New Cuticular Hydrocarbon Phenotypes of *Reticulitermes* (Isoptera: Rhinotermitidae) from the United States

by

Michael I. Haverty[1], Lori J. Nelson[1] & Brian T. Forschler[2]

ABSTRACT

Cuticular hydrocarbon mixtures of *Reticulitermes* samples from Georgia, New Mexico, Arizona, and Nevada were characterized. We identified 10 new, distinct hydrocarbon phenotypes in *Reticulitermes:* 4 from Georgia, 1 from New Mexico, 4 from Arizona, and 1 from Nevada. The 4 new phenotypes from Georgia have a preponderance of olefins in common with several previously described phenotypes (GA-D, GA-E, GA-F, GA-G, GA-H, GA-I) from Georgia. The most abundant components in these profiles are olefins with 27 or 29 carbons. These phenotypes all key to *R. hageni* based on the size of the soldiers. Phenotypes from New Mexico and Nevada and 2 from Arizona have many similarities to each other and to a previously reported phenotype from California (CA-D). The characteristics they share include a number of C25 compounds, C25 and C27 olefins, several 5-methylalkanes and 5,17-dimethylalkanes. They are distinguished by quantitative differences and presence or absence of late-eluting olefins. Of the other 2 phenotypes from Arizona, one resembles that of a commonly seen California phenotype (CA-A'). The other phenotype from Arizona contains an abundance of olefins, though a distinct set that separates it from any type collected in Georgia. It also contains a homologous series of 5,17-dimethylalkanes. On the basis of cuticular hydrocarbons alone we suggest that there are 4 additional, undescribed taxa of *Reticulitermes* in Georgia, 1 new taxon from New Mexico, 3 or 4 new taxa from Arizona, and 1 new taxon from Nevada. Therefore, these new discoveries further emphasize that the taxonomy of *Reticulitermes* in North America is in need of revision.

Key words: Chemotaxonomy, cuticular hydrocarbons, hydrocarbon variability, Isoptera, *Reticulitermes,* subterranean termites

INTRODUCTION

In our previous studies of the chemotaxonomy of *Reticulitermes* we

[1]Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture, P.O. Box 245, Berkeley, CA 94701 USA
[2]Department of Entomology, 413 Biological Sciences Building, University of Georgia, Athens, GA 30602-2603 USA

identified 11 distinct cuticular hydrocarbon phenotypes from Georgia and 5 from California (Haverty *et al.* 1996, Haverty and Nelson 1997). These discoveries were from geographic areas that were reported to have only 3 or 2 species, respectively: *R. flavipes* (Kollar), *R. virginicus* Banks, and *R. hageni* Banks in Georgia and *R. hesperus* Banks and *R. tibialis* Banks in California (Nutting 1990, Weesner 1965, 1970). The basic theme of our previous studies was that the diversity of cuticular hydrocarbon phenotypes indicate the presence of several undescribed taxa in *Reticulitermes*.

We have continued our surveys in Georgia, and have now included samples from New Mexico, Arizona, and Nevada. Nathan Banks (in Banks and Snyder 1920) originally described 5 new species of *Reticulitermes* (*R. claripennis* Banks, *R. tibialis*, *R. hesperus*, *R. humilis* Banks, and *R. tumiceps* Banks), with keys to alates and soldiers, except for *R. tumiceps*. All of these species were reported to occur in either New Mexico, Arizona, or Nevada; *R. tibialis* was reported from all three of these states. Later, in his catalog, Snyder (1949), synonymized *R. tumiceps* and *R. humilis* with *R. tibialis*, without officially revising the genus. The assignment of the alleged *R. claripennis* sample from Tucson, AZ (Banks and Snyder 1920, fig. 32) is not known. Until very recently, most termite biologists accepted the synonymies of Snyder (1949) as reiterated by Snyder (1954) and reinforced by Weesner (1970) and Nutting (1990).

We report here additional discoveries of unique cuticular hydrocarbon phenotypes of *Reticulitermes* from Georgia, New Mexico, Arizona, and Nevada. These additions can be evaluated along with other suites of diagnostic characters (morphology, soldier defense secretions, and genetic characters) to further elucidate the taxonomy of this economically important genus.

METHODS

The majority of our samples of *Reticulitermes* were collected from infested pieces of wood on the ground, that we were confident represented different colonies (at least 50m apart). These serendipitous collections, as well as collections from our permanent field sites, provided the samples reported in this study (Table 1). Voucher specimens for each colony were placed in 70% ethanol and are maintained by the authors.

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 hours. Extraction, separation, characterization, quantification, and labeling of cuticular hydrocarbons were identical to that

Table 1. Collection localities for *Reticulitermes* samples used to characterize cuticular hydrocarbon phenotypes from Georgia, New Mexico, Arizona, and Nevada, U.S.A.

Colony[a]	Locality	County, State
Phenotype GA-K		
Chris 10*	Bledsoe	Spalding, Georgia
121B*	Westbrook Farm	Spalding, Georgia
Joey 1*	Blairsville	Union, Georgia
Hilltop 9*	Blairsville (Georgia Mountain Expt. Station)	Union, Georgia
Poison Ivy*	Blairsville (Georgia Mountain Expt. Station)	Union, Georgia
Phenotype GA-L		
Big House 25*	Sapelo Island	McIntosh, Georgia
Big House 34*	Sapelo Island	McIntosh, Georgia
Timber Dock 1*	Sapelo Island	McIntosh, Georgia
High Point 7*	Sapelo Island	McIntosh, Georgia
Pine 6*	Sapelo Island	McIntosh, Georgia
Phenotype GA-M		
ATH 4*	Athens	Clarke, Georgia
ATH 12*	Athens	Clarke, Georgia
Phenotype GA-N		
Mary 4*	Sapelo Island	McIntosh, Georgia
Mary 7*	Sapelo Island	McIntosh, Georgia
Big House 11*	Sapelo Island	McIntosh, Georgia
High Point 5*	Sapelo Island	McIntosh, Georgia
Pine 3*	Sapelo Island	McIntosh, Georgia
Pine 4*	Sapelo Island	McIntosh, Georgia
Phenotype NM-A		
NM1*	Rio Grande Nature Ctr., Albuquerque	Bernalillo, New Mexico
NM2*	Rio Grande Nature Ctr., Albuquerque	Bernalillo, New Mexico
Phenotype AZ-A		
RETIC AZ1*	Second Mesa	Navajo, Arizona
RETIC AZ2*	Second Mesa	Navajo, Arizona
Phenotype AZ-B		
RETIC AZ4*	Second Mesa	Navajo, Arizona
RETIC GC-1*	North Rim, Grand Canyon	Coconino, Arizona
Phenotype AZ-C		
RETTUMZ1*	Santa Catalina Mts. (in oak)	Pima, Arizona
RETTUMZ2*	Santa Catalina Mts. (in pine)	Pima, Arizona
RETTUMAZ*	Palisades Ranger Sta., St. Catalina Mts.	Pima, Arizona
AZ-98-1	W. Turkey Cr. Campground, Chiracahua Mts.	Cochise, Arizona
AZ-98-12	Rustler Park Campground, Chiracahua Mts.	Cochise, Arizona
MH1	Mt. Hopkins, Santa Rita Mtns.	Santa Cruz, Arizona
AZ-98-14	Noon Creek Picnic Area, Pinaleno Mtns.	Graham, Arizona
AZ-98-17	Arcadia Campground, Pinaleno Mtns.	Graham, Arizona

Table 1. Collection localities for *Reticulitermes* samples used to characterize cuticular hydrocarbon phenotypes from Georgia, New Mexico, Arizona, and Nevada, U.S.A. (continued)

Colony[a]	Locality	County, State
Phenotype AZ-D		
RETICAZ3*	Hwy 89, 16km east of Jacob Lake	Coconino, Arizona
Phenotype NV-A		
NV-2*	Lockwood, nr Truckee River	Storey, Nevada
NV-5*	Wadsworth	Washoe, Nevada
NV-7	Pyramid Lake	Washoe, Nevada
NV-12	Jct. Hwy 445 & 447 @ Pyramid Lake	Washoe, Nevada
NV-14	Indian Head Rock @ Pyramid Lake	Washoe, Nevada

[a]Each collection is from a discrete colony which is numbered or given a unique codename or abbreviated title. Collections marked with an asterisk were used to calculate the percentages of each hydrocarbon for the 10 cuticular hydrocarbon phenotypes.

reported for *Reticulitermes* from Georgia (Haverty *et al.* 1996) and California (Haverty and Nelson 1997). Chromatograms from termite samples were sorted on the basis of hydrocarbon mixtures and assigned to a particular hydrocarbon phenotype, designated by a letter. Within a hydrocarbon phenotype, we selected individual samples that displayed excellent chromatographic separation of the cuticular hydrocarbons for presentation of quantitative data. A code number, representing

Table 2. Relative amounts of normal alkanes found in the cuticular hydrocarbons of each of 10 hydrocarbon phenotypes of *Reticulitermes* collected in Georgia, New Mexico, Arizona, and Nevada.[a]

	Reticulitermes Hydrocarbon Phenotype									
Hydrocarbon[b]	GA-K	GA-L	GA-M	GA-N	NM-A	AZ-A	AZ-B	AZ-C	AZ-D	NV-A
n-C22	1	0	0	0	0	0	0	0	0	0
n-C23	5	5	3	5	0	0	4	0	4	4
n-C24	2	2	2	2	2	2	3	0	3	3
n-C25	4	5	4	4	4	6	6	2	5	6
n-C26	1	2	3	0	2	2	0	2	0	0
n-C27	2	3	4	2	0	2	1	4	0	1
n-C28	0	0	0	0	0	0	0	2	0	0
n-C29	1	1	0	0	0	0	0	2	0	0
n-C31	0	0	0	0	0	0	0	1	0	0

[a]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%.
[b]Presented in order of elution.

Table 3. Relative amounts of monoenes found in the cuticular hydrocarbons of each of 10 hydrocarbon phenotypes of *Reticulitermes* collected in Georgia, New Mexico, Arizona, and Nevada.[a]

		Reticulitermes Hydrocarbon Phenotype									
Hydrocarbon[b]	ECL[b]	GA-K	GA-L	GA-M	GA-N	NM-A	AZ-A	AZ-B	AZ-C	AZ-D	NV-A
C23:1	22.70	0	0	0	0	0	0	0	0	1	0
C25:1	24.70	2	3	0	2	2	4	4	0	2	5
C25:1	24.80	2	2	0	0	1	2	1	0	0	4
C25:1	25.15	0	0	0	0	2	0	0	0	0	0
C27:1	26.30	0	0	0	0	2	1	2	0	0	2
C27:1	26.70	4	7	3	3	3	3	2	5	0	2
C27:1	26.85	1	0	2	0	0	0	0	2	0	1
C27:1	27.14	0	0	0	0	0	0	0	2	0	0
C28:1	27.70	1	3	0	0	0	0	0	0	0	0
C29:1	28.70	3	7	0	3	0	0	0	7	0	0
C29:1	29.15	0	0	0	0	0	0	0	1	0	0
C30:1	29.70	0	0	0	0	0	0	0	2	0	0
C31:1	30.70	1	1	0	0	0	0	0	3	0	0
C33:1	32.70	1	0	0	0	0	0	0	0	0	0
C35:1	34.70	1	0	0	0	0	0	0	0	0	0
C37:1	36.55	1	0	0	0	0	0	0	0	0	0
C37:1	36.70	2	0	3	0	0	0	0	0	0	0
C39:1	38.70	1	0	0	0	2	0	0	0	0	0

[a]See Table 2.
[b]See Table 2. ECL = Equivalent chain length (approximate).

a range in percentage (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%), was assigned to each hydrocarbon rather than an exact percentage (Tables 2-8). The presence of co-eluting compounds precluded exact quantification of many individual hydrocarbons. These ranges are identical to those used in Haverty and Nelson (1997).

RESULTS

New Cuticular Hydrocarbon Phenotypes of *Reticulitermes*

We identified 4 new, distinct cuticular hydrocarbon phenotypes from our collections of *Reticulitermes* from Georgia (Tables 2-8; Figs. 1-4). As in Haverty *et al.* (1996) and Haverty and Nelson (1997), we assigned letters to the new hydrocarbon phenotypes from Georgia in the order that they were determined (K through N); these letters do not necessarily indicate phylogenetic relationships among phenotypes. We also characterized one new phenotype from New Mexico (NM-A), four from Arizona (AZ-A, AZ-B, AZ-C, and AZ-D), and one from Nevada (NV-A).

Table 4. Relative amounts of dienes found in the cuticular hydrocarbons of each of 10 hydrocarbon phenotypes of *Reticulitermes* collected in Georgia, New Mexico, Arizona, and Nevada.[a]

		Reticulitermes Hydrocarbon Phenotype									
Hydrocarbon[b]	ECL[b]	GA-K	GA-L	GA-M	GA-N	NM-A	AZ-A	AZ-B	AZ-C	AZ-D	NV-A
C25:2	24.75	0	0	0	0	0	0	4	0	o	0
C25:2	25.35	0	0	0	0	4	4	5	0	5	6
C25:2	25.50	0	0	0	0	2	0	3	0	4	3
C26:2	25.60	1	0	0	2	0	0	0	0	0	0
C26:2	26.30	0	0	3	0	3	2	2	0	0	2
C27:2	26.30	0	0	0	0	2	0	0	0	0	0
C27:2	26.75	6	0	0	7	3	4	2	0	0	1
C27:2	27.10	0	0	3	0	0	0	0	0	0	0
C27:2	27.20	1	0	0	1	0	0	0	0	0	0
C27:2	27.35	0	0	7	0	6	5	3	3	0	2
C27:2	27.50	1	0	0	0	2	3	0	0	0	2
C28:2	27.60	1	0	0	3	0	0	0	0	0	0
C28:2	27.75	2	0	0	0	0	0	0	0	0	0
C28:2	28.30	0	0	3	0	0	0	0	0	0	0
C29:2	28.50	3	3	0	0	1	0	0	3	0	0
C29:2	28.75	6	0	0	6	0	0	0	0	0	0
C29:2	29.01	0	0	5	0	0	0	0	0	0	0
C29:2	29.30	0	0	5	0	1	2	0	0	0	0
C30:2	29.50	1	0	0	0	0	0	0	2	0	0
C30:2	29.70	1	0	0	0	0	0	0	0	0	0
C31:2	30.40	0	0	0	0	0	0	0	5	o	0
C31:2	30.50	2	2	0	0	0	0	0	0	0	0
C31:2	30.70	2	0	0	0	0	0	0	0	0	0
C31:2	31.35	0	0	1	0	0	0	0	0	0	0
C32:2	31.40	0	0	0	0	0	0	0	2	0	0
C33:2	32.40	1	2	0	0	0	0	0	4	0	0
C35:2	34.40	2	2	0	0	0	0	0	3	0	0
C37:2	36.40	4	0	2	0	3	2	0	1	0	2
C39:2	38.50	2	0	0	0	4	3	0	2	0	3
C41:2		0	0	0	0	2	0	0	3	0	0
C43:2		0	0	0	0	0	0	0	2	0	0
C45:2		0	0	0	0	0	0	0	2	0	0

[a]See Table 2.
[b]See Table 2. ECL = Equivalent chain length (approximate).

Hydrocarbon classes observed include normal alkanes, olefins (alkenes, alkadienes, alkatrienes, alkatetraenes, and an alkapentaene), and mono- and dimethylalkanes. Normal alkanes occurred in all phenotypes; either *n*-C23, *n*-C25, or *n*-C27 is the most abundant (ca. 3.0% to 20.0% of the total hydrocarbon component); *n*-alkanes with an even number of carbons are usually present in small amounts (Table 2).

Eighteen different monoenes, 32 dienes, 21 trienes, 2 tetraenes, and

Table 5. Relative amounts of trienes, tetraenes, and pentaenes found in the cuticular hydrocarbons of each of 10 hydrocarbon phenotypes of *Reticulitermes* collected in Georgia, New Mexico, Arizona, and Nevada.[a]

		Reticulitermes Hydrocarbon Phenotype									
Hydrocarbon[b]	ECL[b]	GA-K	GA-L	GA-M	GA-N	NM-A	AZ-A	AZ-B	AZ-C	AZ-D	NV-A
Trienes											
C25:3	25.98	0	0	0	0	3	4	3	0	0	3
C25:3	26.07	0	0	0	0	1	3	2	0	5	4
C26:3	26.10	0	0	0	0	2	0	0	0	0	0
C27:3	26.28	0	0	0	1	0	0	0	0	0	0
C27:3	27.00	0	0	0	0	6	3	0	0	0	1
C26:3	27.15	0	0	0	0	1	0	0	0	0	0
C28:3	28.01	0	0	3	0	0	0	0	0	0	0
C27:3	28.03	0	0	0	0	0	3	0	0	0	3
C27:3	28.24	0	0	0	0	0	2	0	0	0	0
C29:3	28.31	3	0	0	0	0	0	0	0	0	0
C29:3	28.42	5	0	3	4	0	0	0	0	0	0
C29:3	29.00	0	0	7	0	3	2	0	0	0	0
C31:3	30.36	1	0	0	0	0	0	0	0	0	0
C31:3	30.44	3	0	0	3	0	0	0	0	0	0
C31:3	31.01	0	0	3	0	0	0	0	0	0	0
C33:3	32.40	0	0	0	2	0	0	0	0	0	0
C35:3	34.40	1	0	0	2	0	0	0	0	0	0
C37:3	36.38	3	0	0	1	2	2	0	0	0	2
C37:3	37.01	0	0	5	0	0	0	0	0	0	0
C39:3	38.25	1	0	0	0	3	3	0	0	0	3
C39:3	39.01	0	0	4	0	0	0	0	0	0	0
Tetraenes											
C39:4	37.90	0	0	0	0	3	2	0	0	0	2
C41:4		0	0	0	0	2	0	0	0	0	0
Pentaene											
C39:5		0	0	0	0	0	1	0	0	0	2

[a]See Table 2.
[b]See Table 2. ECL = Equivalent chain length (approximate).

one pentaene were identified (Tables 3-5). No particular olefin appeared to be ubiquitous. The diversity of the olefins found in the 10 cuticular hydrocarbon phenotypes discussed in this paper was roughly equivalent to that of the *Reticulitermes* previously characterized from the southeastern United States (Haverty *et al.* 1996). Olefins were detected in all 10 of the new cuticular hydrocarbon phenotypes reported in this paper, but were least abundant in phenotype AZ-D. Phenotype AZ-D had small amounts of two monoenes (C23:1 and C25:1); phenotypes GA-L and AZ-C had no trienes, tetraenes, or pentaenes. Numerous,

Table 6. Relative amounts of terminally branched monomethyl alkanes found in the cuticular hydrocarbons of each of 10 hydrocarbon phenotypes of *Reticulitermes* collected in Georgia, New Mexico, Arizona, and Nevada.[a]

	Reticulitermes Hydrocarbon Phenotype									
Hydrocarbon[b]	GA-K	GA-L	GA-M	GA-N	NM-A	AZ-A	AZ-B	AZ-C	AZ-D	NV-A
2-meC22	2	1	0	2	0	0	0	0	0	0
2-meC23	3	2	2	3	0	1	2	0	2	2
3-meC23	3	2	2	3	0	0	2	0	2	2
2-meC24	4	4	4	5	5	5	5	0	4	5
3-meC24	0	0	0	0	2	0	0	0	0	0
5-meC25	0	0	0	0	2	3	3	0	0	3
2-meC25	2	2	3	3	4	4	4	2	0	4
3-meC25	3	4	4	4	5	5	5	4	4	5
6-meC26	0	0	0	0	0	2	2	0	0	2
2-meC26	0	0	3	0	3	3	2	5	0	2
3-meC26	0	0	0	0	0	2	0	0	0	0
5-meC27	0	0	0	0	2	2	1	3	0	1
2-meC27	0	0	2	0	2	1	0	3	0	0
3-meC27	1	0	2	0	2	2	1	4	0	1
5-meC29	0	0	0	0	0	0	0	2	0	0
2-meC29	0	0	0	0	0	0	0	2	0	0
3-meC29	0	0	0	0	0	0	0	2	0	0

[a,b]See Table 2.

separable isomers of the alkenes, alkadienes, and alkatrienes were found among the 10 phenotypes. For example, C27:1, C25:2, and C29:2 each had 4 distinct isomers, and C27:2 had 6 distinct isomers (Tables 2 and 3). These isomers had nearly identical spectra, displaying the same molecular ion, but had different retention times.

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* (Brown *et al.* 1996). The diversity of the olefin components within all *Reticulitermes* phenotypes, as determined by differing equivalent chain length estimates, exceeds that reported for *R. flavipes* (Howard *et al.* 1978) and *R. virginicus* (Howard *et al.* 1982). In the future, double bond location and stereochemistry may prove critical for chemosystematic and behavioral studies.

Terminally branched monomethylalkanes were common with 2-meC24 and 3-meC25 being abundant (> 3.0% of the total hydrocarbon component) in nearly all phenotypes. 5-Methylalkanes were not very abundant in these cuticular hydrocarbon phenotypes and never occurred in excess of 3.0% of the total cuticular hydrocarbon component (Table 6). Internally branched monomethylalkanes were found in all

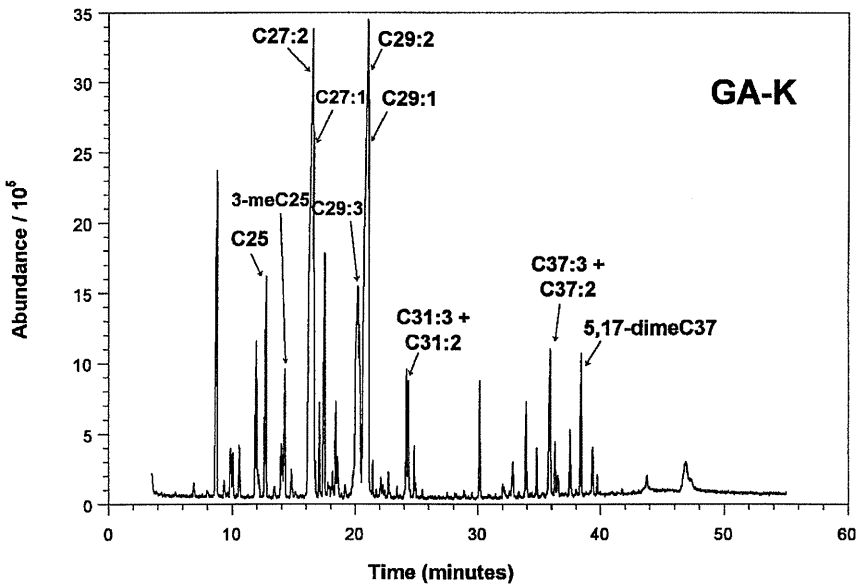

Fig. 1. Total ion chromatogram of cuticular hydrocarbons from Phenotype GA-K specimens from Bledsoe, Spalding County, Georgia.

phenotypes; in phenotypes NM-A, AZ-A, AZ-B, AZ-D, and NV-A the isomeric mixture 9-; 11-; 13-MeC25 was among the most abundant compounds (Figs. 5-7,9,10). Internally branched monomethylalkanes with 35 or more carbons in the parent chain, when present, were often paired with internally branched dimethylalkanes of the same parent chain length and occurred as a homologous series (Tables 7 and 8; Figs. 5-7, 9,10).

Dimethylalkanes were present in all phenotypes except GA-M, but seldom did a single dimethylalkane exceed 3.0% of the total cuticular hydrocarbon component. Internally branched dimethylalkanes with 3 methylene units between methyl branches and 5,17-dimethylalkanes were most common (Table 8).

Additional Phenotypes from Georgia

Hydrocarbon phenotypes GA-K, GA-L, GA-M, and GA-N were collected from disparate locations in Georgia (Table 1). All 4 phenotypes key to *R. hageni* on the basis of soldier morphology using the key in Scheffrahn and Su (1994). These phenotypes had not previously been collected, but for now, we cannot say whether or not they are rare. On a recent collecting trip in Athens, GA, 4 of 12 colonies we collected were phenotype GA-M.

All but one of the Georgia hydrocarbon phenotypes identified as *R.*

hageni on the basis of soldier morphology (GA-D, E, F, G, H, I, K, L, M, and N) possess numerous olefin components in their hydrocarbon mixture; GA-J does not (Haverty *et al.* 1996). Phenotype GA-K is much like GA-H because C27:2 (ECL = 26.75) and C29:2 (ECL = 28.75) are the predominant hydrocarbons (Table 4; Fig. 1). However, the later eluting hydrocarbon components are very similar to GA-E, comprised primarily of dienes and trienes (Tables 4, 5, 7, and 8; Fig. 1), rather than a homologous series of internally branched mono- and dimethylalkanes as found in GA-H (Haverty *et al.* 1996).

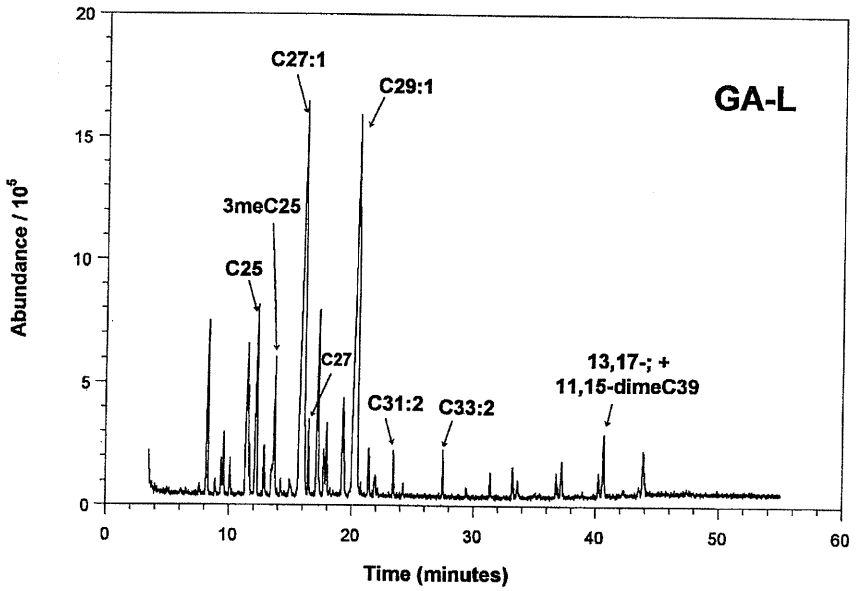

Fig. 2. Total ion chromatogram of cuticular hydrocarbons from Phenotype GA-L specimens from Sapelo Island, McIntosh County, Georgia.

Phenotype GA-L is most similar to phenotypes GA-E and GA-I because the predominant hydrocarbons are C27:1 and C29:1 (Table 3; Fig. 2). We do not know whether these monoenes are the same positional isomers for all 3 phenotypes. GA-L and GA-I both produce a homologous series of late-eluting internally branched mono- and dimethylalkanes, however, GA-L makes these compounds with 39 and 41 carbons in the parent chain, while GA-I does not. GA-L and GA-I also produce C31:2, C33:2, and C35:2; GA-I makes these olefins in large quantities (> 3% of the total hydrocarbon component), whereas GA-L does not. GA-L and GA-E differ mostly in the composition of late-eluting components (carbon number > 35). GA-L has mostly a homologous series of internally branched mono- and dimethylalkanes (Tables 7 and 8; Fig.

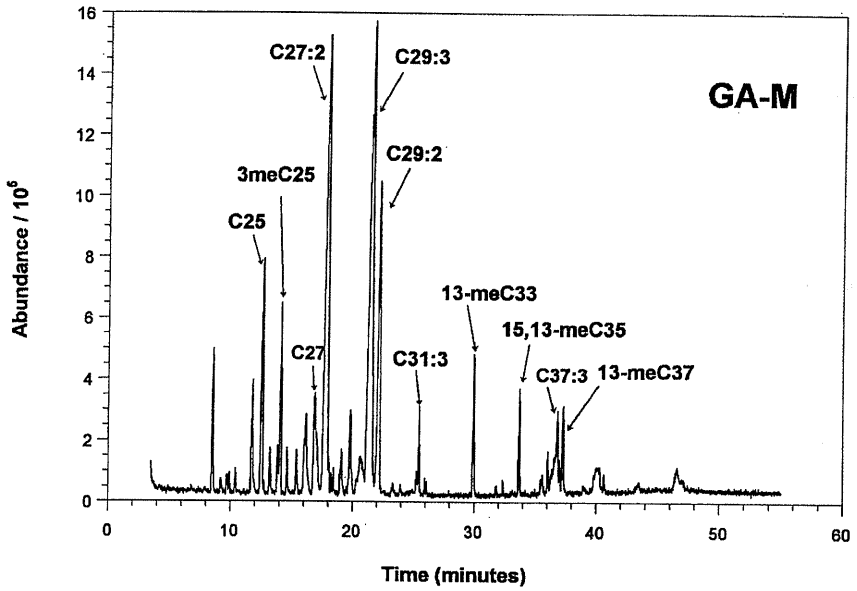

Fig. 3. Total ion chromatogram of cuticular hydrocarbons from Phenotype GA-M specimens from Athens, Clarke County, Georgia.

2), whereas GA-E produces monoenes and dienes and an internally branched monomethylalkane paired with a 5,17-dimethylalkane (Haverty *et al.* 1996).

Phenotype GA-M is readily distinguished by the extremely abundant isomeric mixture of C29:3 (Table 5; Fig. 3). The predominant isomer, which has an equivalent chain length of 29.00, is not found in any other *Reticulitermes* characterized from Georgia to date. GA-M does not have the late-eluting homologous series of either internally branched mono- and dimethylalkanes or the paired internally branched monomethylalkane and 5,17-dimethylalkane.

Phenotype GA-N most closely resembles GA-G with C27:2 (ECL = 26.75) and C29:2 (ECL = 28.75) being the predominant hydrocarbons. However, GA-N produces a series of trienes (Table 5; Fig. 4) that are absent in GA-G (Haverty *et al.* 1996).

Phenotypes from New Mexico, Arizona, and Nevada

Recent serendipitous collections from higher elevations (1750 m) in these 3 southwestern states produced a set of hydrocarbon phenotypes (NM-A, AZ-A, AZ-B, and NV-A) that fit a pattern roughly similar to that of CA-D. These cuticular hydrocarbon mixtures elute as 2 main clusters of hydrocarbons. The first includes predominantly C25 components, such as *n*-C25, 11-; 13-meC25, and C25:2 (ECL = 25.35), as well as

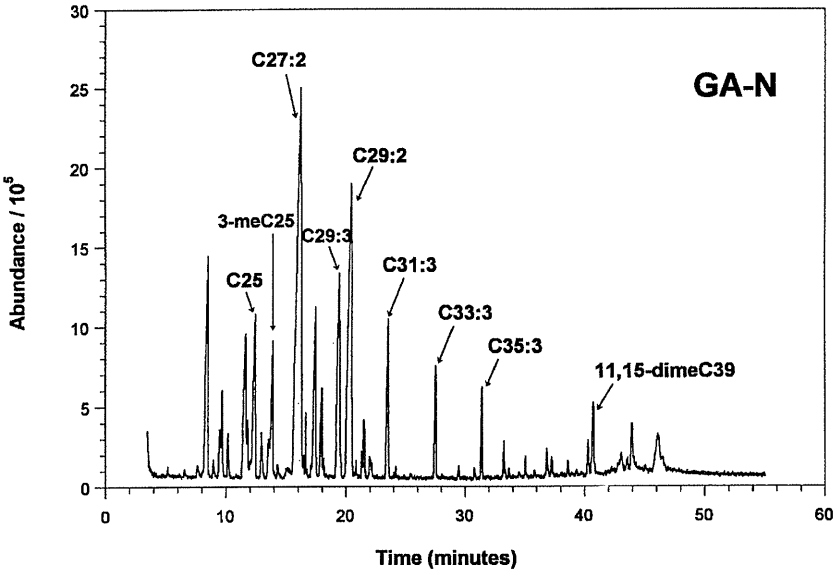

Fig. 4. Total ion chromatogram of cuticular hydrocarbons from Phenotype GA-N specimens from Sapelo Island, McIntosh County, Georgia.

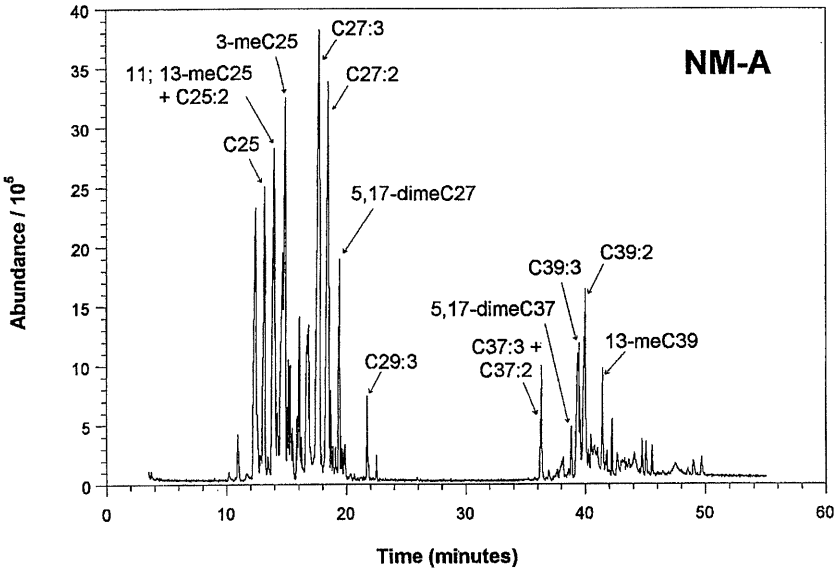

Fig. 5. Total ion chromatogram of cuticular hydrocarbons from Phenotype NM-A specimens from Albuquerque, Bernalillo County, New Mexico.

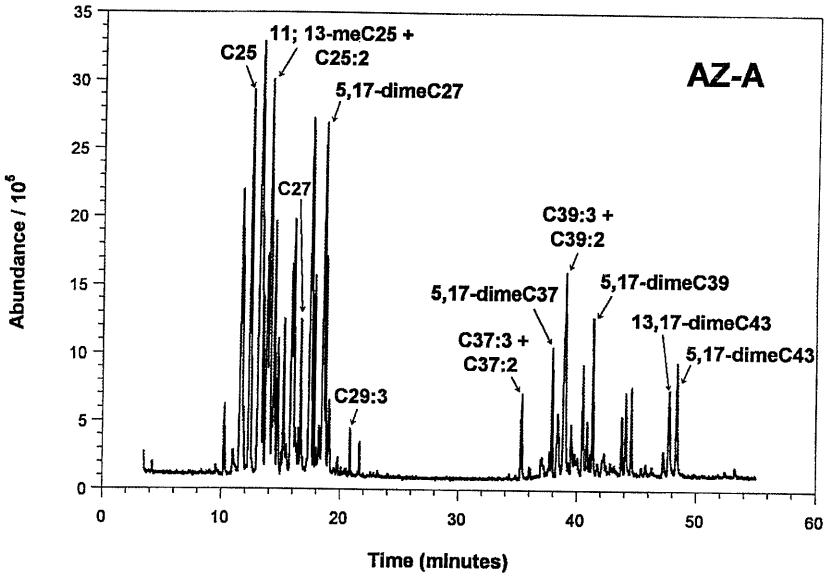

Fig. 6. Total ion chromatogram of cuticular hydrocarbons from Phenotype AZ-A specimens from Second Mesa, Hopi Indian Reservation, Navajo County, Arizona.

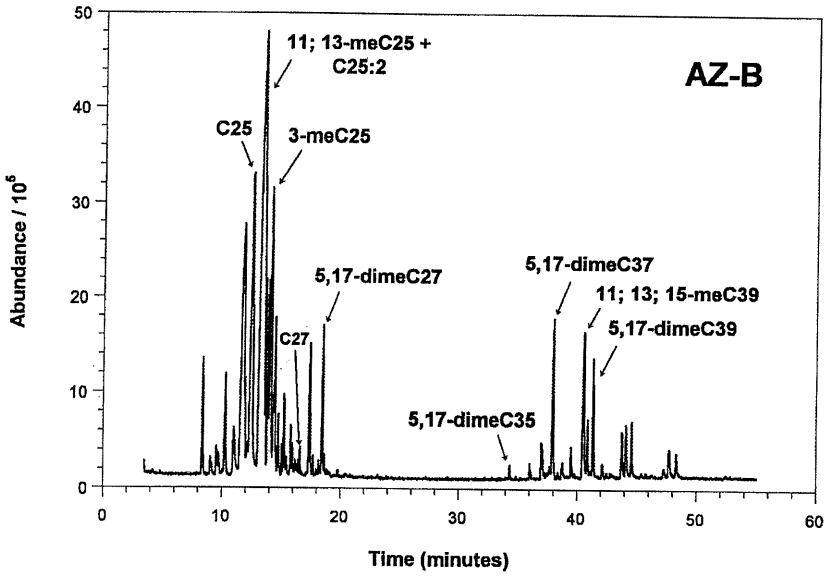

Fig. 7. Total ion chromatogram of cuticular hydrocarbons from Phenotype AZ-B specimens from Second Mesa, Hopi Indian Reservation, Navajo County, Arizona.

Table 7. Relative amounts of internally branched monomethylalkanes found in the cuticular hydrocarbons of each of 10 hydrocarbon phenotypes of *Reticulitermes* collected in Georgia, New Mexico, Arizona, and Nevada.[a]

	Reticulitermes Hydrocarbon Phenotype									
Hydrocarbon[b]	GA-K	GA-L	GA-M	GA-N	NM-A	AZ-A	AZ-B	AZ-C	AZ-D	NV-A
9-; 11-meC23	1	1	2	2	0	0	2	0	3	2
11-; 12-meC24	0	0	0	0	0	2	3	0	3	3
9-; 11-; 13-meC25	2	2	2	2	4	5	6	0	6	6
7-meC25	0	0	0	0	3	3	2	0	0	0
11-; 12-; 13-meC26	0	2	0	0	0	3	2	0	0	2
11-; 13-meC27	1	4	0	4	0	5	0	1	0	3
7-meC27	0	0	0	0	0	2	0	0	0	0
11-; 13-; 15-meC29	1	3	0	3	0	0	0	2	0	0
7-meC29	0	0	0	0	0	0	0	2	0	0
11-; 12-; 13-; 14-meC31	0	0	1	0	0	0	0	2	0	0
11-; 13-; 15-; 17-meC33	2	1	3	1	0	0	0	2	0	0
11-; 13-; 15-; 17-meC35	3	3	3	2	0	0	0	0	3	0
12-; 14-meC36	0	0	0	0	0	0	0	0	2	0
11-; 13-; 15-; 17-meC37	2	2	3	2	0	2	2	0	4	3
12-; 13-; 14-meC38	0	0	0	0	0	0	1	0	0	0
11-; 13-; 15-; 17-meC39	0	2	0	0	3	3	4	0	3	3
11-; 13-; 15-; 17-meC40	0	0	0	0	0	0	1	0	0	0
11-; 13-; 15-; 17-meC41	0	2	0	0	2	2	2	0	2	3
11-; 13-; 15-; 17-meC43	0	0	0	0	0	2	1	0	0	1

[a,b]See Table 2.

varying amounts of C27 components; the second includes late eluting components with carbon numbers in the parent chain ranging from 35 to 43. 5,17-Dimethylalkanes are present in both clusters. This group of phenotypes also includes another California phenotype, CA-B, which produces many of these same components, in particular an abundance of 5,17-dimethylalkanes. However, CA-B is easily distinguished from the other phenotypes by its complete lack of olefins.

Phenotypes NM-A and AZ-A came from collections in the Great Basin Desert (Figs. 5 and 6). These 2 phenotypes are similar, with differences mostly quantitative. Their predominant hydrocarbons have 25 and 27 carbons in the parent chain. Both produce significant quantities (> 3%) of a variety of hydrocarbon classes: dienes, trienes, terminally and internally branched monomethylalkanes, and 5,17-dimeC27 (Tables 4-8). The later eluting compounds are comprised primarily of dienes, trienes, a homologous series of internally branched mono- and dimethylalkanes, and 5,17-dimethylalkanes (Tables 4, 5, 7, 8). The major differences between NM-A and AZ-A are the greater abundance of C27:3 (ECL =

Table 8. Relative amounts of dimethylalkanes found in the cuticular hydrocarbons of each of 10 hydrocarbon phenotypes of *Reticulitermes* collected in Georgia, New Mexico, Arizona, and Nevada.[a]

	Reticulitermes Hydrocarbon Phenotype									
Hydrocarbon[b]	GA-K	GA-L	GA-M	GA-N	NM-A	AZ-A	AZ-B	AZ-C	AZ-D	NV-A
9,13-; 11,15-dimeC25	0	0	0	0	0	0	0	0	6	3
5,15-; 5,17-dimeC25	0	0	0	0	2	2	3	0	0	3
3,7-dimeC25	0	0	0	0	0	0	1	0	0	0
6,18-dimeC26	0	0	0	0	0	2	0	0	0	0
5,17-dimeC26	0	0	0	0	0	1	0	0	0	0
4,16-dimeC26	0	0	0	0	0	1	0	0	0	0
11,15-dimeC27	0	3	0	3	0	0	0	0	0	0
5,17-dimeC27	0	0	0	0	4	5	3	2	0	2
11,15-dimeC29	0	2	0	3	0	0	0	0	0	0
5,17-dimeC29	0	0	0	0	0	0	0	3	0	0
6,18-dimeC30	0	0	0	0	0	0	0	3	0	0
5,17-dimeC31	0	0	0	0	0	0	0	3	0	0
6,16-dimeC32	0	0	0	0	0	0	0	1	0	0
5,17-dimeC33	0	0	0	0	0	0	0	2	0	0
11,13-dimeC35	0	1	0	1	0	0	0	0	0	0
11,15-; 13,17-dimeC35	0	2	0	1	0	0	0	0	4	0
5,17-dimeC35	2	0	0	0	0	0	2	1	0	0
11,15-; 12,16-dimeC36	0	0	0	0	0	0	0	0	3	0
6,18-dimeC36	0	0	0	0	0	0	1	0	0	0
11,13-dimeC37	0	1	0	1	0	0	0	0	0	0
11,15-; 13,17-dimeC37	0	2	0	2	0	0	0	0	4	0
7,X-dimeC37	0	0	0	0	0	2	0	0	0	0
5,17-; 5,15-dimeC37	3	0	0	0	3	3	4	2	0	3
6,18-; 6,16-dimeC38	0	0	0	0	2	2	2	0	0	1
5,17-dimeC38	0	0	0	0	0	1	1	0	0	0
15,17-dimeC39	0	0	0	2	0	0	0	0	0	0
11,15-; 13,17-dimeC39	0	3	0	3	2	2	2	0	3	2
7,X-dimeC39	0	0	0	0	0	1	0	0	0	0
5,17-dimeC39	0	0	0	0	2	3	4	3	0	3
11,15-; 13,17-dimeC41	0	3	0	3	2	3	3	0	3	3
5,17-dimeC41	0	0	0	0	2	2	3	3	0	3
11,15-; 13,17-; 15,19-dimeC43	0	0	0	0	2	3	2	0	0	3
5,17-dimeC43	0	0	0	0	1	3	3	3	0	2

[a,b]See Table 2.

27.00) in NM-A and the lack of 9-; 11-; 13-meC27 in NM-A, while AZ-A produces 2 isomers of C27:3 (ECL = 28.03 and 28.24) not detected in NM-A.

Phenotype AZ-B differs from NM-A and AZ-A because it lacks the later eluting dienes, trienes, and tetraenenes, and produces smaller amounts

of hydrocarbons with 27 carbons in the parent chain (Tables 4-8; Figs. 5-7). These traits are shared with CA-D, although CA-D produces much more 5,17-dimeC25 than AZ-B.

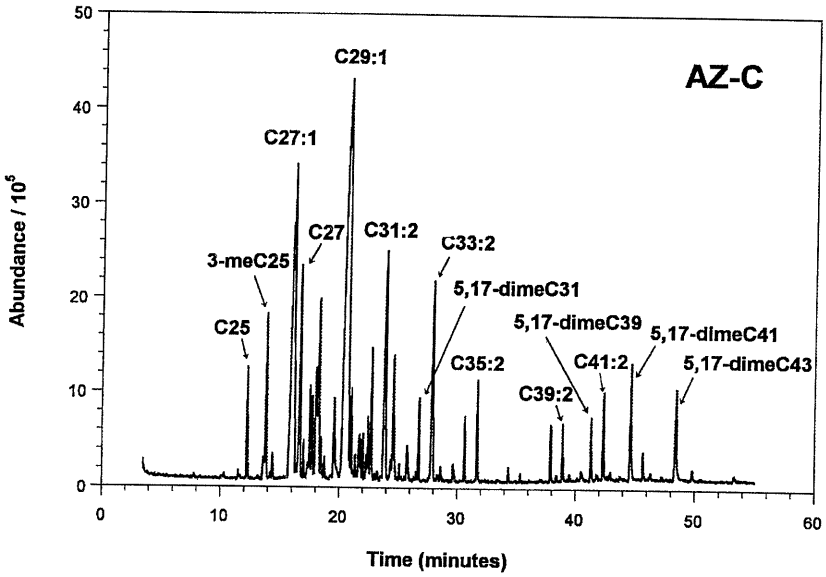

Fig. 8. Total ion chromatogram of cuticular hydrocarbons from Phenotype AZ-C specimens from Palisades Ranger Station, Santa Catalina Mountains, Pima County, Arizona.

Phenotype AZ-C is the most unusual of the western *Reticulitermes* and is more similar to GA-E (Haverty *et al.* 1996) than to any other western phenotype (Fig. 8). The mixture of hydrocarbons from AZ-C is composed primarily of olefins; C29:1 is the most abundant, and with C27:1, C31:2, and C33:2, predominates the hydrocarbon mixture (Tables 3 and 4). There is a homologous series of both dienes and 5,17-dimethylalkanes, beginning at C29 and ending with C43 (plus C45:2), almost exclusively with odd-numbered parent chains.

Phenotype AZ-D is distinguished by the absence of any 5-methylalkanes, 5,17dimethyalkanes, and late-eluting dienes, trienes, or tetraenes (Fig. 9). The profile most closely resembles that of CA-A' (Haverty *et al.* 1996).

Phenotype NV-A from Nevada resembles AZ-B in that it produces smaller quantities of C27 hydrocarbons, however it does produce the later-eluting dienes and trienes absent in AZ-B (Tables 4-8; Figs 7 and 10).

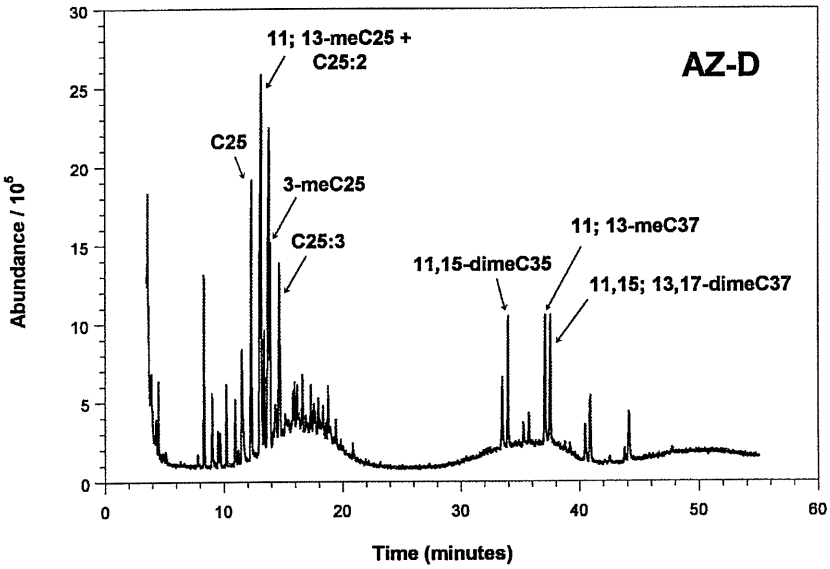

Fig. 9. Total ion chromatogram of cuticular hydrocarbons from Phenotype AZ-D specimens from 16 km east of Jacob Lake, Coconino County, Arizona.

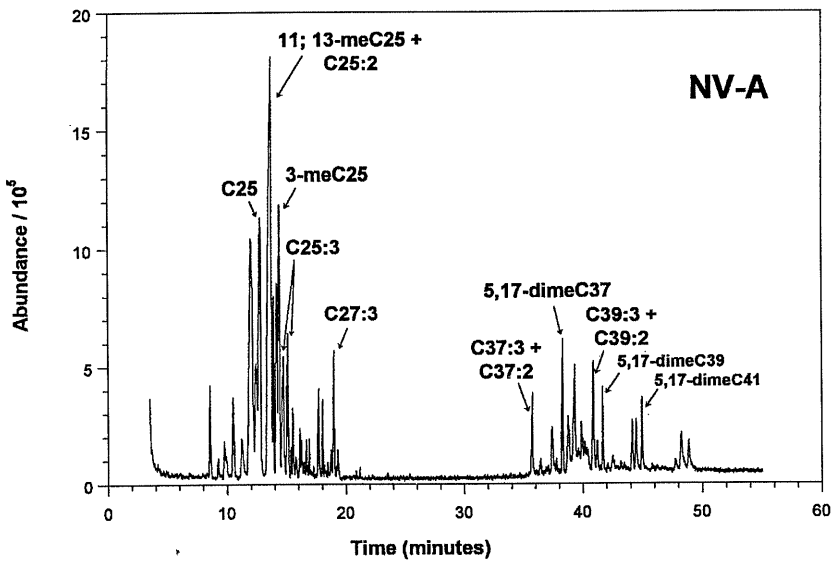

Fig. 10. Total ion chromatogram of cuticular hydrocarbons from Phenotype NV-A specimens from Wadsworth, Washoe County, Nevada.

DISCUSSION

The discovery of these additional cuticular hydrocarbon phenotypes of *Reticulitermes* now brings the total number to 26 in a genus with only 6 recognized species in North America. Additional surveys could possibly elucidate even more phenotypes. The discrepancy between described hydrocarbon phenotypes and described species might cause one to question the species specificity of cuticular hydrocarbon patterns (Howard and Blomquist 1982). However, the repeated appearance of these patterns in *Reticulitermes*, from disparate geographic locations (Haverty *et al.* 1991, 1996, Haverty and Nelson 1997), and the growing body of evidence on the taxonomic value of cuticular hydrocarbons for termites (see citations in Haverty and Nelson 1997), give us confidence that these phenotypes very likely represent distinct taxa.

In the southeastern United States *R. flavipes* and *R. virginicus* clearly appear to be the predominant species of *Reticulitermes.* The great majority of our collections from wood on/in the ground produce specimens that key to either *R. flavipes* or *R. virginicus.* Those from monitoring stations key to either *R. flavipes* or *R. hageni; R. virginicus* is seldom collected from monitoring stations. Specimens that key to *R. flavipes* (Scheffrahn and Su 1994) consist of 3 hydrocarbon phenotypes: GA-A; GA-B, and what appeared to be an intermediate 'GA-AB, so far found only on Sapelo Island and the nearby coastal area of Georgia (Haverty *et al.* 1991, 1996, Howard *et al.* 1978). Whether these patterns represent variation in the same species or 2 or 3 distinct species awaits additional studies of morphology, soldier defense secretions, and genetics. Specimens identified as *R. virginicus,* by both soldier and alate keys (Scheffrahn and Su 1994), consist of a single cuticular hydrocarbon profile which occurs over an extensive range (Haverty *et al.* 1991, 1996, Howard *et al.* 1982). The greatest diversity in cuticular hydrocarbon patterns occurs in the phenotypes that key to *R. hageni* based on soldier size.

In a previous paper (Haverty *et al.* 1996) we reported 7 cuticular hydrocarbon phenotypes that key to *R. hageni* based on soldier morphology (Scheffrahn and Su 1994). Phenotype GA-D keys *to R. hageni* on the basis of both soldiers and alates, however for 2 of these other phenotypes for which we have soldiers and alates from the same colony, the alates key to *R. virginicus.* The 4 new cuticular hydrocarbon phenotypes reported in this paper also key *to R. hageni* based on soldiers, but we do not yet have alates from these same colonies. The keys to soldiers of *Reticulitermes,* regardless of whether you use Weesner (1965), Nutting (1990), Scheffrahn and Su (1994), or the older

references Banks and Snyder (1920) or Snyder (1954), consider all *Reticulitemes* with soldiers pronotum width below 0.7mm to represent *R. hageni* (Scheffrahn and Su 1994). We suspect there are several undescribed species in GA-E through GA-N; only further studies of morphology, soldier chemistry, and genetics will validate our suspicions.

An additional point of interest is the diversity of cuticular hydrocarbon phenotypes we have collected in one relatively small, geographic area: Sapelo Island, McIntosh County, Georgia. This island is 64km S of Savannah, Georgia, is 11km long and 5km wide, is ca. 2km from the mainland, and covers an area of ca. 4,100 hectares. On this island we have collected numerous colonies of GA-AB *(R. flavipes),* GA-C *(R. virginicus),* and 2 or more collections each of GA-F, GA-G, GA-J, GA-L, and GA-N (all *R. hageni).* This diversity exceeds what we have reported from the Institute of Forest Genetics near Placerville, California, with 3 cuticular hydrocarbon phenotypes of *Reticulitermes* on a 4 hectare site (Haverty and Nelson 1997). How these phenotypes partition the habitat is not clear at this time. Phenotypes GA-F, GA-G, GA-J, GA-L, and GA-N have been collected in monitoring stations, whereas only GA-F and GA-L have been collected from wood on the ground.

Previous records of cuticular hydrocarbon phenotypes from Arizona divided them into 2 groups called Type III and Type IV (Haverty *et al.* 1991). The 3 collections from Arizona called Type III roughly match AZ-B because 2-meC24, *n*-C25, 11-; 13-meC25, and 3-meC25 are the predominant hydrocarbons, along with 5,17-dimethylalkanes. Neither AZ-B, NV-A, nor the Type III have as much of the significant C27 components found in NM-A and AZ-A. So until we gather additional samples with concomitant data on soldier chemistry and worker (pseudergate) genetics we cannot yet determine whether we are observing tremendous phenotypic variation of a single species or numerous, closely related taxa/species.

Phenotype AZ-C (labeled Type IV in Haverty *et al.* (1991)) is very distinct and consistent in its cuticular hydrocarbon mixtures. One of us (MIH) has collected this phenotype from numerous locations in the higher elevations of central to southern Arizona. It occurs only at the higher elevations (>1,750m) and is displaced by a different *Reticulitermes* (thought to be *R. tibialis* by Snyder (1949, 1954)) in the riparian areas in the lower elevations. Phenotype AZ-C is likely the same as *R. tumiceps* Banks because it is the only phenotype of *Reticulitermes* collected in the elevational range of the type locality of *R. tumiceps* (Stratton, AZ) at 6,000 to 7,000ft on the north side of the Santa Catalina Mountains, Pima County, Arizona (Banks and Snyder 1920). A collection from this

type locality would confirm our contention.

The characterization of collections of *Reticulitermes* into numerous distinct cuticular hydrocarbon phenotypes reemphasizes the need to focus attention on revision of this genus in North America. It is highly likely that many of these phenotypes represent distinct species and will result in a 2- to 4-fold increase in the number of species. It is also possible that, in some cases, we are simply recording phenotypic variation in the cuticular hydrocarbon composition of various species. Whether the former or the latter or both scenarios are real will have to await analysis of correlated morphological, genetic, and soldier chemistry information. This will only be possible if all of these parameters can be sampled from the same colonies or foraging groups.

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REFERENCES

Banks, N. & T.E. Snyder. 1920. A revision of the Nearctic termites. Bull. U.S. National Museum 108: 1-228.

Brown, W. V. J.A.L. Watson, & M.J. Lacey. 1996. A chemotaxonomic survey using cuticular hydrocarbons of some species of the Australian harvester termite genus *Drepanotermes* (Isoptera: Termitidae). Sociobiology 27: 199-221.

Haverty, M.I. & L.J. Nelson. 1997. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California indicate undescribed species. Comp. Biochem. Physiol. 118B: 869-880.

Haverty, M. I., B. T. Forschler, & L.J. Nelson. 1996. An assessment of the taxonomy of *Reticulitermes* (Isoptera: Rhinotermitidae) from the southeastern United States based on cuticular hydrocarbons. Sociobiology 28: 287-318.

Haverty, M. I., L.J. Nelson, & M. Page. 1991. Preliminary investigations of the cuticular hydrocarbons of North American *Reticulitermes* and tropical *Coptotermes* (Isoptera: Rhinotermitidae) for chemosystematic studies. Sociobiology 19: 51-76.

Howard, R. W. & G.J. Blomquist. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Ann. Rev. Entomol. 27: 149-172.

Howard, R. W., C.A. McDaniel, & G.J. Blomquist. 1978. Cuticular hydrocarbons of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). J. Chem. Ecol. 4: 233-245.

Howard, R. W., C.A. McDaniel, D.R. Nelson, G.J. Blomquist, L.T. Gelbaum, &

L.H. Zalkow. 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species- and caste-recognition cues. J. Chem. Ecol. 8: 1227-1239.

Nutting, W. L. 1990. Insecta: Isoptera. *In* Soil Biology Guide, D.L. Dindal, ed. John Wiley & Sons, Inc., New York pp. 997-1032.

Scheffrahn, R. H. & N.-Y. Su. 1994. Keys to soldier and winged adult termites (Isoptera) of Florida. Florida Entomol. 77: 460-474.

Snyder, T. E. 1949. Catalog of the termites (Isoptera) of the world. Smithsonian Misc. Collections vol. 112.

Snyder, T. E. 1954. Order Isoptera. The termites of the United States and Canada. National Pest Control Association, New York.

Weesner, F. M. 1965. Termites of the United States. The National Pest Control Association, Elizabeth, New Jersey.

Weesner, F. M. 1970. Termites of the Nearctic Region. *In* Biology of Termites, Vol. I. ed F.M. Weesner & K. Krishna. Academic Press, New York and London, pp. 477-525.