]	7.7
n-C27	27.00	3.6	4.4	5.9	3.8	6.7	5.2	11.0	9.1	4.8	4.4	7.2	6.1	6.0
5-MeC27	27.50	0.6	0.7	0.9	0.8	0.3	0.5	1.1	1.2	0.4	0.6	0.6	1.2	0.7
2- or 4-MeC27	27.60	2.6	3.4	3.8	2.7	3.3	3.0	3.5	2.9	2.6	2.8	2.3	2.1	2.9
3-MeC27	27.73	5.7	6.6	6.5	5.8	5.4	5.5	4.8	4.7	5.6	5.8	5.4	4.2	5.5
n-C28	28.00	0	1.1	1.9	0.8	0.9	0.7	0	0	0	0.8	1.0	0	0.6
C29:2	28.69	11.8	3.0	5.0	2.2	3.9	5.2	1.4	2.5	2.0	3.4	1.4	2.0	3.7
C29:1	28.70	34.4	44.8	43.5	48.8	38.8	38.3	38.3	38.4	54.4	49.1	54.0	44.6	44.0
n-C29	29.00	1.4	2.1	2.1	2.2	3.3	2.3	1.9	1.4	2.6	2.3	3.7	1.0	2.2
C30:2	29.45	1.7	0	0	0	0	0	0	0	0	0	0	0	0.1
5-MeC29	29.50	0.6	1.0	0	1.2	1.3	1.2	0.5	0	0.9	0.9	0.7	0.5	0.7
2- or 4-MeC29	29.60	0	0	0	0.4	0.4	0.3	0	0	0	0.4	0.4	0.6	0.2
3-MeC29	29.75	0.9	1.3	0	1.5	1.2	1.0	0	0	1.5	1.4	1.7	0.4	0.9
5,17-DimeC29	29.83	1.5	0	0	0	0.7	0.7	0	0	0.4	0.5	0.4	0	0.4
C31:2	30.69	9.9	7.5	7.7	6.5	7.8	9.1	3.7	4.1	4.2	5.7	3.0	3.7	6.1
C31:1	30.70	1.9	1.4	1.6	1.3	1.4	1.3	1.7	1.4	1.4	1.5	1.7	1.8	1.5
Unk(m/e 432)	31.46	3.5	6.1	5.9	5.6	9.0	9.1	0	0	4.8	5.2	2.9	0*	4.3
5,17-DimeC31	31.80	1.7	0	0	0.8	1.2	1.2	0	0	0.5	0.9	0.6	0*	0.6
C33:2	32.69	3.4	2.8	1.8	2.0	2.1	2.1	2.3	2.5	1.4	1.7	1.2	1.5	2.1
5,17-DimeC33	33.80	1.0	0	0	0	0	0	0	0	0	0.4	0.5	0	0.2
5,17-DimeC41	41.80	0	2.0	1.7	3.9	1.1	1.0	2.1	1.6	1.2	1.0	1.3	1.1	1.5
5,17-DimeC43	41.80	0	3.9	4.2	4.0	4.1	4.8	3.1	2.5	3.3	3.6	3.2	2.5	3.3

[a] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC25 = 3-methylpentacosane.
[b] ECL = equivalent chain length.
[c] Sample content and locality in Table 1. Values of 0 may mean that these minor components are absent in a particular sample or simply in quantities below our level of detectability.
[d] These components coeluted in this particular sample. Quantities were assigned proportional to these two peaks in the other 11 samples.

Table 6. Percent hydrocarbon composition from colonies of *Reticulitermes* flavipes (Kollar) collected from Massachusetts, Florida and Mississippi.[a]

Hydrocarbon[b]	ECL[c]	BLT1	BLT2	BLT3	BLT4	NYS1	NYS2	NYS3	LSU2	Mean
n-C23	23.00	1.3	1.2	1.5	2.7	0.5	0.3	1.0	5.9	1.8
11-C23	23.39	1.2	0.8	1.4	1.7	0.9	0.9	0	1.1	1.0
2- or 4-MeC23 + 3-MeC23[e]	23.65	0.8	0.8	1.0	0.9	0.6	0.5	0.8	2.5	1.0
n-C24	24.00	1.0	1.7	1.3	1.2	0.7	0.6	1.1	3.3	1.4
11-;12-MeC24[d]	24.36	1.6	1.7	1.6	2.0	0.8	1.4	0.7	1.0	1.4
2-MeC24 + 3-MeC24 + C25:1[e]	24.63	12.3	14.5	13.8	12.0	21.5	19.4	26.7	28.6	18.6
n-C25	25.00	9.9	10.1	9.4	8.6	8.1	6.0	12.3	11.2	9.5
9-;11-;13-MeC25[d] + 7,9-C25:2	25.38	41.7	43.7	43.3	46.0	34.5	38.6	34.0	9.5	36.4
2- or 4-MeC25[f]	25.60	4.6	4.5	3.8	2.5	5.9	4.5	4.6	2.7	4.1
3-MeC25	25.73	7.5	6.8	8.2	4.4	6.2	6.9	6.4	7.3	6.7
n-C26	26.00	0	0	0	0	0	0	0	0.5	0.1
11-;12-;13-MeC26[d]	26.34	2.2	1.2	1.0	1.0	1.3	4.5	1.1	0	1.6
2- or 4-MeC26	26.63	1.4	0.5	0.3	0.2	0.5	1.2	0.3	0	0.5
n-C27	27.00	0	0	0	0	0.2	0	0	0	<0.1
11-;13-MeC27[d]	27.35	0	0	0.6	0.6	0	1.2	0.9	0	0.4
11-;12-MeC34[d]	34.35	0	0	0	0.9	0.2	0	0	0	0.1
12,16-DimeC34	34.60	0	0	0	0	0.1	0	0	0	<0.1

Table 6. Continued.

Hydrocarbon[b]	ECL[c]	BLT1	BLT2	BLT3	BLT4	NYS1	NYS2	NYS3	LSU2	Ave.
11-;13-MeC35[d,f]	35.31	1.9	1.4	2.0	2.4	4.6	3.9	2.2	3.6	2.8
11,15-DimeC35[f]	35.60	0.3	0.2	0	0.2	3.8	3.4	2.4	3.0	1.7
7,17-DimeC35[f]	35.70	0	0	0	0	0	0	0	0.9	.1
5,17-DimeC35[f]	35.80	0	0	0	0	0	0	0	2.2	.3
11-;12-;13-MeC36[d,f]	36.35	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.6	.3
11,15-;12,16-DimeC36[e]	36.60	0	0	0	0.1	0.3	0.2	0.2	0	.1
11-;13-;15-MeC37[d,f]	37.30	3.3	2.7	2.4	2.9	2.1	1.4	1.1	4.2	2.5
11,15-DimeC37[f]	37.59	3.6	2.5	3.6	4.0	3.2	3.0	2.4	5.0	3.4
7,17-DimeC37[f]	37.70	0	0	0	0	0	0	0	0.9	.1
5,17-DimeC37	37.80	0	0	0	0	0	0	0	2.7	.3
11-;12-;13-MeC38[d]	38.30	0.4	0.3	0.2	0.3	0.1	0	0	0	.2
12,16-DimeC38	38.60	0.2	0.2	0.1	0.2	0.2	0	0.1	0	.1
11-;13-MeC39[d]	39.30	2.5	2.4	1.9	2.7	0.4	0.2	0.2	1.0	1.4
11,15-DimeC39	39.60	1.5	1.5	1.8	1.9	1.7	1.2	0.9	0.8	1.4
5,17-DimeC39	39.80	0	0	0	0	0	0	0	1.1	.1
11-;13-MeC41[d]	41.30	0.3	0.5	0.1	0.4	0.2	0	0	0	.2
11,15-DimeC41	41.60	0.2	0.4	0.3	0.5	1.0	0.4	0.4	0	.4
5,17-DimeC41	41.80	0	0	0	0	0	0	0	0.4	.1

[a] Sample content and locality in Table 1. Values of 0 may mean that these minor components are absent in a particular sample or simply in quantities below our level of detectability.
[b] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC25 = 3-methylpentacosane.
[c] ECL = equivalent chain length.
[d] An isomeric mixture. The 11-methyl isomer is predominant.
[e] These components co-elute in this peak.
[f] Not identified by Howard et al. (1978, 1980).

Table 7. Mean percent hydrocarbon composition from three colonies of *Reticulitermes* virginicus (Banks) from Idlewild Experiment Station, Clinton, Louisiana.

Hydrocarbon[a]	ECL[b]	Percent	Hydrocarbon[a]	ECL[b]	Percent
n-C23	23.00	4.9	11-MeC29	29.32	0.6
11-MeC23	23.39	0.7	11,15-DimeC29[c]	29.60	1.2
2-MeC23	23.65	1.4	*n*-C30[c]	30.00	0.2
3-MeC23	23.73	1.5	3,7-DimeC29[c]	30.13	0.2
n-C24	24.00	2.5	11-MeC30[c]	30.32	0.6
11-MeC24	24.36	1.4	11,15-DimeC30[c]	30.60	0.5
2- or 4-MeC24	24.63	12.3	11-MeC31	31.32	6.4
C25:1	24.70	2.8	11,15-DimeC31	31.60	4.9
n-C25	25.00	7.6	11-MeC32[c]	32.35	1.2
11-MeC25	25.38	11.9	12,16-DimeC32[c]	32.60	0.4
2- or 4-MeC25	25.60	3.1	11-MeC33	33.34	6.6
3-MeC25[c]	25.73	5.3	11,15-DimeC33	33.57	1.6
n-C26	26.00	1.1	11-MeC34[c]	34.35	0.7
11-MeC26	26.34	1.0	12,16-DimeC34[c]	34.60	0.1
2-MeC26	26.63	1.4	11-MeC35	35.31	4.8
n-C27	27.00	1.0	11,15-DimeC35	35.60	2.1
11-MeC27	27.35	2.7	11-MeC36[c]	36.35	0.5
11,15-DimeC27[c]	27.60	0.3	11-MeC37	37.30	2.5
3-MeC27[c]	27.73	0.2	11,15-DimeC37	37.59	0.7
n-C28	28.00	0.6	11-MeC39	39.30	0.4
n-C29	29.00	0.4			

[a] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC25 = 3-methylpentacosane.
[b] ECL = equivalent chain length.
[c] Not identified by Howard *et al.* (1982a,b).

different from Types I, II, and III. In October, 1990, one of us (MH) made a collecting trip in central and southern Arizona specifically to collect *Reticulitermes* and verify our earlier collection of one specimen of this unique phenotype. We discovered that this phenotype is common in the higher elevations of Arizona (>1750m) from Williams, Arizona, to the Santa Catalina Mountains just north of Tucson, Arizona. The Type IV phenotype is characterized primarily by the abundance of the alkenes and alkadienes (C27:1, C29:1, C29:2, C30:2, C31:1, C31:2, and C33:2) which constitute a majority of the cuticular hydrocarbons (Table 5). From these results we infer that there are probably at least three species of *Reticulitermes* in western North America.

We have divided these *Reticulitermes* samples from Oregon, California, and Arizona into four hydrocarbon phenotypes, but do

so with the caveat that this is not a definitive division. From our experience with the dampwood termites (Haverty *et al.* 1988) we know that some minor components are often absent or present in quantities that are not detectable. Because of this, and the minor differences between Types I and II, we suspect that these two phenotypes are either minor variations of the same species or species that are very closely related.

We have also collected distinctly different phenotypes in the same location. Types I and III were both collected in the Coast Range within 20 km of each other near Willits, California (samples 744 and 745). Given the disparate collection sites of Type III, more "sympatric" collections in California are likely. Types III and IV were also gathered in the same area, <30m apart (samples PSCT1 and PSCT3), on the Prescott National Forest near Prescott, Arizona. It would be very interesting to investigate the ecological relationships and distributions of these two phenotypes or species: Type III, which has often been assumed to be *R. tibialis*, occurs in the lower (<1750m) elevations, especially in association with riparian zones (Haverty and Nutting 1976) and Type IV, which has also been assumed to be *R. tibialis*, but may actually be the synonymized *R. tumiceps* (Banks and Snyder 1920), occurs in forested areas primarily in dead wood of ponderosa pine, *Pinus ponderosa* Laws.

In addition to the collections of *Reticulitermes* from the western United States, we have reexamined the hydrocarbon mixtures of *R. flavipes* and *R. virginicus* with those published in the literature (Howard *et al.* 1978, 1980, 1982a,b). Initially we did this to assist colleagues verify species diagnoses of colonies of these two species that they were using in studies of feeding excavations of subterranean termites (Delaplane and La Fage 1990). We were able to identify blind samples, 3 separate colonies of each species, solely by comparing the cuticular hydrocarbons we identified with those reported in the literature. However, the hydrocarbon components we identified did not correlate exactly with the components reported by Howard *et al.* (1978, 1980, 1982a,b).

We did not recognize significant quantities of hydrocarbons that elute before tricosane in either *R. flavipes* or *R. virginicus* nor did we find C23:1 or C23:2 reported for *R. virginicus* (Howard 1978, 1980, 1982a,b). We did identify numerous late-eluting

Table 8. Mean percent composition of hydrocarbons from workers of five species of *Coptotermes*.[a]

	Coptotermes species[c]				
Hydrocarbon[b]	form	curv	acin	lact	test
n-C23	---	---	---	0.3	---
9-;11-MeC23	---	---	---	0.3	---
2-MeC23	---	---	---	0.8	---
n-C24	---	---	---	0.5	---
9-;10-;11-;12-;13-MeC24	---	---	---	0.8	---
2-MeC24	---	---	0.3	1.0	---
C25:1	---	---	tr	---	---
n-C25	0.9	tr	1.8	4.3	---
9-;11-;13-MeC25[d]	0.3	8.2	2.2	49.2	---
2-MeC25	8.3	7.9	10.6	11.0	---
3-MeC25	0.5	---	3.1	5.5	---
n-C26	1.2	0.7	2.2	0.9	---
9-;11-;13-MeC26[d]	0.8	3.8	tr	4.3	---
2-MeC26	4.1	3.5	---[g]	0.7	---
C27:1	---	---	51.6[g]	---	---
3-MeC26	---	---	---	0.2	---
n-C27	8.9	4.7	4.3	0.1	---
9-;11-;13-;15-MeC27[d]	27.1	47.4	9.4	0.5	---
7-MeC27	---	---	2.1	---	---
2-MeC27 + 9,13-DimeC27	23.8	14.9[f]	2.5[f]	0.1[f]	8.1[f]
3-MeC27	7.2	4.5	2.8	0.1	---
C28:1	---	---	0.8	---	---
n-C28	1.2	1.0	---	---	---
3,7-DimeC27	---	---	tr	---	---
9-;11-;13-;15-MeC28[d]	2.9	1.7	tr	---	---
2-MeC28	---	---	0.5	---	---
C29:1	---	---	1.8	---	---
n-C29	---	0.5	tr	---	---
9-;11-;13-;15-MeC29[d] + 13,15-DimeC29[e]	12.8	1.2[f]	0.5[f]	---	49.0[f]
2-MeC29	---	---	---	---	17.1
11-;12-;13-MeC30[d]	---	---	---	---	6.4
11-;13-;15-MeC31[d]	---	---	---	---	19.4
13,17-DimeC31	---	---	---	0.3	---
11-;13-;15-;15-;17-MeC33	---	---	---	0.5	tr
13,17-;15,19-DimeC33	---	---	---	0.5	---
12-;14-MeC34	---	---	---	0.2	---
12,16-MeC34	---	---	---	0.1	---
11-;13-;15-;17-MeC35	---	---	---	1.5	---
11,15-;13,21-DimeC35	---	---	---	1.6	---
12-;13-;14-;15-;16-; 17-MeC36	---	---	---	0.7	---
12,16-DimeC36	---	---	---	0.7	---
11-;13-;15-;17-;19-MeC37 + 15,17-DimeC37	---	---	---	0.5	---
11,15-;13,17-DimeC37	---	---	---	5.1	---
12-;14-;15-;16-;17-; 18-MeC38	---	---	---	0.6	---
12,16-DimeC38	---	---	---	0.5	---
11-;13-;15-;16-MeC39 + 15,17-DimeC39	---	---	---	1.7	---
11,15;13,17-DimeC39	---	---	---	1.0	---

mono- and dimethyl alkanes (Tables 6 and 7) which were not characterized by Howard *et al.* (1978, 1980, 1982a,b). This is most likely because we had available a gas chromatograph/mass spectrometer with capillary column capability, whereas the early work by Howard and his colleagues was done with lower resolution packed columns. Late-eluting compounds separate quite well on our system and occur in sufficient quantities to provide characteristic mass spectra.

Recently we characterized the cuticular hydrocarbons from colonies of *C. formosanus* from four different geographic locations in the United States (Haverty *et al.* 1990a). Concurrently, McDaniel (1990) characterized the hydrocarbons from nine colonies of *C. formosanus* collected from Lake Charles, Louisiana, and one from Honshu, Japan. McDaniel identified numerous mono- and dimethylalkanes, which occurred in trace amounts, that were not detectable in our samples (see Table 1, McDaniel [1990]). He also identified a very unique trimethylalkane, 13,15,17-TrimeC29, in trace amounts: we did not detect this compound. The major constituents in our samples and in McDaniel's samples agree with a few exceptions.

We identified three hydrocarbons that McDaniel (1990) did not detect (*n*-C28; 9,13-DimeC27; and 13,15-DimeC29). The latter two unique hydrocarbons co-elute with a monomethylalkane: 2-MeC27 + 9,13-DimeC27 and 9-;11-;13-;15-MeC29 + 13,15-DimeC29 (Table 8). Of the five *Coptotermes* species we have investigated thus far, these coeluting dimethylalkanes are unique

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Table 8 cont. (footnotes)

[a] Hydrocarbons are quantified as the mean percent of the total hydrocarbon composition.

[b] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g. 3-MeC25 = 3-methylpentacosane.

[c] 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.

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

[e] Because of incomplete separation of these hydrocarbons both peaks are included as one value for *C. formosanus*.

[f] Only the monomethylalkane occurs in this species.

[g] 2-MeC26 co-elutes with C27:1.

to *C. formosanus* and could be considered diagnostic for this species. Analogous, but different, dimethylalkanes have also been identified in *Coptotermes* from Australia (Brown *et al.* 1990). McDaniel (1990) distinguished two hydrocarbons that we did not detect (15-;16-;17-;18-MeC41 and 13,17-;15,19-DimeC41). We apparently did not allow our hydrocarbon analyses of *C. formosanus* to run long enough to detect hydrocarbons with an equivalent chain length greater than 41.

We were able to separate colonies from all four sites we sampled in the United States on the basis of the relative abundance of cuticular hydrocarbon components (Haverty *et al.* 1990a). We found the population from Lake Charles to be very different from those from the three other locations (Hallandale, Florida, New Orleans, Louisiana, and Honolulu, Hawaii). We feel that our results support the assertion that the introduction of *C. formosanus* into Louisiana was from two separate sources (La Fage 1987). Our results suggested that *C. formosanus* from Hallandale, Florida, New Orleans, Louisiana, and Lake Charles, Louisiana, are not related to those from Honolulu, Hawaii (at least the insects we sampled from the Manoa Valley in Honolulu), and probably originated from other geographical locations.

A preliminary survey of the hydrocarbon composition of four additional species of *Coptotermes* demonstrates clear qualitative differences from *C. formosanus* (Table 8). *C. curvignathus* Holmgren from Thailand is the most similar of these four species to *C. formosanus*. Nearly all of the components in the hydrocarbon profiles of these two species are shared (Table 8). Two relatively minor components, *n*-C25 and 3-MeC25, 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-DimeC27 and 13,15-DimeC29 in *C. curvignathus*.

In our characterization of the hydrocarbons of *C. acinaciformis* (Froggatt) from Darwin, N.T., 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, N.T., Australia, as reported by Brown *et al.* (1990), except that we did not identify any hydrocarbons that eluted after 13-MeC29. The most dramatic

difference that we, and Brown *et al.* (1990), found in *C. acinaciformis* is the huge C27:1 peak (ca. 52% 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 agree dramatically with hydrocarbon profiles of *C. lacteus* from Batemans Bay and Canberra, Australia (Brown *et al.* 1990). Several minor components (2-MeC28, *n*-C29, 2-MeC29 and 3-MeC29) were not detected from our workers nor were they found by Brown *et al.* (1990) in soldiers or nymphs of the colonies they examined. One intriguing difference is our identification of two dimethylalkanes (15,17-DimeC37 and 15,17-DimeC39) that coelute with their corresponding internally branched monomethylalkanes (X-MeC37 and X-MeC39). Hydrocarbons with the methyl branches separated by a single methylene group are not common in insects (Blomquist *et al.* 1987). However, this type of compound has been identified in two species of *Coptotermes* (Haverty *et al.* 1990a, Table 8).

Finally, we report the hydrocarbon profile from a single colony of *C. testaceus* (L.) from Trinidad. We recognize, of course, that characterization of a species on the basis of one colony is unwise, yet we feel the hydrocarbon profile of this colony is so striking that it warrants mention. *C. testaceus* is notably different from *C. formosanus* and the *Coptotermes* species from Southeast Asia and Australia. All of the hydrocarbon components are terminally-branched monomethylalkanes (ca. 25 percent 2-MeC27 and 2-MeC29) or internally-branched monomethylalkanes: no *n*-alkanes, alkenes, 3-; 5-; or 7-methylalkanes were identified (Table 8). Therefore, we can conclude with reasonable certainty that none of the four *Coptotermes* populations we have studied in the United States was misidentified as *C. testaceus*.

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? (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, as well as morphology, behavior, and genetics, 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 (Bagnères *et al.* 1991, Brown *et al.* 1990, Clément *et al.* 1985, 1986, Haverty *et al.* 1988, 1990b, Howard *et al.* 1978, 1982a, 1988; Watson *et al.* 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 *et al.* 1988) to identify a new diagnostic morphological character for *Zootermopsis* species (Thorne and Haverty 1989). Where morphology cannot be used to separate hydrocarbon phenotypes, we have been able to use inter- and intra-phenotype agonistic behavior to corroborate cuticular hydrocarbon phenotypes (Haverty and Thorne 1989). Geographical races of *Coptotermes formosanus* can be distinguished on the basis of the concentrations of individual hydrocarbon components (Haverty *et al.* 1990a). Hydrocarbons have been used to separate sympatric populations of two species of *Nasutitermes* in Trinidad (Haverty *et al.* 1990b). 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 *et al.* 1990, Watson *et al.* 1989). Our results with the western *Reticulitermes* and those of Watson *et al.* (1989) and Brown *et al.* (1990) support this conclusion.

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