Journal of Chemical Ecology, Vol. 16, No. 5, 1990

CUTICULAR HYDROCARBONS OF FOUR POPULATIONS OF *Coptotermes formosanus* SHIRAKI[1] IN THE UNITED STATES
Similarities and Origins of Introductions

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(Received July 14, 1989; accepted September 18, 1989)

Abstract—The degree of similarity among cuticular hydrocarbon profiles of four populations of *Coptotermes formosanus* Shiraki in the United States is reported. Sixteen individual or isomeric mixtures of hydrocarbons were identified by gas chromatography–mass spectrometry. Hydrocarbon components consist of *n*-alkanes, 2-methylalkanes, 3-methylalkanes, internally branched monomethylalkanes on carbons 9–15, and dimethylalkanes. The predominant hydrocarbons have 27 carbons in the parent chain. Methyl-branched hydrocarbons are more abundant than *n*-alkanes. No qualitative differences were apparent in the hydrocarbon components of workers or soldiers from any of the four populations. Quantitative differences in the hydrocarbon components separate castes and populations into different concentration profiles. Stepwise discriminant analysis and canonical discriminant analysis were used to choose and display seven hydrocarbon components for workers and three for soldiers that best reveal the differences among populations. Within-population variation is low compared to the differences among populations. These 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.

Key Words—Cuticular lipids, chemotaxonomy, biogeography, founder principle, *Coptotermes formosanus*, Isoptera, Rhinotermitidae, insect quarantine, insect integument, canonical discriminant analysis.

[1] Isoptera: Rhinotermitidae.

INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is considered to be one of the most voracious subterranean termites and a serious threat to wood in structures throughout its range. In addition to buildings, *C. formosanus* attacks live trees, creosoted transmission poles, structural pilings, and even underground utility cables. No accurate estimate of the economic impact of *C. formosanus* is available; it likely exceeds $100 million annually in the United States alone. Even though the common name implies that this species is from Formosa (Taiwan), *C. formosanus* is considered to be indigenous to mainland China (Kistner, 1985). It was introduced into Japan prior to 1600 and subsequently into Hawaii before 1907 (Swezey, 1914). Since its introduction into Hawaii, *C. formosanus* has been imported to Guam, Midway, Sri Lanka, Taiwan, South Africa, and the mainland United States (Su and Tamashiro, 1987).

Although many of the infestations in the continental United States were first noticed after 1965, *C. formosanus* colonies were most likely established following World War II. Active movement of military goods from the Pacific Theater to storage facilities in port cities of Houston and Galveston, Texas; New Orleans and Lake Charles, Louisiana; and Charleston, South Carolina, probably resulted in the establishment of *C. formosanus* in North America (Beal, 1987). There has been a significant "spread" of *C. formosanus* to other southern port cities including greater Fort Lauderdale, Florida; Memphis, Tennessee; Baton Rouge, Louisiana; and Mobile, Alabama. Because of the capacity of *C. formosanus* to infest wooden watercraft and wooden products transported on marine vessels, initial introductions have been near cities involved in shipping (La Fage, 1987).

Since 1980, indications of established infestations inland suggest this species may now be transported in infested wood products via domestic surface commerce (Chambers et al., 1988; La Fage, 1987; Sponsler et al., 1988). Despite the overall success of *C. formosanus* in infesting new areas, distribution is generally confined to regions where average temperatures for the coldest month do not fall below 4°C, and mean minimum daily temperatures for the same month are not less than 0°C. This includes locations ca. 36° north or south of the equator (Su and Tamashiro, 1987; Beal, 1987), and means potential for infestation of the highly populated, coastal metropolitan area of Los Angeles, California, north to the San Francisco Bay area.

With this potential threat to the most populous state in the United States, in addition to new locations along the entire Gulf Coast and much of the Atlantic Coast, effective quarantine procedures are imperative. Knowledge of how introductions of *C. formosanus* have been spread will help regulatory agencies formulate and/or reexamine quarantine policies and procedures. Has *C. formosanus*

been introduced numerous times from Asia or Hawaii, or once introduced, has it spread from port to port via domestic, maritime, or surface commerce? In this paper we report the identification of the hydrocarbon components in the cuticular wax of *C. formosanus* from four geographical locations. These baseline data are analyzed to determine whether similarities among cuticular hydrocarbon profiles can reveal the origin(s) of recent infestations of *C. formosanus*.

METHODS AND MATERIALS

Termites were collected from four geographically separated locations: Hallandale, Florida; New Orleans and Lake Charles, Louisiana; and Honolulu, Hawaii. Foraging parties from colonies were collected from the soil in Florida as described by Su and Scheffrahn (1986) or in Hawaii as described by Tamashiro et al. (1973). The seven different colonies from Florida all came from a single location (up to 5 km apart) in Hallandale. Six of the seven colonies from Honolulu were from the University of Hawaii campus; another came from Kaneoe on the island of Oahu. Hollow centers of live and dead trees were the source of *C. formosanus* from New Orleans and Lake Charles. Groups of workers (and soldiers from Lake Charles only) were removed from standing trees in these latter two sites as described by Waller and La Fage (1987). All collections were made within a 14-month period: New Orleans in August 1986, Lake Charles in November 1986, Hallandale in April 1987, and Honolulu in October 1987.

Termites were separated from wood debris and carton material, frozen, and subsequently dried in a desiccator. They were held dry at room temperature until hydrocarbons were extracted. Cuticular lipids were extracted by immersing 50–100 termites, as a group, in 10 ml of hexane for 10 min. After the termites were extracted, hydrocarbons were separated from other components and identified by gas chromatography–mass spectrometry (GC-MS) (Page et al., 1990). Routine quantification of cuticular hydrocarbon components was achieved with a Hewlett Packard 5890 gas chromatograph; all operating conditions were the same as described by Haverty et al. (1988), except that oven temperature was increased at 3°C/min to 320°C, with a final holding time of 11 min.

For each of the four *Coptotermes* populations, summary statistics (mean and standard deviation of the percent of each hydrocarbon component) were calculated separately for workers and soldiers. Similarity of hydrocarbon patterns or differences between the four populations was determined by stepwise discriminant analysis to select discriminating variables, followed by canonical discriminant analysis to provide two-dimensional displays of the chosen variables. The purpose of the discriminant analysis was to define a subset of quan-

titative variables (in this case percentage of each hydrocarbon component) that best revealed the differences among the populations. The program that we used (STEPDISC) employs stepwise selection of variables (SAS, 1985). Canonical discriminant analysis is a dimension-reduction technique related to principle component analysis and canonical correlation that derives a linear combination of quantitative variables (percentage of hydrocarbon components) that summarizes between-class variation. The program that we used (CANDISC) computes and tests Mahalanobis distances for pairwise comparisons of the populations (SAS, 1985). We assume that these distances are indicative of the degree of genetic similarity between populations.

RESULTS AND DISCUSSION

GC-MS analyses of the cuticular wax of representative samples of *C. formosanus* (one group of workers and/or soldiers from each location) indicate that all colonies and both castes from all four locations contain the same hydrocarbon components. All of the 16 major hydrocarbon components and/or isomeric mixtures (mean percent $\geq 0.1\%$ of the total hydrocarbon mixture) were characterized (Table 1, Figure 1). Hydrocarbon components consist of homologous series of *n*-alkanes, 2-methylalkanes, 3-methylalkanes, and single-component and isomeric mixtures of internally branched monomethylalkanes and dimethylalkanes. No unsaturated components were identified. With the exception of one dimethylalkane, hydrocarbon structures were previously identified in other insects by the authors (Haverty et al., 1988; Page et al., 1990).

The *n*-alkane composition is a continuous series from *n*-pentacosane to *n*-octacosane with *n*-heptacosane predominating. Both 3-methylalkanes and 2-methylalkanes were found. 3-Methylheptacosane (peak 11) occurs in significant quantities ($\geq 5\%$) whereas 3-methylpentacosane occurs in small amounts except in the colonies from Lake Charles, Louisiana. It is difficult to distinguish 2-methylalkanes from 4-methylalkanes (Blomquist et al., 1987); however, all are identified as 2-methylalkanes because there was no strong carbonium ion pair at M-71 : M-72, indicating that the 4-methylalkanes were not present (Table 1). 2-Methylalkanes are quite predominant in the hydrocarbon profiles of *C. formosanus*; they usually are much more abundant than the corresponding *n*-alkane of the same chain length. No single-component 5- or 7-monomethyalkanes are present in *C. formosanus*. However, significant quantities ($>0.5\%$ total hydrocarbon) of other internally branched monomethylalkanes occur in this species. The most abundant hydrocarbon component in the cuticular wax of *C. formosanus* is peak 9, a mixture of 9-, 11-, 13-methylheptacosane. Only two dimethylalkanes are produced by *C. formosanus*: 9,13-dimethylheptacosane and 13,15-dimethylnonacosane. The former coelutes with 2-methylheptacosane in

TABLE 1. PERCENT COMPOSITION OF HYDROCARBONS FROM WORKERS AND SOLDIERS OF *Coptotermes formosanus* SHIRAKI FROM FOUR DISJUNCT LOCATIONS IN THE UNITED STATES[a]

		Workers				Soldiers	
Peak[b]	Hydrocarbon[c]	Hallandale (N = 7)	New Orleans (N = 5)	Lake Charles (N = 9)	Honolulu (N = 7)	Lake Charles (N = 10)	Honolulu (N = 4)
1	*n*-C25	0.9 (0.3)	1.6 (0.3)	1.2 (0.4)	0.9 (0.1)	1.6 (0.5)	1.7 (0.9)
2	9-, 11-MeC25[d]	0.4 (0.4)	0.6 (0.3)	1.5 (0.2)	0.3 (0.4)	1.1 (0.5)	0.0 (0.0)
3	2-MeC25	8.6 (0.8)	7.8 (0.9)	14.7 (1.9)	8.3 (1.7)	8.5 (1.6)	4.0 (2.4)
4	3-MeC25	0.4 (0.3)	0.6 (0.1)	2.4 (0.5)	0.5 (0.3)	1.8 (0.5)	0.2 (0.3)
5	*n*-C26	1.0 (0.2)	2.1 (0.3)	1.2 (0.3)	1.2 (0.6)	2.1 (0.4)	3.1 (1.2)
6	9-, 11-, 13-MeC26[d]	1.3 (0.1)	1.3 (0.2)	1.5 (0.1)	0.8 (0.5)	1.2 (0.7)	0.1 (0.3)
7	2-MeC26	3.3 (0.4)	4.0 (0.4)	4.6 (0.6)	4.1 (0.6)	3.1 (0.4)	2.5 (0.8)
8	*n*-C27	4.9 (0.9)	5.9 (1.3)	4.1 (0.9)	8.9 (3.7)	11.0 (3.7)	27.7 (13.9)
9	9-, 11-, 13-MeC27[d]	31.7 (1.9)	29.8 (3.6)	28.5 (2.7)	27.1 (2.9)	25.4 (3.6)	17.9 (3.9)
10	2-MeC27 + 9,13-DimeC27	22.9 (1.4)	22.2 (2.1)	16.9 (1.6)	23.8 (2.0)	16.2 (2.6)	18.3 (6.2)
11	3-MeC27	6.5 (0.4)	6.8 (0.6)	7.8 (0.9)	7.2 (0.4)	8.9 (1.5)	6.4 (1.9)
12	*n*-C28	0.9 (0.5)	1.7 (0.2)	0.4 (0.2)	1.2 (0.6)	0.9 (0.5)	2.9 (0.9)
13	9-, 11-, 13-, 15-MeC28[d]	3.3 (0.3)	3.1 (0.3)	3.0 (0.4)	2.9 (0.2)	3.1 (0.8)	2.1 (1.2)
14	9-, 11-, 13-, 15-MeC29 + 13,15-DimeC31[d,e]	13.8 (0.7)	12.5 (0.9)	12.3 (2.0)	12.8 (2.3)	15.0 (5.5)	12.9 (1.9)

[a] Hydrocarbons are quantified as the mean percent (standard deviation) of the total hydrocarbon component.
[b] Peak numbers refer to peaks identified in Figure 1.
[c] This shorthand uses a descriptor for the location of the methyl group (X-Me) and the total number of carbons (CXX) in the hydrocarbon component, excluding methyl branch(es).
[d] A mixture of positional isomers; two or more components coelute in this peak.
[e] Because of incomplete separation of peaks 14 and 15 during routine quantification of components, both peaks are included as one value.

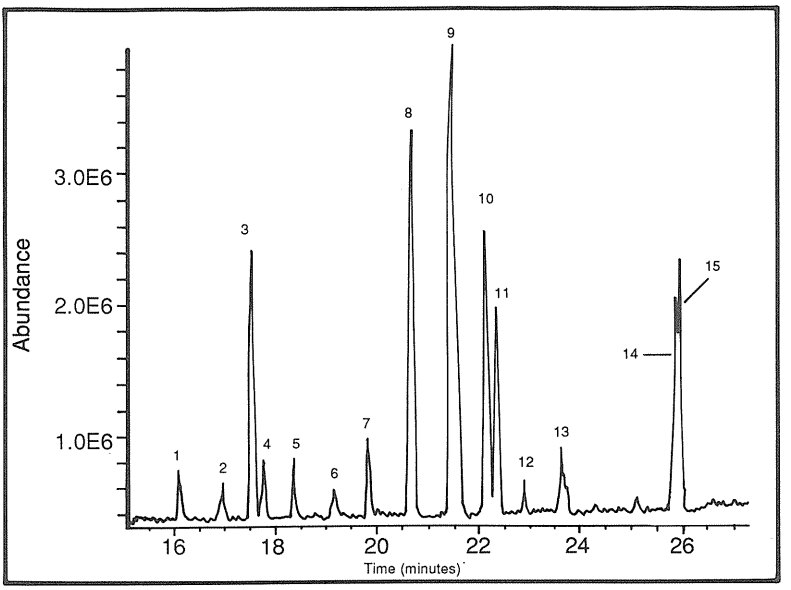


FIG. 1. Total ion chromatogram of the cuticular hydrocarbons from *Coptotermes formosanus* Shiraki collected from Honolulu, Hawaii. Numbers identify peaks listed in Tables 1 and 2.

peak 10 and occurs as a single isomer. The latter elutes as a shoulder of the 9-, 11-, 13-methylnoncosane peak (Figure 1).

The mass spectrum of peak 15 (Figure 2) is interpreted as arising from 13,15-dimethylnonacosane. Hydrocarbons with the methyl branches separated by a single methylene group are not common in insect hydrocarbons (Blomquist et al., 1987). The M-15 ion at *m/z* 421 indicates a hydrocarbon with 31 carbons. The larger fragments, which have a high odd/even ratio at *m/z* 267/266 and 239/238, are consistent with cleavage external to the two branching methyl groups (Figure 2). The fragments at *m/z* 224/225 and 196/197 have an even-to-odd ratio greater than one, consistent with cleavage internal to each methyl branch. The observed equivalent chain length (ECL) of this component (29.47) is slightly less than the ECL of this compound reported from *Solenopsis invicta* Buren (Nelson et al., 1980). However, dimethylalkanes with one methylene group separating the methyl substituents are known to produce diastereoisomers with different elution times yet nearly identical mass spectra (Pomonis et al., 1980). We examined a synthesized standard, 9,11-dimethylheptacosane, provided by J.G. Pomonis. On our system, two separate peaks eluted from this standard with ECLs of 27.4 and 27.7. The mass spectra of these two peaks

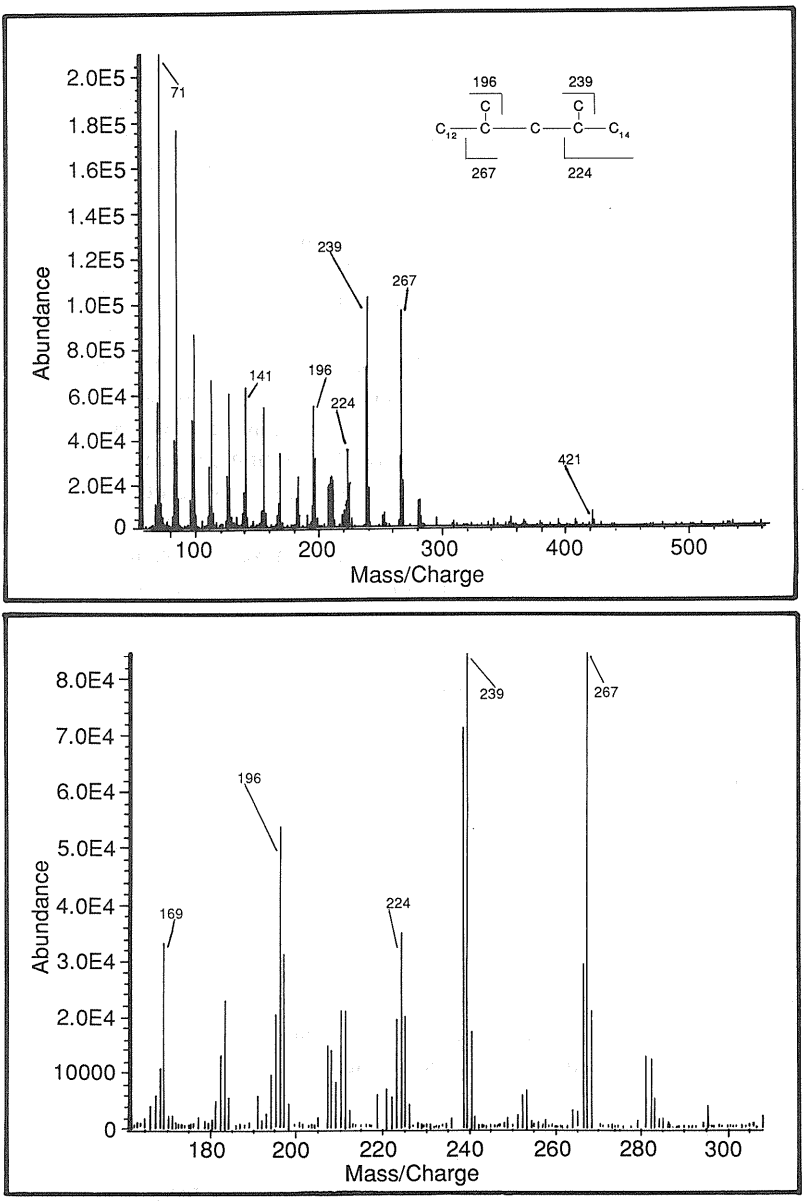


FIG. 2. EI mass spectrum of peak 15, Figure 1, identified as 13,15-dimethylnonacosane.

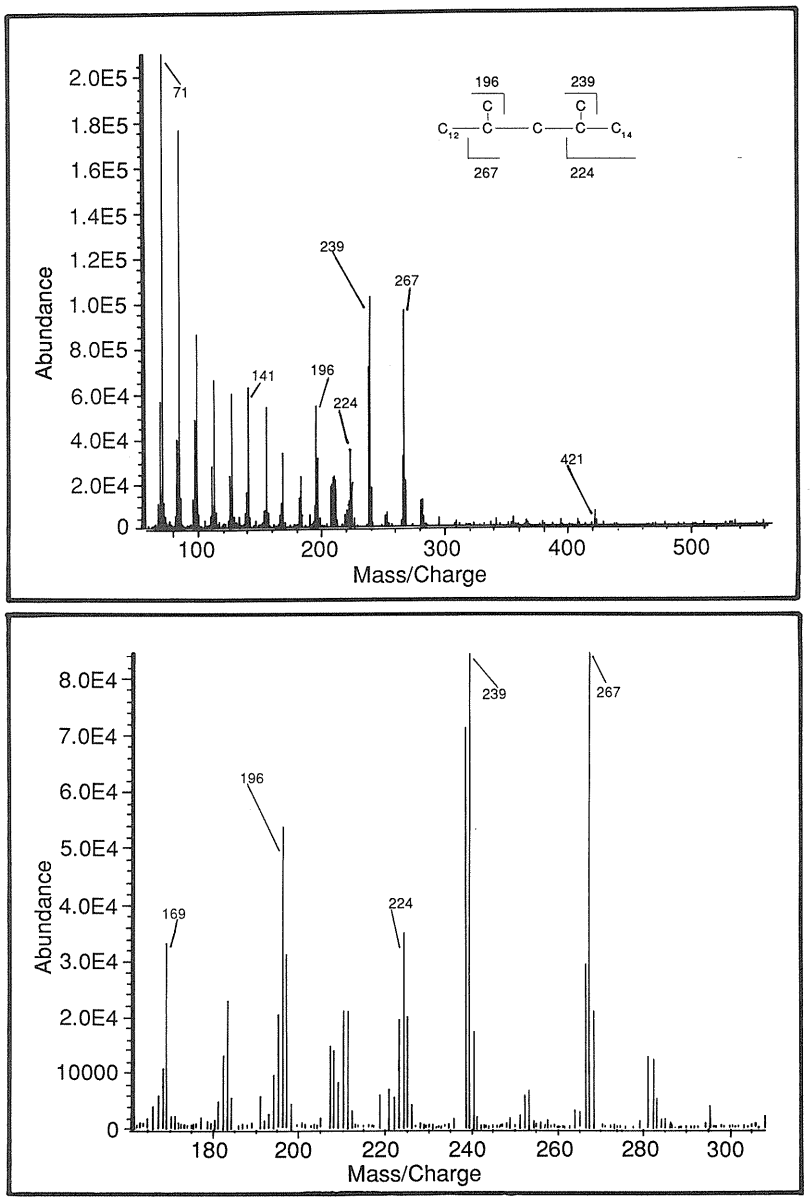
were identical. Insects apparently make one or the other diastereomer: *C. formosanus* makes the early-eluting compound (confirmation provided by D.R. Nelson and J.G. Pomonis).

The cuticular hydrocarbons of *C. formosanus*, as is the case for many insect species, consist of complex mixtures of straight-chain and methyl-branched saturated components (Blomquist et al., 1987). The relatively large number of potential components, ease of analysis, and species-specific compositions make them attractive characters for use in chemotaxonomy (Carlson and Bolten, 1984; Carlson and Brenner, 1988; Gastner and Nation, 1986; Haverty et al., 1988; Howard et al., 1988; Lockey, 1982; Page et al., 1990; Vander Meer, 1986).

Description of the cuticular hydrocarbons of other species of *Coptotermes* would be a logical extension of this work, but our goal in this study was not to test the taxonomic status of the introductions of *C. formosanus* into the continental United States. Rather, we examine the potential for using this set of quantitative chemical characters to determine the similarity between the hydrocarbon profiles within and among the sampled geographic locations. Our underlying assumption is 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.

Discriminant analysis of the proportions of the cuticular hydrocarbons of workers identified seven hydrocarbon components that reasonably separate the four populations: peaks 2–8 (Table 1, Figure 1). For the two locations for which we had soldiers, only three hydrocarbon components were necessary: peaks 3, 4, and 14. With these hydrocarbon variables, we were able to display the differences with plots of hydrocarbon proportions along two axes of canonical discriminant space (Figure 3) and to test the similarity between locations. Statistical analysis of Mahalanobis distances for all possible comparisons of workers from these four geographic locations indicates that all distances are statistically different from zero (Table 2). The same is true of the distance of hydrocarbon profiles of soldiers from Lake Charles and Hallandale; the estimated Mahalanobis distance is 6.0 and is statistically different from zero at the 0.0001 level. In other words, 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. Only slight overlap occurred between the Hallandale and Honolulu populations using just the first two canonical variates.

Carlson and Brenner (1988) used GC-MS followed by principal component analysis to identify and analyze the cuticular hydrocarbons of *Blattella germanica* (L.) from several distant geographical locations and from museum specimens. Their primary objective was to develop a technique for identifying immatures of the closely related species, *B. germanica* and *B. asahinai* Mizukubo, for which no diagnostic morphological characters exist. For *B. german-*



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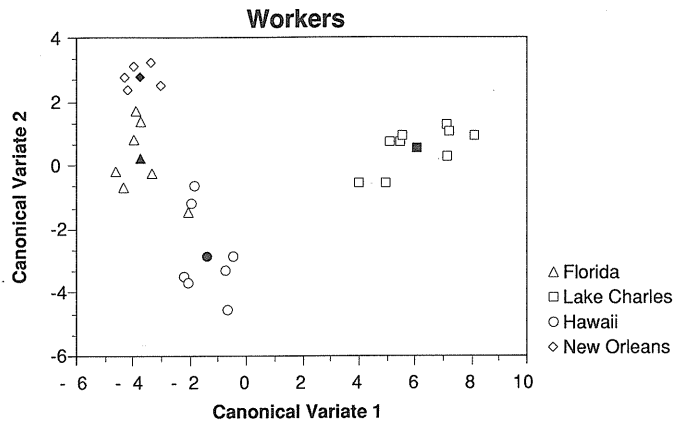

FIG. 3. Plot of colonies of *C. formosanus* from four different locations along two axes of canonical discriminant space. Open characters represent actual data points; closed characters represent the mean value for the population.

ica, they found "no appreciable difference . . . in patterns of individuals from the populations examined. . . ." The chromatographic patterns of field-collected specimens from Washington, D.C., Alaska, Florida, and other Gulf Coast states were similar to those of New Caledonia from museum collections. Thus for *B. germanica*, analysis of concentrations of cuticular hydrocarbons does not appear to be useful for determining the origin of populations, at least from the sample reported by Carlson and Brenner (1988).

This is the first use of cuticular hydrocarbons to ascertain similarities or differences between populations (in our case collections of separate colonies) of the same species from disjunct geographic locations. From our previous

TABLE 2. MAHALANOBIS DISTANCE BETWEEN POPULATIONS OF WORKERS OF *Coptotermes formosanus* SHIRAKI FROM FOUR DISJUNCT LOCATIONS IN THE UNITED STATES[a]

Location	Honolulu	Lake Charles	New Orleans
Hallandale	4.64	9.92	4.19
Honolulu		8.29	6.11
Lake Charles			10.22

[a] Probabilities of Mahalanobis distance test the difference between each value and zero. All distances in this table are statistically greater than zero at the 0.005 level.

experience (Haverty et al., 1988), we know that within a species or hydrocarbon phenotype of the dampwood termites, *Zootermopsis* (Hagen), there are statistically significant differences between colonies or castes for many of the hydrocarbon components in each species' profile. No single component, however, can be used to separate all four of the geographic locations of *C. formosanus*. Stepwise discriminant analysis allows us to use a selection of the hydrocarbon components to separate these geographic locations.

We believe that the utility of cuticular hydrocarbons for determining similarity of insect populations has promise. Clearly, the population from Lake Charles is very different from those from the other locations. This would support 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. These "invasions" likely originated from elsewhere in the Pacific Theater. The affinity of the infestation from Hallandale, Florida, is least clear. The hydrocarbon profile is significantly different from both Honolulu and New Orleans. Given that the site of this infestation is in an area of eastern Florida frequented by large, ocean-going pleasure craft, either origin is likely. 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.

This study points to the need for additional information and sampling. We need to analyze the variation in hydrocarbon components of *C. formosanus* over a greater geographical range. The samples used in this study are from relatively small areas within their respective geographic locations. This cannot be helped in Hallandale or Lake Charles because of the restricted nature of the local distribution. Similarity within a geographic area may, in fact, simply represent a founder event. A much more dispersed sample could be taken in Hawaii, all over the island of Oahu and other locations on the neighboring islands, where *C. formosanus* is now established (Tamashiro et al., 1987). Such a biogeographical study would greatly assist the evaluation of hydrocarbon profiles as an indicator of the origin of *C. formosanus* populations.

We cannot yet rule out that the differences in cuticular hydrocarbon profiles among the four populations of *C. formosanus* may be due to diet (Prestwich, 1983). Hydrocarbons are almost exclusively manufactured by insects (Blomquist et al., 1987); however, we do not know how food source affects biosynthesis of the various components in cuticular hydrocarbons or if differences in concentration may result. The insects from Lake Charles were collected from the interior of bald cypress, *Taxodium distichum* Rich., whereas those from New Orleans came from a variety of living trees (see La Fage, 1987). The colonies from Hallandale and Honolulu were collected from traps in the soil

and could have been feeding on numerous structural materials as well as other vegetative sources.

An increasing body of knowledge is accumulating that indicates hydrocarbon profiles are species-specific (Carlson and Brenner, 1988; Howard et al., 1982, 1988; Haverty et al., 1988; Page et al., 1990; Vander Meer, 1986). Qualitative differences in cuticular hydrocarbons have led us and our colleagues to identify a new subspecies, or possibly species, of *Zootermopsis* (Haverty and Thorne, 1989) and to find a morphological character for unequivocal identification of the three extant species of *Zootermopsis* (Thorne and Haverty, 1989). Cuticular hydrocarbons will help us corroborate or contradict current taxonomy based on the morphology and feeding behavior of the refractory genus of cone beetles, *Conophthorus* Hopkins (Page et al., 1990). In this paper we have introduced yet another biologically meaningful use of cuticular hydrocarbon data. We anticipate that the combination of GC-MS with discriminant or cluster analysis followed by canonical discriminant analysis will prove more powerful and precise than GC-MS analyses alone in resolving statistical comparisons of proportions or ratios of pairs of individual hydrocarbon components (Carlson and Bolten, 1984; Carlson and Service, 1980; Howard et al., 1988). Such clustering techniques will assist in separating closely related populations within a species or closely related and morphologically indistinguishable species when no qualitative differences in hydrocarbon profiles are apparent (Carlson and Brenner, 1988; Howard et al., 1988).

Thus far, isozyme analysis has provided limited information on the relatedness of *C. formosanus* populations in Florida, Louisiana, and Hawaii (A.K. Korman, personal communication). Additional potential methods of examining similarity among populations would be to decipher the genetic code (DNA hybridization) or to characterize end products of the genetic code, such as morphology, cuticular hydrocarbons, and agonistic behavior (Su and Haverty, in preparation). From one or more of these methods we should be able to establish relationships among populations of *C. formosanus* throughout its distribution. Future studies will involve further characterization of the cuticular hydrocarbons of additional collections of *C. formosanus*, and additional species within this genus, to evaluate quantitatively intra- and interspecific variation in hydrocarbon patterns. It is hoped, further study will clarify and/or validate the use of cuticular hydrocarbons for separating species or determining the origin of introduced populations of the Formosan subterranean termite.

Acknowledgments—The authors are extremely thankful to A.K. Korman, N.-Y. Su, R. Yamamoto, and J.P. La Fage who supplied the collections of *C. formosanus*. We appreciate the assistance given by J.A. Baldwin in helping us understand the multivariate analyses that were the key to this research project. J.G. Pomonis, D.R. Nelson, and G.J. Blomquist concurred in our diagnosis of peak 15 based on their independent evaluation of the mass spectrum. G.J. Blomquist

and B.L. Thorne provided helpful reviews of early versions of the manuscript. Their suggestions greatly improved the manuscript.

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