Cuticular Hydrocarbons: Species and Population-Level Discrimination in Termites[1]

Michael I. Haverty Marion Page Barbara L. Thorne Pierre Escoubas[2]

Abstract: Hydrocarbons in the cuticle of insects are essential in protecting them from desiccation. The vast variety of hydrocarbons synthesized by insects and the apparent species-specificity of cuticular hydrocarbon mixtures make them excellent taxonomic characters for separating species within termite genera. Hydrocarbon phenotypes of dampwood termites, Zootermopsis, correspond to species diagnoses. A morphological character, the subsidiary tooth of the right mandible, was found that correlates exactly with the hydrocarbon phenotypes of the three described species of Zootermopsis. Two distinct hydrocarbon phenotypes within Z. nevadensis have been used to identify a new subspecies, Z. n. nuttingi, which is morphologically indistinguishable from its conspecific, Z. n. nevadensis. This subspecies designation was corroborated with studies of agonistic behavior. Preliminary data suggest that cuticular hydrocarbons might be similarly used to sort specimens and to search for morphological characters within the subterranean termite genus Reticulitermes. This may facilitate a revision of this important genus. Cuticular hydrocarbon profiles enabled population-level discrimination of the Formosan subterranean termite, Coptotermes formosanus, from different geographic locations.

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

Alkanes occur in all insect surface lipids investigated so far. n-Alkanes generally range from 21 to 36 carbon atoms, and an odd number of carbons predominate. Terminally-branched and internally-branched monomethylalkanes are also prevalent in insect surface lipids and range from simple compositions, in which only one compound is present, to complex mixtures (Blomquist and Dillwith 1985). 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, or three double bonds, have been characterized in about one-half of the insect species examined to date. 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).

HYDROCARBONS AS TAXONOMIC CHARACTERS

Moore (1969) was the first to report the composition of cuticular hydrocarbons in a termite, Nasutitermes exitiosus (Hill) (Nasutitermitidae). He found the majority of the hydrocarbons to be paraffins from C_{24} to C_{47} with compounds with an odd number of carbons in the parent chain predominating. Blomquist and others (1979) and Howard and others (1978, 1980 and 1982) completely characterized the cuticular hydrocarbons of three termite species. They found that R. flavipes (Kollar). R. virginicus (Banks) (Rhinotermitidae), and Z. angusticollis (Hagen) (Termopsidae) possess drastically different hydrocarbon profiles, and all three of these profiles differ markedly from those reported earlier for N. exitiosus (Hill). Those investigators postulated that hydrocarbons might serve as semiochemical cues for caste and species recognition. On the basis of these early results, Howard and Blomquist (1982) hypothesized that each insect species had a mixture of cuticular hydrocarbons that was peculiar to that species and potentially useful as taxonomic characters.

Insects synthesize most if not all of their complement of cuticular hydrocarbons de novo (Blomquist and Dillwith 1985). 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 attractive characters for use in chemotaxonomy (see references in Haverty and others 1989 and Page and others 1990).

[1]Presented at the Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use, September 13, 1989, Bend, Oregon.

[2]Project Leader and Principal Research Entomologist, respectively, Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley, Calif.; Adjunct Assistant Professor, Department of Biology, Northeastern University, Boston, Mass., and Research Associate, Museum of Comparative Zoology, Harvard University, Cambridge, Mass.; Research Associate, Plant-Animal Interactions Research Group, Mizutani Plant Ecochemicals Project, Research Development Corporation of Japan, Eniwa-shi, Hokkaido, Japan.

We consider hydrocarbons to be unnecessary for higher order taxonomic separations. Obvious and consistent morphological characters will usually suffice. If there are qualitative differences among species within a genus, a reasonable goal would be to develop a dichotomous key to the species. Useful hydrocarbons should be abundant, not minor 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, rare or of insignificant quantities in one or a few. Furthermore, they should have a unique elution time so that they do not coelute 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. From a collection of these hydrocarbons, many different dichotomous keys are possible.

For the most part we have been corroborating existing taxonomies that are based on morphological, genetic and/or behavioral characteristics. For example, we have helped unravel species complexes, identified sibling species and substantiated recent synonymies in cone beetles, genus Conophthorus (Haverty and others 1989, Page and others 1990). 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.

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

These serendipitous discoveries have encouraged us to continue our work on termites as well as other groups of economically important forest insects. We have found that characterization of cuticular hydrocarbons often leads to subsequent biological or chemical studies which clarify taxonomic questions.

CHARACTERIZATION OF CUTICULAR HYDROCARBONS

Detailed descriptions of collection and extraction procedures for specific termite species have been published elsewhere (Blomquist and others 1979, Howard and others 1978, 1982, 1988, Haverty and others 1988, 1990). Cuticular lipids are extracted by immersing 15 to 500 termites (depending on size and total quantity of cuticular hydrocarbon), as a group, in 2 to 10 ml of hexane for 10 min. After extraction, hydrocarbons are separated from other components by pipetting the extract and an additional 2 to 8 ml of hexane through 3 cm of activated BioSil-A in Pasteur pipette mini-columns. Hydrocarbon extracts are evaporated to dryness under a stream of nitrogen and redissolved in 30 to 100 p of hexane for GC-MS analyses. When termites are extracted in the field, the hexane extracts can be allowed to fully evaporate. Dried extracts can then be easily shipped to the laboratory for chromatographic analysis. These field-dried extracts are redissolved in 10 ml of hexane and processed as usual. If termites are frozen, they should be removed from the freezer and allowed to warm to ambient temperature before extraction. After extraction, insects should be stored in 70 percent ethanol for later identification by morphological features and to serve as voucher specimens. All voucher specimens should be deposited in an appropriate reference museum.

All of our extractions were accomplished as described above. Our gas chromatography-mass spectrometry (GC-MS) analyses have been performed on a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 5970B Mass Selective Detector interfaced with Hewlett Packard Chemstation computer. The GC-MS was equipped with a fused silica capillary column (30 m x 0.2 mm ID, HP-1) and operated in split mode (with a split ratio of 8:1 to 20:1). Each mixture is analyzed by a temperature program from either 150°C, or 200°C to 320°C at 3°C/min with a final hold of 10 to 20 minutes. Electron impact (EI) mass spectra were obtained at 70 eV. n-Alkanes were identified by comparing their retention times and mass spectra with external standards (n-C_{22}, n-C_{24}, n-C_{28}, and n-C_{32}). Alkenes and methyl-branched alkanes were tentatively identified by calculating their equivalent chain lengths; mass spectra of methylalkanes were interpreted as described by Blomquist and others (1987).

SORTING SPECIMENS BY HYDROCARBON MIXTURES

The best example of the use of cuticular hydrocarbons as taxonomic characters for termites is the study we initiated with the dampwood termites, Zootermopsis (Haverty and others 1988). In preliminary work we collected what we tentatively identified as colonies of Z. angusticollis (Hagen) from the vicinity of Burney, Calif., in the Lassen National Forest, and extracted their cuticular hydrocarbons. We identified the same hydrocarbon components as published by Blomquist and others (1979), but in markedly different proportions. This was our first clue that considerable intraspecific variation might exist in the mixtures of cuticular hydrocarbons of Zootermopsis, and prompted us to examine this genus more thoroughly.

The current taxonomy of the genus Zootermopsis recognizes three species: Z. nevadensis, Z. angusticollis, and Z. laticeps (Banks) (Emerson 1933, Sumner 1933, Weesner 1970). We identified four consistent, unique cuticular hydrocarbon phenotypes from the three described species. Phenotype I (Z. nevadensis) contains large amounts (ca. 10 percent of the total hydrocarbon) of the symmetrical dimethylalkane 5, 17-dimethylheneicosane (fig. 1). This dimethylalkane is not found in any other phenotype (table 1).

Phenotype II (Z. angusticollis) has the most complex hydrocarbon mixture, sharing many of the components eluting before n-nonacosane with at least one of the other three phenotypes (Haverty and others 1988). However, phenotype II possesses many unique compounds in quantities greater than 1 percent (table 1): an isomeric mixture of 9- and 11-methylheneicosane; 3,11- and 3, 13-dimethylheneicosane; 3, 11- and 3, 13-dimethyltricosane; 2- or 4-methyloctacosane and 2- or 4-methyihentriacontane.

Table 1—Diagnostic hydrocarbons identified from four phenotypes of Zootermopsis (from Haverty and others 1988)

		Phenotype[1]			
Hydrocarbon[2]	ECL[3]	I	II	III	IV
9-, 11-MeC_{21}	21.37	0	++	0	Tr
2- or 4-MeC_{21}	21.62	0	0	0	++
5,17-$DimeC_{21}$	22.04	+++	0	0	0
3,11 ; 3,13-$DimeC_{21}$	22.11	0	+++	0	0
n-$C23_{23:1}$	22.70	0	0	++	++
3,11-, 3,13-$DimeC_{23}$	24.10	0	++	0	0
2- or 4-MeC_{28}	28.60	0	++	0	0
2- or 4-$MeC3_{31}$	31.75	0	++	0	0
7,11-$DimeC_{35}$	35.70	0	0	0	++
7,13-$DimeC_{39}$	38.53	0	0	0	++

[1] A triple + indicates > 5.0 percent of the total hydrocarbon component and ++ from 1.0 to 5.0 percent of the total. Trace (Tr) components appear infrequently or consistently in very small quantities (< 0.5 percent of the total). A zero indicates the hydrocarbon was never identified for the phenotype.

[2] Carbon number is the total number of carbons in the parent chain, excluding the methyl groups.

[3] ECL = Equivalent chain length.

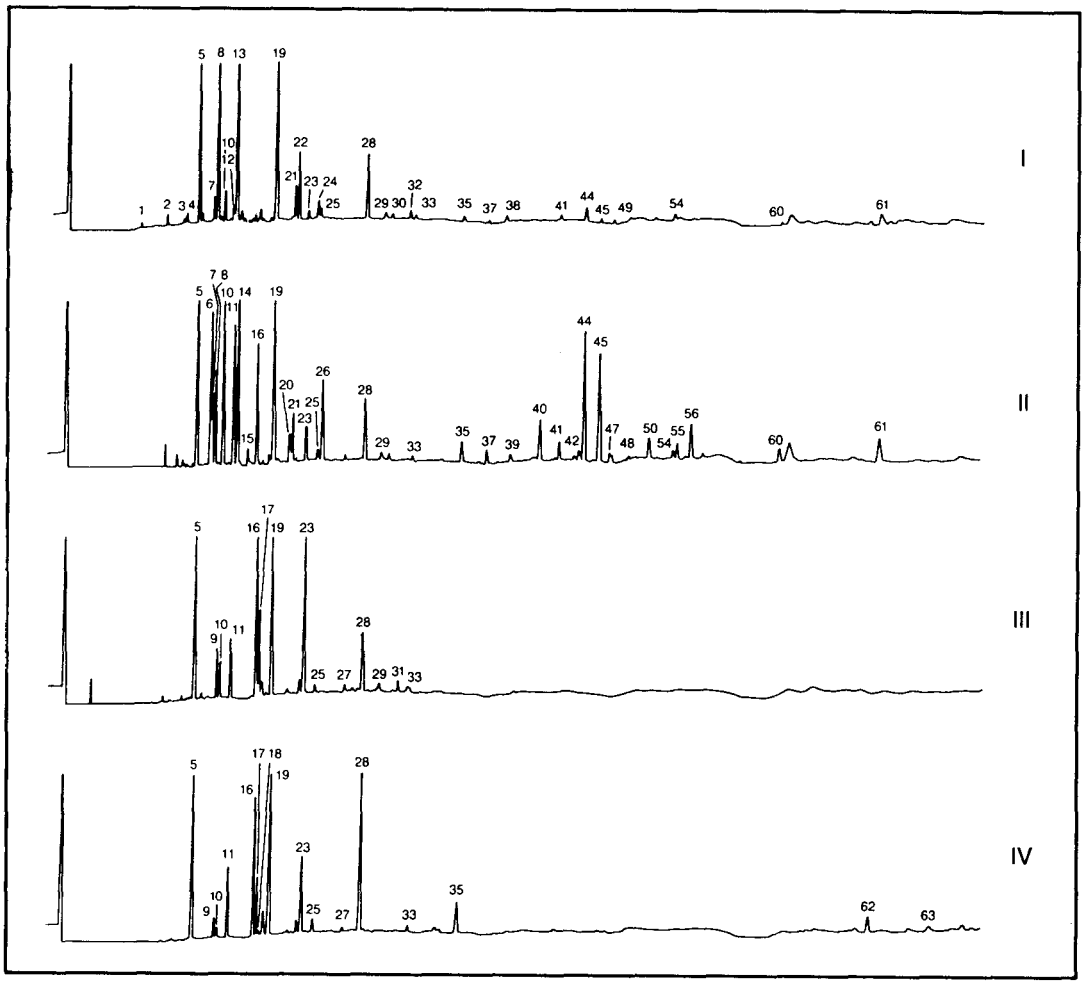

Figure 1—Gas chromatograms of the surface hydrocarbons of four phenotypes of Zootermopsis. Numbers identify peaks characterized by Haverty and others (1988).

Phenotype III (Z. nevadensis) clearly contains the simplest hydrocarbon profile. Only 10 hydrocarbon components are present in significant quantities (greater than 1.0 percent of the total hydrocarbon fraction). This is the only phenotype we collected from the Pacific Coast with an olefin (tricosene). All of the other major (>5.0 percent) or significant (>1.0 percent) components of Phenotype III are shared with at least one of the other phenotypes (Haverty and others 1988).

Phenotype IV (Z. laticeps) is also fairly simple, with only 13 significant (1.0 percent of total hydrocarbon) components, and is quite similar to Phenotype III. The major differences are the three unique compounds: 2- or 4- methylheneicosane; 7, 11-dimethylpentatriacontane and 7,13-dimethylheptatriacontane (table 1).

Identification of four consistent, unique cuticular hydrocarbon phenotypes from three extant species was in conflict with the species-specific hypothesis put forward by Howard and others (1978, 1982). If cuticular hydrocarbon profiles are truly species-specific, then there is at least one new, undescribed species of Zootermopsis. It appears that Z. angusticollis and Z. laticeps are valid species, but Z. nevadensis is a complex of at least two species or one species with polymorphic hydrocarbon chemistry. Clarification of specific status of each phenotype required many different approaches. The approaches we investigated were correlation of hydrocarbon chemistry and external morphology from an extensive geographic collection (Thorne and Haverty 1989), bioassays of aggressive behavior (Haverty and Thorne 1989), isoenzyme analysis (Korman and others, 1991), and DNA hybridization (Broughton 1989).

CORRELATION OF HYDROCARBONS WITH EXTERNAL MORPHOLOGY

Determination of Zootermopsis species on the basis of morphology is often equivocal, especially in juvenile or small colonies. However, by sorting specimens on the basis of cuticular hydrocarbon phenotypes, we found a character on the right mandible, the shape and position of the subsidiary tooth, that enables unequivocal diagnosis of Z. angusticollis, Z. nevadensis and Z. laticeps (Thorne and Haverty 1989). This small subsidiary tooth is present at the base of the anterior edge of the first marginal tooth on the right mandible of nonsoldiers of all genera within the Termopsidae. Shape and position of the subsidiary tooth can be consistently and accurately used to diagnose both sexes of all nonsoldier morphs of Z. angusticollis and Z. nevadensis.

Because the subsidiary tooth character does not rely on a series of soldiers (relatively rare in colonies or collections) or alates (only present seasonally and absent from most samples or collections), it can be used to determine virtually any collection of Zootermopsis. Large larvae or nymphs are almost always abundant, and dissection of several individuals will not reduce the value of the collection. The mandible structure does not enable morphological discrimination of the two Z. nevadensis hydrocarbon phenotypes. However, agonistic behavior which involves social interactions between individuals, including fighting, fleeing, or submitting, provides a biological model for species discrimination.

AGONISTIC INTERACTIONS BETWEEN HYDROCARBON PHENOTYPES

Termites show a wide range of agonistic behaviors when interacting with other termites from a different colony of the same or a different species. Aggressive encounters are often particularly dramatic because of defensive morphological modifications of the soldier caste and effective use of strong mandibles by the pseudergate or worker castes. Interspecific aggression is common in termite-termite interactions. Intraspecific aggression (between colonies) has also been shown in a variety of other termites. A few documented cases exist, however, in which aggression between conspecific termites is absent; i.e., where individuals from different colonies can be mixed with little or no reaction (see references in Haverty and Thorne 1989).

Three experiments were conducted to assess agonistic interactions between hydrocarbon phenotypes (Haverty and Thorne 1989). We hypothesized that meetings between termites from colonies of like phenotypes would be more passive than encounters between individuals of different phenotypes. In the first series of experiments we assessed the agonistic response of soldiers from each of the three hydrocarbon phenotypes with nymphs from each phenotype. In the second we assessed the intra-caste agonistic behavior of either pseudergates or soldiers from hydrocarbon phenotypes I, II, and III. In the third we assessed the intra-caste agonistic behavior of pseudergates or soldiers from hydrocarbon phenotype IV with those like castes from phenotypes I, II, and III.

Our results demonstrated that soldiers or pseudergates seldom attack individuals of the same hydrocarbon phenotype. Phenotype II (Z. angusticollis) is typically aggressive toward phenotype III (Z. nevadensis), but not always aggressive against phenotype I (Z. nevadensis). The variation in response is dependent on which castes are placed in the bioassay arena: soldier-versus-soldier bouts result in consistent aggression, while pseudergate-versus-pseudergate or soldier-versus-nymphs contacts do not. Both pseudergates and soldiers of Z. laticeps (phenotype IV) respond agonistically toward the other three phenotypes.

Although hydrocarbon phenotypes I and III, both Z. nevadensis, are morphologically indistinguishable, agonistic behavioral responses between them are not equivalent to I-versus-I or III- versus-III behavioral responses. We interpret the lack of avoidance or aggressive behavior within each of the two phenotypes of Z. nevadensis and the significant avoidance and aggressive behavior between phenotypes as definite evidence of discrimination between disparate hydrocarbon phenotypes (fig. 2). These agonistic bioassays along with data on distinct hydro--carbon patterns and geographic distributions serve as the basis for designating two subspecies of Z. nevadensis: Z. n. nevadensis, (Hagen) and Z. n. nuttingi (Haverty and Thorne), ssp. nov (Haverty and Thorne 1989).

HYDROCARBONS OF RETICULITERMES

The morphology of the subterranean termites in the family Rhinotermitidae is quite variable among colonies within the

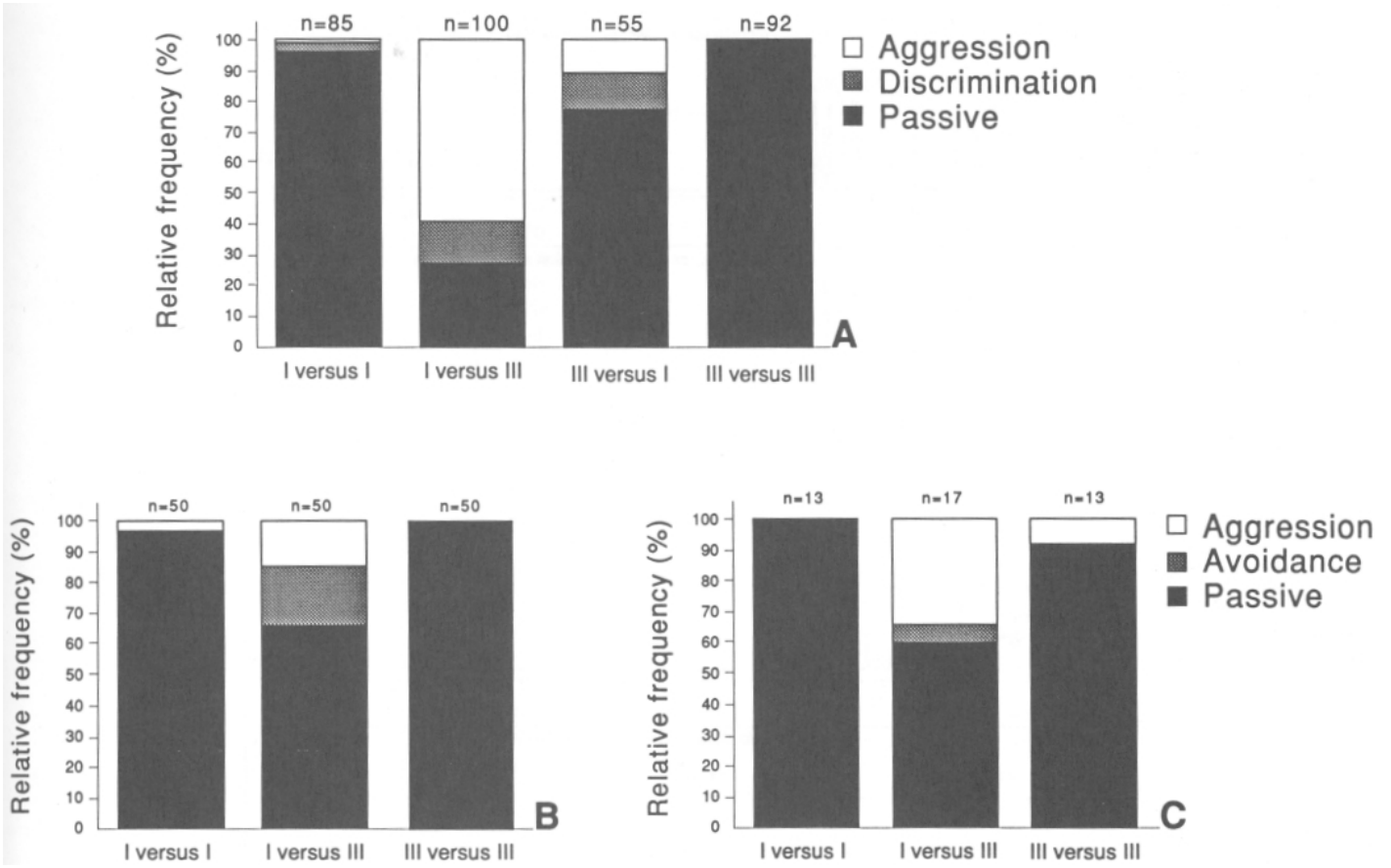

Figure 2—Agonistic behavior between Zootermopsis hydrocarbon phenotypes I and III: A. Soldier (first Roman numeral) vs. nymphs (second Roman numeral) of like or different phenotypes; B. Pseudergates vs. pseudergates; C. Soldier vs. soldier. The number of observations and relative frequency of each class of behavior are shown. Behaviors are passive, avoidance, and aggression.

same species. It is readily acknowledged that the genus Reticulitermes is in need of revision. "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..." (Weesner 1970). We have characterized the cuticular hydrocarbons of "species" of Reticulitermes from the western United States, R. tibialis Banks and R. hesperus Banks, as well as those from two species common to the eastern United States, R. flavipes (Kollar) and R. virginicus (Banks) (fig. 3). Examination of our profiles and those published in the literature reveals 80 hydrocarbons from these species of Reticulitermes from the southern and western United States. Of these hydrocarbons, we consider at least 21 to be of diagnostic value (table 2).

We have collected Reticulitermes in California from areas suspected to have only R. hesperus or R. tibialis, Cloverdale and Owens Lake, respectively. We found that the cuticular hydrocarbon profiles of workers from these two areas are very similar, with the primary difference being one hydrocarbon substitution: 11, 15-dimethylpentacosane in R. hesperus and 2- or 4-methylpentacosane in R. tibialis (table 2, fig. 3). This similarity of hydrocarbon mixtures indicates that these two collections are either the same species or closely related. Our collections of "R. tibialis" from two different locations in Arizona, Fairbank and Prescott, exhibit extremely different cuticular hydrocarbon profiles which are also different from our collections of either R. hesperus or R. tibialis from California (fig. 3). From these results we infer that there are probably at least three species of Reticulitermes in western North America.

ORIGIN OF INTRODUCED SPECIES

Another way to use data on cuticular hydrocarbons is to relate the similarity of hydrocarbon profiles to the origin of introduced termite species. To test this approach we examined the cuticular hydrocarbon profiles of four geographically distant populations of the Formosan subterranean termite, Coptotermes formosanus Shiraki, in the United States (Haverty and others 1990). This species is considered to be one of the most voracious subterranean termites and a serious threat to wood in structures throughout its range. It was introduced into Japan before 1600 and subsequently into Hawaii 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 after World War II in the port cities of Houston and Galveston, Texas; New

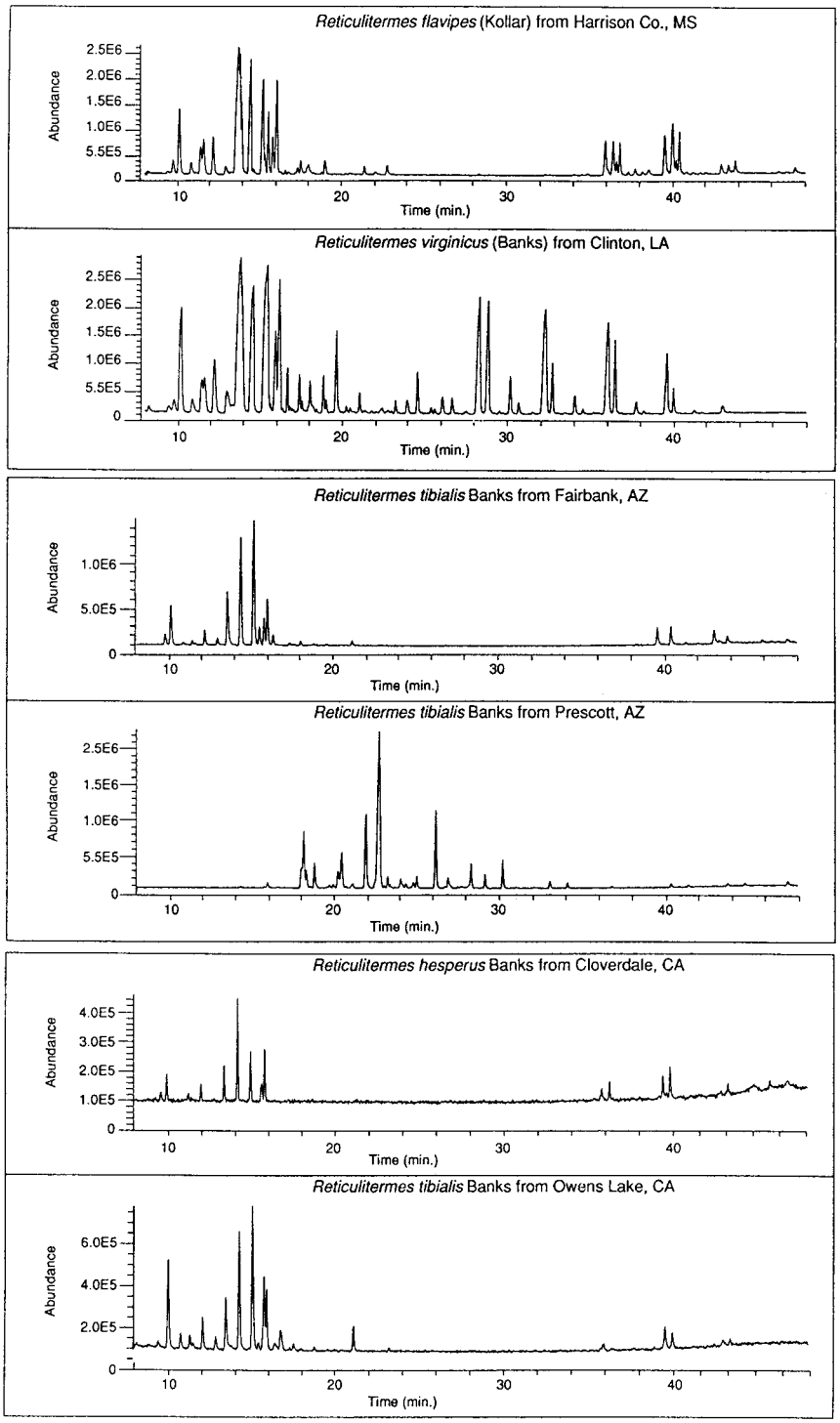

Figure 3—Gas chromatograms of the surface hydrocarbons of four "species" of Reticulitermes.

Orleans and Lake Charles, Louisiana; and Charleston, South Carolina (Beal 1987). There has been a significant "spread" of C. formosanus to other southern port cities including greater Fort Lauderdale, Florida, Memphis, Tennessee, Baton Rouge, Louisiana, and Mobile, Alabama. Similarity or difference in hydrocarbon patterns between the four populations (Hallandale, Florida; New Orleans, Louisiana; Lake Charles, Louisiana; and Honolulu, Hawaii) was determined by stepwise discriminant analysis to select discriminating variables (proportion of a given hydrocarbon), followed by canonical discriminant analysis to provide two-dimensional displays of the chosen variables. GC-MS analyses of the cuticular wax of representative samples of C. formosanus indicate that all colonies and both castes from all four locations contain the same sixteen hydrocarbon components. We examined this set of quantitative chemical characters to determine the similarity between the hydrocarbon profiles

Table 2—Diagnostic hydrocarbons for four species of Reticulitermes[1]

Hydrocarbon[4]	ECL[5]	Reticulitermes species[2,3]					
		flav	virg	AZ1	AZ2	CA1	CA2
n-C_{23}	23.00	+++	++	+++	o	+++	+++
$C_{25:1}$	24.70	+++	+++	o	o	o	o
$C_{25:2}$	25.40	+++	+++	o	o	o	o
11,15-DimeC_{25}	25.60	o	o	o	o	o	+++
2- or 4-MeC_{25}	25.70	++	++	++	o	+++	o
5,17-DimeC_{25}	25.90	o	o	++	o	o	o
$C_{27:1}$	26.70	o	o	o	+++	o	o
$C_{29:2}$	28.69	o	o	o	+++	o	o
$C_{29:1}$	28.70	o	o	o	+++	o	o
$C_{31:2}$	30.69	o	o	o	+++	o	o
11-; 15-MeC_{31}	31.40	o	+++	o	0	o	o
11,15-DimeC_{31}	31.64	o	++	o	o	o	o
11-; 13-; 15-; 17-MeC_{33}	33.30	o	+++	o	o	o	o
11,15-; 11,21-; 13,17-DimeC_{33}	33.57	o	++	o	o	o	o
11-; 13-; 15-; 17-MeC_{35}	35.31	++	++	o	o	++	o
11,15-DimeC_{35}	35.60	++	++	o	o	++	o
7,17-DimeC_{35}	35.70	+	o	o	o	o	o
5,17-DimeC_{35}	35.80	++	o	o	o	o	o
11-; 13-; 15-; 17-; 19-MeC_{37}	37.30	++	++	++	o	+++	++
11,15-DimeC_{37}	37.59	++	+	o	o	+++	++
5,17-DimeC_{37}	37.80	++	o	++	o	o	o

[1] Hydrocarbons listed for each species are all from chemical analyses by Page, Haverty, and Escoubas.

[2] Reticulitermes "species": flav=R. flavipes, virg=R.virainicus, AZ1=R. tibialis from Fairbank, Ariz., AZ2 = R. tibialis from Prescott, Ariz., CA1 = R. hesperus from Cloverdale, Calif., CA2 = R. tibialis from Highway 35 near Owens Lake, Calif.

[3] A triple + indicates ≥ 5.0 percent of the total, ++ from 1.0 to 5.0 percent of the total and + from 0.5 to 1.0 percent of the total hydrocarbon component. Some trace (tr.) components appear infrequently or consistently in very small quantities (< 0.5 percent of the total). A zero indicates the hydrocarbon was never identified for the phenotype.

[4] Carbon number is the total number of carbons in the parent chain, excluding the methyl groups.

[5] ECL = Equivalent chain length.

within and among the sampled geographic locations. Our underlying assumption was that colonies of C. formosanus with quantitatively similar cuticular hydrocarbon profiles are likely to be more closely related, i.e., originating from the same geographical source, than those that are less similar.

Colonies of C. formosanus from each of the four geographic locations can be separated from colonies from all of the other sites on the basis of concentrations of cuticular hydrocarbon components. Discriminant analysis of the proportions of the cuticular hydrocarbons of workers identified seven hydrocarbon components that reasonably separate the four populations. For the two locations for which we had soldiers, only three hydrocarbon components were necessary. No single component, however, can be used to separate C. formosanus from the four geographic locations. Stepwise discriminant analysis allowed us to use a selection of the hydrocarbon components to effectively distinguish C. formosanus from these geographic locations (fig. 4).

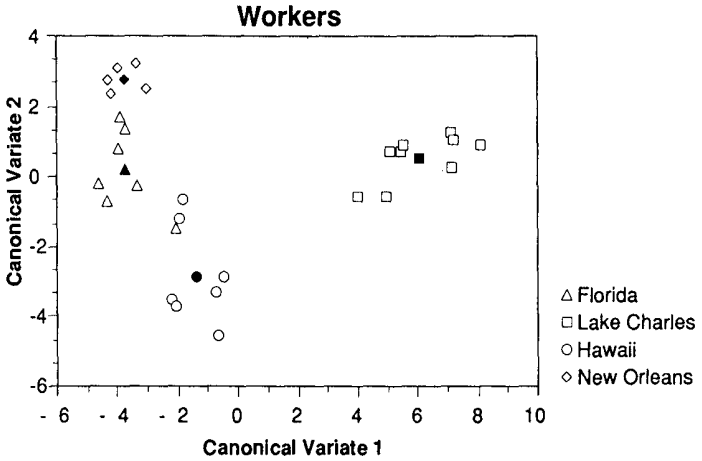

Figure 4—Plot of colonies of C. formosanus from four different locations along two axes of canonical discriminant space. Open characters represent actual data points; closed characters represent the mean value for the population.

Clearly the population from Lake Charles is very different from those from the other locations. This difference supports the assertion that the introduction of C. formosanus into Louisiana was from two separate sources (La Fage 1987). It also appears that the Honolulu population, at least the insects we sampled from the Manoa Valley in Honolulu, was probably not the original source of infestations in either New Orleans, Lake Charles or Hallandale. The affinity of the infestation from Hallandale, Florida, is least clear. The hydrocarbon profile is significantly different from that of both Honolulu and New Orleans.

Unfortunately, with our present data base, cuticular hydrocarbon profiles have not allowed us to determine whether the more recent infestation in Hallandale, Florida, resulted from international or domestic, maritime or surface commerce. Our results suggest that C. formosanus from Hallandale, Florida, New Orleans, Louisiana, and Lake Charles, Louisiana, are not related to those from Honolulu, Hawaii, and probably originated from other geographical locations.

CONCLUSIONS

Our experience, thus far, in applying knowledge of cuticular hydrocarbon composition to the identification of termite species has been encouraging. We have used hydrocarbons to sort specimens initially to identify a new diagnostic morphological character for Zootermopsis species. Where morphology cannot be used to separate hydrocarbon phenotypes, we have been able to use inter- and intraphenotype agonistic behavior to corroborate cuticular hydrocarbon phenotypes. Geographical races of Coptotermes formosanus can be distinguished even on the basis of the concentrations of individual hydrocarbon components.

There are still some unresolved questions to be addressed before we consider 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 the mixture of hydrocarbons? (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.

Currently we rely on gross quantities of chemical insecticides applied to the soil for protection of wooden structures from our native subterranean termites. This practice will likely be deemed as unacceptable soon. Future species-specific control strategies for any subterranean termite will require an understanding of the foraging, feeding, and interspecific behaviors of the species to be controlled, and this necessitates accurate identification of the species.

It is readily acknowledged that Reticulitermes is the most economically important genus of termites in the United States and Europe, yet the genus is in desperate need of revision. There should be a significant effort to revise the genus Reticulitermes in North America and two important groups of pantropical termites: Coptotermes and Nasutitermes. These latter two genera are among the most economically important groups in the world. This revision will require collaboration among the termite scientists throughout the world, and will involve studies of morphology, behavior, genetics and, of course, cuticular hydrocarbons. We consider cuticular hydrocarbons to constitute a very useful set of characters that can provide insight into the taxonomy of termites for which classical morphometric analyses have failed.

ACKNOWLEDGMENTS

The experiments reported here would not have been possible without numerous collections of termite foraging groups and colonies. For their help in gathering the specimens used in these studies we thank David Azuma, Patrick Hahn, Jill Haverty, Peter Haverty, Jeffery La Fage, Bryce McPherson, Timothy Myles, Lori Nelson, William Nutting, Rebecca Rosengaus, Michael Rust, Rudolf Scheffrahn, Timothy Schowalter, Nan-Yao Su, and Robin Yamamoto. The diligence of Lori Nelson in processing the numerous hydrocarbon samples is greatly appreciated. We are most thankful to Gary Blomquist for helping us understand hydrocarbon chemistry and analysis. David Wood provided resources to assist in the collection and extraction of the western Reticulitermes species described. This work was supported, in part, by an Internal Competitive Grant from the Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley, California, and by National Science Foundation grant BSR-8607407 to B.L.T., by research scholarships to P.E. from the French government (Programme LAVOISIER), E.I. DuPont de Nemours, Inc., and the Philippe Foundation.

REFERENCES

Beal, Raymond H. 1987. Introductions of Coptotermes formosanus Shiraki to the continental United States. In: Tamashiro, M.; Su, N.-Y., eds. Biology and control of the Formosan subterranean termite. Research Extension Series 083. Honolulu: University of Hawaii; 48-53.

Blomquist, Gary J.; Dillwith, Jack W. 1985. Cuticular lipids. In: Kerkut, G.A.; Gilbert, L.I., eds. Comprehensive insect physiology, biochemistry and pharmacology. Vol. 3. Integument, respiration and circulation. Oxford: Pergamon; 117-154.

Blomquist, Gary J.; Howard, Ralph W.; McDaniel, C.A. 1979. Structure of the cuticular hydrocarbons of the termite Zootermopsis angusticollis (Hagen). Insect Biochemistry 9:371-374.

Blomquist, Gary J.; Nelson, Dennis R.; de Renobales, Mertxe. 1987. Chemistry, biochemistry, and physiology of insect cuticular lipids. Archives of Insect Biochemistry and Physiology 6:227-265.

Broughton, Richard E. 1989. A DNA-DNA hybridization study of the termite genus Zootermopsis (Isoptera). Chico: California State Univ.; 77 p. Master of Science Thesis.

Emerson, Alfred E. 1933. A revision of the genera of fossil and recent Termopsinae (Isoptera). University of California Publications in Entomology 6:165-196.

Hadley, Neil F. 1980. Surface waxes and integumental permeability. American Scientist 68:546-553.

Hadley, Neil F. 1985. The adaptive role of lipids in biological systems. New York: John Wiley and Sons.

Haverty, Michael I.; Nelson, Lori J.; Page, Marion. 1990. Cuticular hydrocarbons of four populations of Coptotermes formosanus shiraki in the United States: similarities and origins of introductions. Journal of Chemical Ecology. 16:1635-1647.

Haverty, Michael I.; Page, Marion; Blomquist, Gary J. 1989. Value of cuticular hydrocarbons for identifying morphologically similar species of pine cone beetles. In: Miller, Gordon E., compiler, Proceedings 3rd cone and seed insects working party conference, Working party S2.07-01, International Union of Forestry Research Organizations; 1988 June 26-30; Victoria, B.C., Canada. Victoria, B.C., Canada: Forestry Canada, Pacific Forestry Centre; 50-62.

Haverty, Michael I.; Page, Marion; Nelson, Lori J.; Blomquist, Gary J. 1988. Cuticular hydrocarbons of dampwood termites, Zootermopsis: intra- and intercolony variation and potential as taxonomic characters. Journal of Chemical Ecology 14: 1035-1058.

Haverty, Michael I.; Thorne, Barbara L. 1989. Agonistic behavior correlated with hydrocarbon phenotypes in dampwood termites, Zootermopsis (Isoptera: Termopsidae). Journal of Insect Behavior 2:223-243.

Howard, Ralph W.; Blomquist, Gary J. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Annual Review of Entomology 27:149-172.

Howard, Ralph W.; McDaniel, C.A.; Blomquist, Gary J. 1978. Cuticular hydrocarbons of the eastern subterranean termite, Reticulitermes flavipes (Kollar)(Isoptera: Rhinotermitidae). Journal of Chemical Ecology 4:233-245.

Howard, Ralph W.; McDaniel, C.A.; Blomquist, Gary J. 1980. Chemical mimicry as an integrating mechanism: cuticular hydrocarbons of a termitophile and its host. Science 210:431-433.

Howard, Ralph W.; McDaniel, C.A.; Nelson, Dennis R.; Blomquist, Gary J.; Gelbaum, Leslie T.; Zalkow, Leon H. 1982. Cuticular hydrocarbons of Reticulitermes virginicus (Banks) and their role as potential species- and caste-recognition cues. Journal of Chemical Ecology 8:1227-1239.

Howard, Ralph W.; Thorne, Barbara L.; Levings, Sally C.; McDaniel, C.A. 1988. Cuticular hydrocarbons as chemotaxonomic characters for Nasutitermes corniger (Motschulsky) and N. ephratae (Holmgren)(Isoptera: Termitidae). Annals of the Entomological Society of America 81:395-399.

Korman, Amy K.; Pashley, Dorothy P.; Haverty, Michael I.; La Fage, Jeffery P. 1991. Allozymic relationships among cuticular hydrocarbon phenotypes of Zootermopsis species (Isoptera: Termopsidae). Annals of Entomological Society of America 84:1-9.

La Fage, Jeffery P. 1987. Practical considerations of the Formosan subterranean termite in Louisiana: A 30-year-old problem. In: Tamashiro, M.; Su, N: Y., eds. Biology and control of the Formosan subterranean termite. Research Extension Series 083. Honolulu: University of Hawaii; 37-42.

Moore, Barry P.1969. Biochemical studies in termites. In: Krishna, K.; Weesner, F.M., eds. Biology of termites, Vol. I. New York: Academic Press; 407-432.

Page, Marion; Nelson, Lori J.; Haverty, Michael I.; Blomquist, Gary J. 1990. Cuticular hydrocarbons of eight species of North American cone beetles, Conophthorus Hopkins. Journal of Chemical Ecology. 16:1173-1198.

Su, Nan-Yao; Tamashiro, Minoru. 1987. An overview of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the world. In: Tamashiro, M.; Su, N: Y., eds. Biology and control of the Formosan subterranean termite. Research Extension Series 083. Honolulu: University of Hawaii; 3-15.

Sumner, Ethel Craig. 1933. The species of the termite genus Zootermopsis Emerson (=Termopsis Hagen). University of California Publications in Entomology 6:197-230.

Thorne, Barbara L.; Haverty, Michael I. 1989. Accurate identification of Zootermopsis species (Isoptera: Termopsidae) based on a mandibular character of non-soldier castes. Annals of the Entomological Society of America 82:262-266.

Weesner, Frances M. 1970. Termites of the Nearctic Region. In: Krishna, K.; Weesner, F.M., eds. Biology of termites, Vol. I. New York: Academic Press; 477-525.