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Cuticular Hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) from Northern California Indicate Undescribed Species

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ABSTRACT. Cuticular hydrocarbon mixtures of *Reticulitermes* samples from disparate locations in northern California were characterized and correlated with species determinations. Literature records indicate that there are two extant species of *Reticulitermes* in northern California: *Reticulitermes hesperus* Banks and *R. tibialis* Banks. We identified five distinct hydrocarbon phenotypes in *Reticulitermes* from northern California. Termites were diagnosed morphologically as *R. tibialis* for all five cuticular hydrocarbon phenotypes based on available keys to soldiers. Commonly accepted distributions of *Reticulitermes* in northern California indicate that our primary collection sites should only produce *R. hesperus*. On the basis of cuticular hydrocarbons alone we suggest that there are two or more undescribed taxa of *Reticulitermes* in northern California, or that described species of *Reticulitermes* occur in northern California, but have never been noticed before. Therefore, we conclude that the taxonomy of *Reticulitermes* in North America is in need of revision. COMP BIOCHEM PHYSIOL 118B;4:869–880, 1997. Published by Elsevier Science Inc.

KEY WORDS. Chemotaxonomy, cuticular hydrocarbons, hydrocarbon variability, Isoptera, morphological correlation, *Reticulitermes*, species delimitation, subterranean termites

INTRODUCTION

According to the most recently published biogeographical information, only two species of *Reticulitermes*, *R. hesperus* Banks and *R. tibialis* Banks, are reported to occur in northern California (22,32,33). *R. hesperus* is thought to occur "along the Pacific Coast into northern Baja California. . . . In the inland valleys of California it coexists with, or is displaced by, *R. tibialis*. The actual limits of its eastward extensions are not clearly defined" (33). *R. tibialis* has been reported to occur in the inland valleys, desert southeast, and the east side of the Sierra Nevada (22,25,33). There is considerable disagreement among authors as to the precise distributions of either *R. hesperus* or *R. tibialis* in California (22,24,25,33). This uncertainty is likely a result of the ambiguity of the taxonomy of this genus in North America.

Most of the studies of the taxonomy of *Reticulitermes* and keys to the species are based on morphology of the alates and soldiers (3,22,28). A key to the species of *Reticulitermes* form the Northwest and Pacific Coastal area of North America (32) separates species based on alate morphology, but does not attempt to separate species based on soldier morphology. The most recent key to the species of North American *Reticulitermes* (22) does include a key to soldiers, however this key is largely based on the original descriptions (3) and subsequent synonymies (27).

A completely different set of characters, cuticular hydrocarbons of workers, has been examined among *Reticulitermes* from the southeastern United States and indicates numerous undescribed species (1,9,16,17). We decided to examine the taxonomy of *Reticulitermes* in northern California with this potentially powerful technique. We report here a chemotaxonomic study using the cuticular hydrocarbons of *Reticulitermes* from northern California. As with our previous study of the *Reticulitermes* of the southeastern United States (9), our primary goal is to make initial separations of groups based on these chemical characters. These separations can then be used to search for additional characters, such as morphology and soldier defense secretions, in future taxonomic studies.

METHODS

Collection of Termites

The majority of our samples of *Reticulitermes* were collected from termite monitoring stations. Permanent field sites were established at the U.S.D.A Forest Service Institute of Forest Genetics (IFG) near Placerville, El Dorado County, at two sites in Novato, Marin County, and one in Kentfield, Marin

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County. Monitoring stations installed at these field sites were checked monthly for termite activity. When present in sufficient numbers (in excess of 100 workers), termite samples were returned to the laboratory. Hydrocarbon phenotypes were determined for each active monitoring station at least once. A total of 75 stations at IFG, and 35 from Marin County, were characterized. For quantification we used from 4 to 8 samples from each hydrocarbon phenotype.

We selected individual samples, from monitoring stations or wood on the ground, that we were confident represented different colonies (at least 50 m apart) within a hydrocarbon phenotype, and that displayed excellent chromatographic separation of the cuticular hydrocarbons. Our permanent field sites and other serendipitous collections of infested wood from 14 additional locations in northern California provided the samples reported in this study (Table 1, Fig. 1). Voucher specimens for each colony were placed in 70% ethanol and are maintained by the authors.

Characterization of Cuticular Hydrocarbon Mixtures

Cuticular hydrocarbon characterizations were completed without prior knowledge of the species determinations. Cuticular hydrocarbons were characterized by extracting samples of 100 to 200 workers from a single colony that had been dried at 70°C for up to 6 hr. Extraction, separation, characterization, quantification, and labeling of cuticular hydrocarbons were identical to that reported for *Reticulitermes* from the southeastern United States (9). Chromatograms from termite samples were sorted on the basis of hydrocarbon mixtures and assigned to a particular hydrocarbon phenotype, designated by a letter. For presentation of quantitative data, a code number was assigned to each hydrocarbon rather than an exact percentage. The presence of co-eluting compounds precluded exact quantification of many individual hydrocarbons. Therefore, these codes represent a given range of percentage values (Tables 2–7). These ranges are narrower, in order to be more descriptive, than previously reported (9).

Determination of Species by Morphology

Morphological determinations of species of *Reticulitermes* were made using a key to soldiers in (22). We used three soldiers from each of three monitoring stations of each of the cuticular hydrocarbon phenotypes. The first character used is maximum and minimum width of the gula (22). The maximum width is the anterior margin of the gula rather than the actual maximum (22). Previous keys (3,28) are less clear and simply refer to the front and middle of the gula. A more explicit measurement of the maximum and minimum widths for characterizing soldier termites has been suggested (21), but the most recent key (22) was used because it was the most well-defined key to *Reticulitermes* of North America.

Length and width of the head capsule was also measured (21). The length of the head (without the mandibles) was measured from the posterior margin to the anterior limit of chitinization of the head capsule (21). The width was measured at its maximum.

RESULTS

Cuticular Hydrocarbon Phenotypes of Reticulitermes *in Northern California*

We identified five distinct cuticular hydrocarbon phenotypes from our collections of *Reticulitermes* from northern California (Tables 2–7; Figs 2–6). We assigned letters to the various hydrocarbon phenotypes in the order that they were determined (A through D); letters do not necessarily indicate phylogenetic relationships among phenotypes. One phenotype, A′, was given this designation because it appeared to be very similar to phenotype A with the addition of two isomers of pentacosatriene.

Species Determination by Morphology

Alates were not collected from any of the 42 colonies used to characterize cuticular hydrocarbons. Specimens representing all five hydrocarbon phenotypes key, in our opinion, to *R. tibialis* based on keys to soldiers (Table 8). The gula measurement used in the key to soldiers (22) was equivocal. For phenotypes A, A′, and D the ratio of the front (maximum width) of the gula to the middle (minimum width) was almost always less than two (Table 8). This would lead us to infer that these specimens are *R. tibialis*. However, for three of the soldiers measured, the ratio was ≥ 2.0. Gula measurements of phenotypes B and C were also equivocal: the gula ratio was >2.0 for four of the nine phenotype B soldiers and for six of the nine phenotype C soldiers (Table 8). This variability did not allow us to make a determination based on this character.

The head capsule of *R. hesperus* has been reported to be at least twice as long as wide (3,22,28). None of the specimens we measured met this criterion (Table 8). Therefore, we can only report that all specimens/colonies reported here key to *R. tibialis*.

Cuticular Hydrocarbons of Reticulitermes *from Northern California*

Hydrocarbon classes observed include normal alkanes, olefins (alkenes, alkadienes, alkatrienes), and mono-, di-, and trimethylalkanes. Normal alkanes occurred in all phenotypes; *n*-C23 and *n*-C25 are generally the most abundant, however in phenotypes B and C, *n*-C25 and *n*-C27 predominate (Table 2). Twenty two different olefins were identified (Table 3), but the diversity was not as extreme as that of the *Reticulitermes* from the southeastern United States (9). No olefins were detected in phenotype B. Numerous, sepa-

TABLE 1. Collection localities for *Reticulitermes* samples used to characterize cuticular hydrocarbon phenotypes from northern California*

Colony†	Source‡	Locality	County
Phenotype A			
Pea Patch East§	ms	Placerville	El Dorado
Wc7§	ms	Placerville	El Dorado
Wk64§	ms	Placerville	El Dorado
Xi21§	ms	Placerville	El Dorado
Yq31§	ms	Placerville	El Dorado
Zp8§	ms	Placerville	El Dorado
L34§	ms	Kentfield	Marin
JBSAC§	wog	Sacramento	Sacramento
RETICMTZ§	wog	Martinez	Contra Costa
RETCAR	wog	Carmichael	Sacramento
RETJBW	wog	Woodland	Yolo
RETLAF	wog	Lafayette	Contra Costa
RETRR1	wog	Russell Reservation, Lafayette	Contra Costa
Phenotype A′			
St25§	ms	Novato	Marin
St63§	ms	Novato	Marin
St87§	ms	Novato	Marin
F167§	ms	Novato	Marin
L55§	ms	Kentfield	Marin
RETHASPS§	wog	Hastings Nature Preserve	Monterey
RETHC1§	wog	Hat Creek	Shasta
RETBLMT§	wog	Black's Mountain Expt. Forest	Lassen
RETG1	ms	San Rafael	Marin
RETCL1	wog	Cloverdale	Sonoma
RETCL6§	wog	Yorkville	Mendocino
RETBMM	wog	Fairfax	Marin
Phenotype B			
Wb36§	ms	Placerville	El Dorado
Wc10§	ms	Placerville	El Dorado
Xm14§	ms	Placerville	El Dorado
Yv34§	ms	Placerville	El Dorado
Zv31§	ms	Placerville	El Dorado
Phenotype C			
PV§	ms	Placerville	El Dorado
Wi74§	ms	Placerville	El Dorado
Yt2§	ms	Placerville	El Dorado
Zn11§	ms	Placerville	El Dorado
Phenotype D			
St21§	ms	Novato	Marin
St116§	ms	Novato	Marin
St253§	ms	Novato	Marin
St314§	ms	Novato	Marin
L5§	ms	Kentfield	Marin
RETUCB§	wog	Berkeley	Alameda
RETTNG1	ms	Tanglewood Estate, Sausalito	Marin
RETRR2	wog	Russell Reservation, Lafayette	Contra Costa
RETICSHA	wog	Sacramento Housing Authority	Sacramento
RETBMC	wog	Oakland	Alameda

*Cuticular hydrocarbon phenotypes were collected primarily from the Institute of Forest Genetics near Placerville, CA, or from one of three sites in Marin County. Additional sites were included to broaden the geographic base for this study.

†Each collection is from a discrete colony that is numbered or given a unique code name or abbreviated title.

‡Termites were colleced from a monitoring station (ms) or wood-on-ground (wog).

§These collections were used to calculate the percentages of each hydrocarbon for the five cuticular hydrocarbon phenotypes.

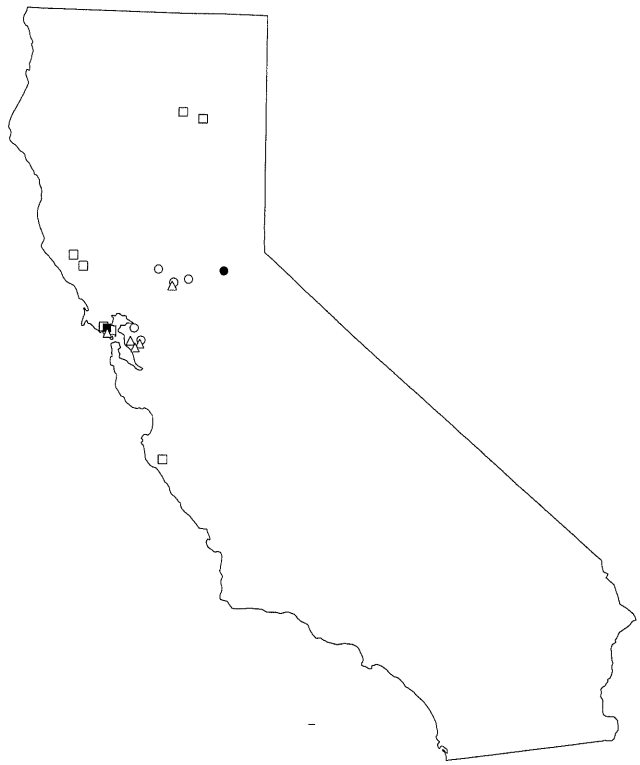

FIG. 1. Collection localities in northern California. Phenotypes A, B, and C collected in Placerville (●). Phenotypes A, A′, and D collected in Kentfield/Novato (■). Other localities A (○), A′ (□), and D (△).

rable isomers of the alkadienes and alkatrienes were found among four of the five phenotypes. For example, C25:2 and C27:2 each had three distinct isomers, and C25:3 had four distinct isomers (Table 3). We did not locate the positions of the double bonds for any of these olefins nor did we observe spectra that would indicate branched alkenes as reported for *Drepanotermes* (6). No particular olefin appeared to be ubiquitous in the four phenotypes with an olefin component.

Terminally branched monomethylalkanes were common with 2-meC24 and 3-meC25 being abundant in nearly all samples. 5-MeC25 was abundant in phenotypes B and D and 5-meC27 occurred in phenotype B in abundant amounts, and in phenotype D in lesser amounts (Table 4). Internally branched monomethylalkanes were found in all phenotypes; in phenotypes A, A′, and C these alkanes were the most abundant compounds (Figs 2, 4, and 5). 9-; 11-; 13-MeC25 was abundant in all phenotypes. Internally branched monomethylalkanes with 35 or more carbons in the parent chain were often paired with an internally branched dimethylalkane of the same parent chain length and occurred as a homologous series (Tables 5 and 6; Figs 2, 4–6).

Dimethylalkanes were abundant in all phenotypes (Table 6). In phenotypes A, A′, and C, the majority of the dimethylalkanes were internally branched with three methylene units between methyl branches. In phenotypes B and D, 5,17-dimethylalkanes were the most abundant type of hydrocarbon. Trimethylalkanes were found in relatively small quantities (<1.0% of the total hydrocarbon) only in phenotypes B and D.

Phenotypes from Placerville (IFG), El Dorado County

Hydrocarbon phenotypes A, B, and C have been collected on our 4-hectare research site (Figures 2, 4, 5). Hydrocarbon phenotype A is the most abundant: 59 of our monitoring stations were characterized as phenotype A, 11 as phenotype B, and only 5 as phenotype C. Phenotype B can be separated from phenotypes A and C by the total absence of olefins in the cuticular hydrocarbons (Table 3). Likewise, phenotype B lacks internally branched dimethylalkanes, such as 11,15-dimeC25, 11,15-dimeC27, 11,15-dimeC35,

TABLE 2. Relative amounts of normal alkanes found in the cuticular hydrocarbons of each of five hydrocarbon phenotypes of *Reticulitermes* collected in northern California*

	Reticulitermes hydrocarbon phenotype				
Hydrocarbon†	**A**	**A′**	**B**	**C**	**D**
n-C21	0	0	0	1	0
n-C22	1	1	0	1	0
n-C23	4	3	1	3	4
n-C24	3	3	2	3	3
n-C25	5	5	4	5	5
n-C26	2	0	2	3	0
n-C27	2	1	3	3	0
n-C28	1	0	2	2	0
n-C29	0	0	2	1	0
n-C30	0	0	1	1	0
n-C31	0	0	2	1	0

*Numbers represent the following ranges of percent of total hydrocarbon: 0 = not detected; 1 = 0.01 to 0.3%; 2 = 0.3 to 1.0%; 3 = 1.0 to 3.0%; 4 = 3.0 to 6.0%; 5 = 6.0 to 10.0%; 6 = 10.0 to 20.0%; 7 = >20.0%.

†Presented in order of elution.

TABLE 3. Relative amounts of olefins found in the cuticular hydrocarbons of each of five hydrocarbon phenotypes of *Reticulitermes* collected in northern California*

Hydrocarbon†	ECL	*Reticulitermes* hydrocarbon phenotype				
		A	A′	B	C	D
C23:1	22.70	2	0	0	0	1
C25:1	24.75	2	2	0	3	0
C26:1	25.70	1	0	0	0	0
C27:1	26.75	0	0	0	4	0
C29:1	28.80	0	0	0	1	0
C25:2	24.75	0	0	0	3	0
C25:2	25.35	6	5	0	0	0
C25:2	25.50	2	3	0	0	2
C26:2	26.30	2	1	0	0	0
C27:2	26.75	0	0	0	5	0
C27:2	27.35	2	2	0	3	0
C27:2	27.50	0	1	0	2	2
C29:2	28.75	0	0	0	1	0
C29:2	29.30	0	0	0	1	0
C25:3	25.98	0	4	0	0	0
C25:3	26.07	0	5	0	0	0
C25:3	26.19	0	0	0	0	4
C25:3	26.47	0	2	0	0	0
C26:3	26.81	0	1	0	0	1
C26:3	27.15	0	1	0	0	0
C27:3	28.03	0	1	0	1	3
C27:3	28.24	0	1	0	2	1

*Numbers represent the following ranges of percent of total hydrocarbon: 0 = not detected; 1 = 0.01 to 0.3%; 2 = 0.3 to 1.0%; 3 = 1.0 to 3.0%; 4 = 3.0 to 6.0%; 5 = 6.0 to 10.0%; 6 = 10.0 to 20.0%; 7 = >20.0%.

†Presented in order of elution. ECL = Equivalent chain length (approximate).

or 13,17-dimeC41, which occur in phenotype A or C in amounts above 3% of the total hydrocarbon component (Table 6). Conversely, the cuticular hydrocarbon mixture of phenotype B contains 5-meC25, 5-meC27, 5,17-dimeC27, a homologous series of 5,17-dimethylalkanes from C33 to C43, and several 5,9,17-trimethylalkanes which are totally absent in phenotypes A and C (Tables 4, 6–7). Phenotypes A and C can be separated by the presence of large quantities (>3%) of C27:1 (ECL = 26.75) and C27:2 (ECL = 26.75) in phenotype C (Table 3). Phenotype C also makes large amounts of 11-; 13-meC27 and 11,15-dimeC27 which are present only in small amounts in phenotype A (Tables 5 and 6). Phenotype A has 9-; 11-meC23 and 9,13-; 11,15-dimeC25 in its hydrocarbon mixture; these compounds occur only in minor amounts or are absent in phenotype C (Tables 5 and 6).

Phenotypes from Novato and Kentfield, Marin County

Two additional hydrocarbon phenotypes have been characterized from our monitoring stations in Marin County: A′ and D (Figs 5 and 6). Phenotype A has also been collected in our monitoring stations in Kentfield. Seventeen of our monitoring stations are occupied by phenotype A′, 14 by phenotype D, and 4 by phenotype A. Phenotype A′ is nearly identical to phenotype A except for the presence of large quantities of two isomers of C25:3 (ECL = 25.98 and 26.07) (Table 3; Figs 2 and 5). Phenotype D can be separated from phenotypes A and A′ by the abundant 5-meC25, numerous 5,17-dimethylalkanes, and several 5,9,17-trimethylalkanes that occur in minor amounts (Tables 3, 6, and 7, Fig. 6). Phenotype D can be separated from phenotype B by the presence of C25:3 (ECL = 26.19) and two isomers of C27:3 (ECL = 28.03 and 28.24), and numerous quantitative differences (Tables 2–7; Figs 3 and 6).

Diagnostic Cuticular Hydrocarbons for Each Phenotype

We have condensed the predominant differences among the hydrocarbon phenotypes of *Reticulitermes* from northern California and identified what we consider diagnostic hydrocarbons (Table 9). These hydrocarbons are abundant, not minor, components (at least 1%, but preferably >5% of the total hydrocarbon). Diagnostic cuticular hydrocarbons should be unique or present in only one or a few species or taxa, or conversely, they should be abundant in most of the species or taxa yet completely absent, rare, or present in insignificant quantities in one or a few. Ideally, they should have a unique elution time so that they do not co-elute with another hydrocarbon in the same species, nor should they elute at a time similar to a different hydrocarbon in a different species (23).

The most dramatic difference among the five hydrocarbon phenotypes is the apparent absence of olefins in pheno-

TABLE 4. Relative amounts of terminally branched monomethyl alkanes found in the cuticular hydrocarbons of each of five hydrocarbon phenotypes of *Reticulitermes* collected in northern California*

	Reticulitermes hydrocarbon phenotype				
Hydrocarbon†	**A**	**A′**	**B**	**C**	**D**
2-meC22	0	1	0	1	0
3-meC22	0	1	0	1	0
5-meC23	0	0	0	0	2
2-meC23	3	3	0	3	2
3-meC23	3	3	0	2	2
6-meC24	0	0	0	0	2
5-meC24	0	0	1	0	2
2-meC24	4	5	3	4	4
3-meC24	2	2	0	0	0
5-meC25	0	0	5	0	5
2-meC25	4	4	2	4	2
3-meC25	4	4	3	4	3
6-meC26	0	0	3	0	2
5-meC26	0	0	2	0	1
4-meC26	0	0	1	0	1
2-meC26	2	1	3	3	2
3-meC26	1	1	1	0	0
5-meC27	0	0	4	0	2
2-meC27	1	1	2	1	0
3-meC27	1	1	0	2	0
6-meC28	0	0	1	0	0
2-meC28	0	0	0	1	0
5-meC29	0	0	1	0	0
5-meC35	0	0	0	0	1

*Numbers represent the following ranges of percent of total hydrocarbon: 0 = not detected; 1 = 0.01 to 0.3%; 2 = 0.3 to 1.0%; 3 = 1.0 to 3.0%; 4 = 3.0 to 6.0%; 5 = 6.0 to 10.0%; 6 = 10.0 to 20.0%; 7 = >20.0%.
†Presented in order of elution.

TABLE 5. Relative amounts of internally branched monomethylalkanes found in the cuticular hydrocarbons of each of five hydrocarbon phenotypes of *Reticulitermes* collected in northern California*

	Reticulitermes hydrocarbon phenotype				
Hydrocarbon†	**A**	**A′**	**B**	**C**	**D**
9-; 11-meC23	4	3	0	1	3
7-meC23	0	0	0	0	2
11-; 12-meC24	3	3	1	2	3
9-; 11- 13-meC25	6	5	4	5	7
7-meC25	0	0	2	2	2
11-; 12-; 13-meC26	2	2	3	3	3
11-; 13-meC27	2	2	5	6	2
7-meC27	0	0	3	1	1
10-; 11-; 12-; 13-meC28	0	0	1	2	0
11-; 13-; 15-meC29	0	0	1	2	0
11-; 13-; 15-; 17-meC33	0	0	3	0	0
11-; 13-; 15-; 17-meC33	0	0	3	0	0
11-; 12-; 13-; 14-meC34	0	0	1	0	0
11-; 13-; 15-; 17-meC35	3	3	3	3	2
12-; 14-meC36	2	2	2	2	2
11-; 13-; 15-; 17-meC37	4	4	3	3	3
12-; 13-; 14-meC38	1	1	0	1	1
11-; 13-; 15-; 17-meC39	3	3	2	3	2
11-; 13-; 15-; 17-meC40	1	1	0	1	0
11-; 13-; 15-; 17-meC41	1	2	0	2	2
11-; 13-; 15-; 17-meC43	0	1	0	2	0

*Numbers represent the following ranges of percent of total hydrocarbon: 0 = not detected; 1 = 0.01 to 0.3%; 2 = 0.3 to 1.0%; 3 = 1.0 to 3.0%; 4 = 3.0 to 6.0%; 5 = 6.0 to 10.0%; 6 = 10.0 to 20.0%; 7 = >20.0%.
†Presented in order of elution.

TABLE 6. Relative amounts of dimethylalkanes found in the cuticular hydrocarbons of each of five hydrocarbon phenotypes of *Reticulitermes* collected in northern California*

	Reticulitermes hydrocarbon phenotype				
Hydrocarbon†	**A**	**A′**	**B**	**C**	**D**
9,13-; 11,15-dimeC25	4	4	0	0	3
5,15-dimeC25	0	0	3	0	6
3,7-dimeC25	2	0	0	0	1
11,15-dimeC26	2	1	0	0	0
6,18-dimeC26	0	0	3	0	2
5,17-dimeC26	0	0	2	0	1
4,16-dimeC26	0	0	2	0	1
11,13-dimeC27	0	0	0	2	0
11,15-dimeC27	1	1	0	5	0
5,17-dimeC27	0	0	6	0	3
3,7-dimeC27	0	0	1	0	0
11,15-dimeC28	0	0	0	2	0
6,18-dimeC28	0	0	2	0	0
5,17-dimeC29	0	0	2	0	0
5,17-dimeC33	0	0	3	0	0
6,18-dimeC34	0	0	2	0	0
5,17-dimeC34	0	0	1	0	0
11,15-; 13,17-dimeC35	4	4	0	2	1
5,17-dimeC35	0	0	4	0	3
11,15-; 12,16-dimeC36	2	2	0	0	1
6,18-dimeC36	0	0	2	0	2
5,17-dimeC36	0	0	1	0	1
11,15-; 13,17-dimeC37	4	4	0	3	2
5,17-; 5,15-dimeC37	0	0	4	0	3
11,15-; 12,16-dimeC38	2	1	0	1	1
6,18-; 6,16-dimeC38	0	0	1	0	2
5,17-dimeC38	0	0	1	0	1
11,15-; 13,17-dimeC39	3	3	0	3	2
5,17-dimeC39	0	0	3	0	3
12,16-; 14,18-dimeC40	1	1	0	1	0
5,17-dimeC40	0	0	1	0	2
11,15-; 13,17-dimeC41	2	2	0	3	2
5,17-dimeC41	0	0	3	0	3
5,17-dimeC42	0	0	1	0	0
11,15-; 13,17-; 15,19-dimeC43	0	1	0	3	2
5,17-dimeC43	0	0	3	0	3

*Numbers represent the following ranges of percent of total hydrocarbon: 0 = not detected; 1 = 0.01 to 0.3%; 2 = 0.3 to 1.0%; 3 = 1.0 to 3.0%; 4 = 3.0 to 6.0%; 5 = 6.0 to 10.0%; 6 = 10.0 to 20.0%; 7 = >20.0%.

†Presented in order of elution.

TABLE 7. Relative amounts of trimethylalkanes found in the cuticular hydrocarbons of each of five hydrocarbon phenotypes of *Reticulitermes* collected in northern California*

	Reticulitermes hydrocarbon phenotype				
Hydrocarbon†	**A**	**A′**	**B**	**C**	**D**
5,9,15-trimeC25	0	0	0	0	2
5,9,17-trimeC27	0	0	2	0	1
5,9,17-trimeC35	0	0	2	0	1
5,9,17-trimeC37	0	0	2	0	1
5,9,17-trimeC39	0	0	1	0	0

*Numbers represent the following ranges of percent of total hydrocarbon: 0 = not detected; 1 = 0.01 to 0.3%; 2 = 0.3 to 1.0%; 3 = 1.0 to 3.0%; 4 = 3.0 to 6.0%; 5 = 6.0 to 10.0%; 6 = 10.0 to 20.0%; 7 = >20.0%.

†Presented in order of elution.

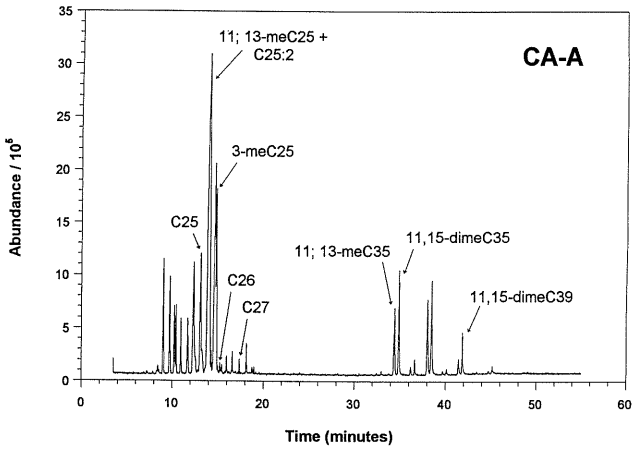

FIG. 2. Total ion chromatogram of cuticular hydrocarbons from Phenotype A specimens from the Institute of Forest Genetics, Placerville, California.

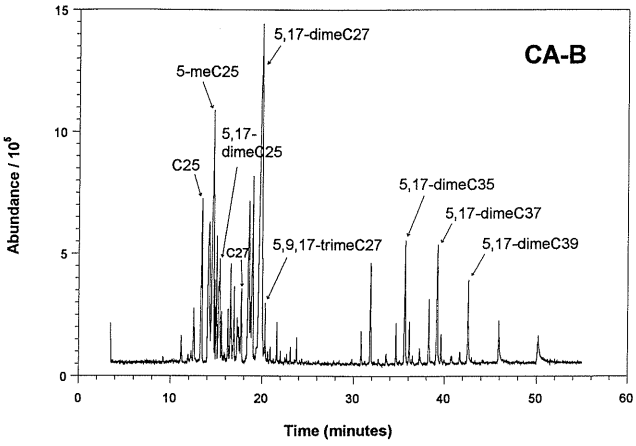

FIG. 3. Total ion chromatogram of cuticular hydrocarbons from Phenotype B specimens from the Institute of Forest Genetics, Placerville, California.

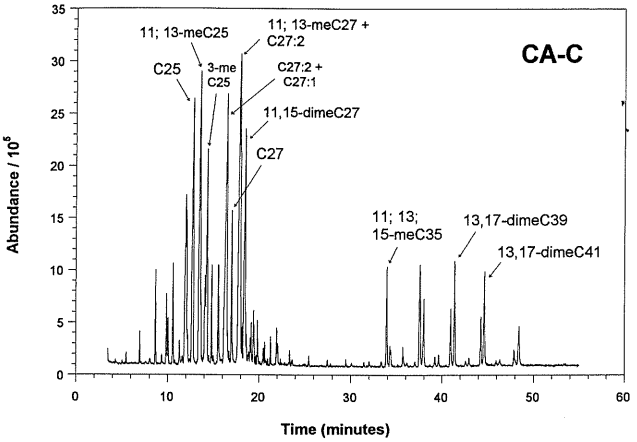

FIG. 4. Total ion chromatogram of cuticular hydrocarbons from Phenotype C specimens from the Institute of Forest Genetics, Placerville, California.

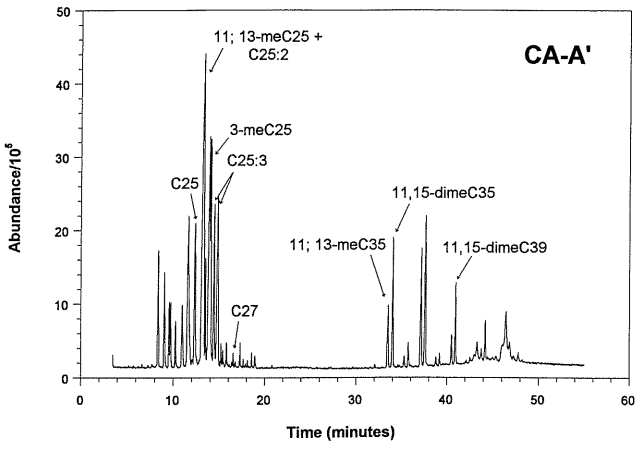

FIG. 5. Total ion chromatogram of cuticular hydrocarbons from Phenotype A′ specimens from Novato, California.

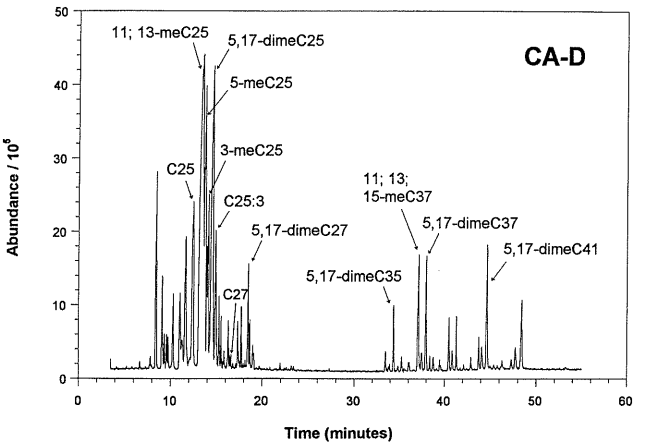

FIG. 6. Total ion chromatogram of cuticular hydrocarbons from Phenotype D specimens from Novato, California.

type B (Table 3). Phenotype D can be separated from the other three hydrocarbon phenotypes by the presence of 5-meC25 and numerous 5,17-dimethylalkanes (Table 4, 6, and 9). Phenotype C can be diagnosed by the abundant quantities of 11,15-dimeC27 and C27:1 and C27:2 (ECL = 26.75) (Table 3, 6, and 9). Phenotypes A and A′ are distinguished by the presence of significant amounts of two different isomers of C25:3 (ECL = 25.98 and 26.07) in phenotype A′. From these diagnostic hydrocarbons we have developed a dichotomous key to the phenotypes (Table 10).

Other Collections in Northern California

Serendipitous collections in other locations in northern California have appended additional locations for phenotypes A, A′, and D (Table 1; Fig. 1). The distributions of these phenotypes do not appear to follow a geographic trend; none of these three seem to be restricted to the coastal areas or to the Sierra Nevada as has been reported

TABLE 8. Measurements of three soldiers from each of three colonies of each of five cuticular hydrocarbon phenotypes of *Reticulitermes* collected in northern California

		Gula (mm)			Head (mm)		
Colony*	**Phenotype**	**Maximum**	**Minimum**	**Ratio**	**Length**	**Width**	**Ratio**
Wc7	A	0.27	0.16	1.69	1.78	1.14	1.56
10/17/96		0.27	0.17	1.59	1.85	1.14	1.62
		0.27	0.17	1.59	1.80	1.09	1.65
Yq29	A	0.30	0.15	2.00	1.73	1.06	1.63
10/17/96		0.30	0.17	1.76	1.73	1.04	1.66
		0.31	0.16	1.94	1.88	1.04	1.81
Zm13	A	0.30	0.17	1.76	1.88	1.14	1.65
10/17/96		0.32	0.17	1.88	2.15	1.14	1.89
		0.32	0.22	1.45	1.93	1.16	1.66
St25	A′	0.27	0.17	1.59	1.88	1.11	1.69
7/9/96		0.35	0.15	2.33	2.02	1.16	1.74
		0.32	0.20	1.60	1.83	1.11	1.65
St35	A′	0.33	0.19	1.74	1.73	1.14	1.52
7/9/96		0.32	0.16	2.00	2.00	1.14	1.75
		0.35	0.20	1.75	1.85	1.06	1.75
L4	A′	0.26	0.17	1.53	1.80	1.11	1.62
9/11/96		0.30	0.22	1.36	1.80	1.14	1.58
		0.27	0.21	1.29	1.53	1.06	1.44
Wc10	B	0.25	0.12	2.08	1.65	0.94	1.76
10/17/96		0.25	0.12	2.08	1.73	0.91	1.90
		0.25	0.12	2.08	1.65	0.94	1.76
Yq23	B	0.26	0.17	1.53	1.60	0.93	1.72
10/17/96		0.25	0.15	1.67	1.60	0.94	1.70
		0.27	0.15	1.80	1.56	0.91	1.71
YD16	B	0.26	0.14	1.86	1.65	0.94	1.76
10/17/96		0.26	0.15	1.73	1.48	0.94	1.58
		0.25	0.12	2.08	1.48	0.86	1.72
Yk27	C	0.27	0.15	1.80	1.70	0.91	1.87
10/17/96		0.26	0.12	2.17	1.73	0.94	1.84
		0.27	0.12	2.25	1.73	0.93	1.86
Yt2	C	0.25	0.12	2.08	1.70	0.94	1.81
9/19/96		0.25	0.12	2.08	1.80	0.99	1.82
		0.30	0.19	1.58	1.73	0.99	1.75
Zn11	C	0.25	0.12	2.08	1.48	0.91	1.63
10/17/96		0.25	0.14	1.79	1.78	0.94	1.89
		0.25	0.12	2.08	1.43	0.86	1.66
St21	D	0.25	0.22	1.14	1.65	1.01	1.63
9/11/96		0.25	0.20	1.25	1.58	1.04	1.52
		0.25	0.22	1.14	1.75	1.11	1.58
St253	D	0.30	0.20	1.50	1.71	1.09	1.57
9/11/96		0.32	0.19	1.68	1.73	1.06	1.83
		0.30	0.17	1.76	1.70	1.04	1.63
L6	D	0.28	0.17	1.65	1.95	1.11	1.76
9/11/96		0.32	0.22	1.45	1.80	1.06	1.65
		0.28	0.21	1.33	1.83	1.09	1.68

*Monitoring station at Placerville, Novato, or Kentfield sites and collection date.

for the dampwood termites, *Zootermopsis* (11,30). Phenotype A, which is common in our IFG site has also been collected in Marin County. Phenotypes A′ and D, which were most common at our Marin County sites have also been collected in Sacramento, Berkeley, Oakland, Fairfax, and Lafayette (Table 1; Fig. 1) and as far north as British Columbia (10).

Phenotype B has only been characterized from one other location: Washoe County, Nevada. This phenotype is not easily confused with any other because of the lack of olefins. Phenotype C has only been characterized from our Sierra Nevada site, and even there it is fairly rare (Table 1; Fig. 1).

DISCUSSION

The cuticular hydrocarbon mixtures of *Reticulitermes* from North America have been reported for *Reticulitermes flavipes*

TABLE 9. Diagnostic cuticular hydrocarbons from five hydrocarbon phenotypes of *Reticulitermes* collected in northern California*

Hydrocarbon†	Hydrocarbon phenotypes				
	A	A′	B	C	D
C25:3 (ECL = 25.98)	0	4	0	0	0
C25:3 (ECL = 26.07)	0	5	0	0	0
C27:1	0	0	0	4	0
C27:2 (ECL = 26.75)	0	0	0	5	0
5-meC25	0	0	5	0	5
5-meC27	0	0	4	0	2
11,15-dimeC27	1	1	0	5	0
5,17-dimeC27	0	0	6	0	3
5,17-dimeC35	0	0	4	0	3
5,17-dimeC37	0	0	4	0	3

*Numbers represent the following ranges of percent of total hydrocarbon: 0 = not detected; 1 = 0.01 to 0.3%; 2 = 0.3 to 1.0%; 3 = 1.0 to 3.0%; 4 = 3.0 to 6.0%; 5 = 6.0 to 10.0%; 6 = 10.0 to 20.0%; 7 = >20.0%.

†Presented in order of elution.

(Kollar) (1,16) and *R. virginicus* (Banks) (17). The characterization of these species was improved with chemical descriptions of an additional species, *R. hageni* Banks, and several undescribed taxa (9). The growing body of knowledge on the taxonomic value of cuticular hydrocarbon mixtures of termites (1–9,11,12,14,15,18–20,29,31) gives us confidence in accepting distinct, repeatable mixtures of cuticular hydrocarbons as descriptive of distinct taxa. It is most likely that the numerous cuticular hydrocarbon phenotypes from the southeastern United States represent distinct species and will result in a 2- or 3-fold increase in the number of *Reticulitermes* species in that geographical area alone. A similar increase in the number of distinct, valid taxa of *Reticulitermes* in western North America is highly likely given the great diversity of biological regions in California alone (34).

The cuticular hydrocarbon mixtures of *Reticulitermes* spp. from western North America were first reported in a preliminary investigation (10). A great majority of these samples reported from California, Oregon, and Arizona consisted of ≤60 workers extracted fresh or freshly unfrozen (*i.e.*, without drying to remove the water in the body or cuticle). The resulting mixtures were relatively dilute and did not resolve many diagnostic components, especially the di- and trienes (13).

Two of the three cuticular hydrocarbon types previously recognized from California (10) likely represent phenotypes A and A′ reported in this present study. Type I was separated from the other types by the absence of C25:1 and C29:1. C25:1 occurs in relatively small amounts in samples of phenotypes A and A′ (Table 3) and may have been missed (10) because the samples were extracted without drying first. Drying samples appears to greatly enhance the extractability of the olefins in termite cuticular hydrocarbons (13).

C29:1 is extremely rare in the cuticular hydrocarbons of *Reticulitermes* from northern California, but is very abundant in *Reticulitermes* (probably *R. tumiceps* Banks) from higher elevations in the southern half of Arizona (10). Thus, the lack of C29:1 was used to separate the Types I, II, and III *Reticulitermes* from the Type IV *Reticulitermes* (10). Unfortunately, the authors of this preliminary study did not recognize that the lack of 5-methyl and 5,17-dimethylalkanes is what separates their Types I and II from Types III and IV (10).

The Type III samples from California and Oregon (10) are most likely phenotype D reported in this study because of the numerous 5-methyl and 5,17-dimethylalkanes and the absence of 5-meC27 and 5,17-dimeC27, which are abundant in phenotype B (Tables 4 and 9). The three samples from Arizona labeled as Type III (10) are likely one or more of the three hydrocarbon phenotypes recently characterized from disparate locations in Arizona which possess a number of 5,17-dimethylalkanes, as well as numerous olefins (Haverty *et al.*, unpublished observations).

The cuticular hydrocarbon mixtures previously reported

TABLE 10. Dichotomous key to the cuticular hydrocarbon phenotypes of *Reticulitermes* collected in northern California

1a	5,17-dimeC27 abundant; olefins absent	Phenotype B
1b	olefins present	2
2a	5-meC25 and 5,17-dimethylalkanes abundant	Phenotype D
2b	5,17-dimethylalkanes absent	3
3a	C27:1, C27:2 (ECL = 26.75), and 11,15-dimeC27 abundant	Phenotype C
3b	C27:1 and 11,15-dimeC27 present ≤ trace amounts	4
4a	C25:3 (two isomers) abundant	Phenotype A′
4b	C25:3 absent	Phenotype A

(10) are consistent with the information contained in this paper; Types I and II correspond to phenotypes A and A′ and some of the Type III samples correspond to phenotype D. Phenotypes B and C are not common and have not been previously reported; phenotype C has only been recorded from our research site near Placerville, California, and phenotype B has only been found at this same site and once east of the Sierra Nevada. They likely represent valid taxa that have not been described. Phenotypes A, A′, and D most likely represent two distinct taxa with A and A′ representing variants of the same species. Preliminary investigations of soldier defense secretions (Haverty *et al.*, unpublished data) indicate that phenotypes A (plus A′), B, C, and D are indeed distinct and probably represent four separate taxa. Measurement of the width of the soldier head capsule provides further evidence that phenotypes A, A′, and D can be separated from phenotypes B and C on the basis of morphology, but separation within these two groups currently requires chemical analysis of the cuticular hydrocarbons or soldier defense secretions.

At this time, we hesitate to assign any of these phenotypes to one of the two species that are supposed to occur in California, *R. hesperus* or *R. tibialis*. All samples key to *R. tibialis*, but occur in areas that are only supposed to be inhabited by *R. hesperus*. Collections of samples from the vicinity of the type locality of *R. hesperus* and *R. tibialis* will help clarify phenotype assignments. It is generally agreed that the genus *Reticulitermes* is in need of revision (9,22, 26,33) and a serious examination of the *Reticulitermes* in western North America is long overdue. Our results demonstrate five distinct hydrocarbon phenotypes among *Reticulitermes* collected from northern California. Studies are in progress to evaluate these phenotypes in light of species groupings. We hope to compile sample series that contain soldiers, alates, and workers for morphological studies, coupled with cuticular hydrocarbon and soldier defense secretion profiles for the same samples. These data, in conjunction with biological and biogeographic observations, will help us to align hydrocarbon phenotypes with described species, and to identify and describe new taxa if warranted.

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