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INTERCASTE, INTERCOLONY, AND TEMPORAL VARIATION IN CUTICULAR HYDROCARBONS OF *Coptotermes formosanus* SHIRAKI (ISOPTERA: RHINOTERMITIDAE)

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Abstract—We characterized the variation in cuticular hydrocarbon mixtures between seven colonies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, from the same population. We report differences between castes, between colonies, and within the population over time to assess seasonality. Colonies of *C. formosanus* from Oahu, Hawaii, were sampled for 25 months. Each month, one sample each of 200 workers, 50 soldiers, nymphs, or alates from each colony was subjected to gas chromatography-mass spectrometry (GC-MS) analysis of the cuticular hydrocarbons. We resolved 39 individual peaks and identified 52 individual or isomeric mixtures of hydrocarbons. Only *n*-alkanes and methyl-branched alkanes occur; no olefins were found. Internally branched monomethylalkanes were the most abundant class of hydrocarbons, representing 45% to 50% of the total. 9-;11-;13-Methylheptacosane accounted for over 30% of the total hydrocarbon for all castes. 2-Methyl- and 3-methylalkanes comprise approximately 30% of the total. Internally branched dimethylalkanes constitute 15% to 20% of the total cuticular hydrocarbon. Only one trimethylalkane, 13,15,17-trimethylnonacosane, was found in small amounts. The hydrocarbon mixtures of all four castes were similar. Quantitative differences in hydrocarbon mixtures among the castes were easily displayed using canonical discriminant analysis. Soldiers and workers are significantly different from one another and from nymphs and alates. Nineteen peaks are statistically significant between workers and soldiers. Nymphs and alates were not statistically different. We detected statis-

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tically significant quantitative differences between colonies in 18 peaks for workers and 12 peaks for soldiers. Each of the colonies of *C. formosanus* can be separated from the others by the proportions of their hydrocarbon components. We detected statistically significant differences between months of the year for 12 peaks for workers and four peaks for soldiers; two peaks each for workers and soldiers showed distinct, seasonal trends. This seasonal shift in proportions of hydrocarbons correlates with the production of alates.

Key Words—Formosan subterranean termite, *Coptotermes formosanus*, founder event, biogeography, populations, seasonal variation, Isoptera, Rhinotermitidae, cuticular hydrocarbons, Hawaiian insects.

INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is one of the most economically important subterranean termites species in the world and is a serious threat to wood in structures wherever it occurs (Su and Scheffrahn, 1990). Even though the common name implies that this species is from Formosa (Taiwan), Kistner (1985) considered *C. formosanus* to be indigenous to mainland China on the basis of its associated termitophiles. This species was introduced into Japan prior to 1600 and was first collected in Hawaii in 1907 (Swezey, 1914), although it may have been present as early as 1869 (Tamashiro et al., 1987). Since its introduction into Hawaii, *C. formosanus* has been reported to be established in Guam, Midway Island, Sri Lanka, Taiwan, South Africa, and the mainland United States (Su and Tamashiro, 1987). Morphological examination and characterization of the cuticular hydrocarbons of recent collections of *Coptotermes* from Guam, however, indicate that a species other than *C. formosanus* is present there (Su, 1994; Su and Scheffrahn, 1996; Haverty, unpublished observations). It is possible that two or more species of *Coptotermes* occur on Guam.

Infestations of *C. formosanus* were first noticed in the continental United States about 1965. It is likely, however, that colonies of *C. formosanus* were established soon after the end of World War II. Storage of *C. formosanus*-infested military goods at facilities near port cities of the United States has been implicated as the means of establishment of *C. formosanus* in North America (Beal, 1987). Once *C. formosanus* was firmly settled in locations such as New Orleans, Louisiana; Galveston, Texas; and Charleston, South Carolina, it began to appear in other port cities in the southeastern United States including Baton Rouge, Louisiana, and Hallandale, Florida (La Fage, 1987).

The analysis of cuticular hydrocarbon components of *C. formosanus* indicates that this species is separable from other species of *Coptotermes* characterized thus far on the basis of qualitative differences in cuticular hydrocarbons (Brown et al., 1990, 1994; Haverty et al., 1991, 1992). Quantitative differences

in cuticular hydrocarbons have been used to separate geographic populations of *C. formosanus* (Haverty et al., 1990a). Analysis of the cuticular hydrocarbons of workers from several colonies each from Hallandale, Florida; New Orleans, Louisiana; Lake Charles, Louisiana; and Honolulu, Hawaii suggests that *C. formosanus* was introduced into the United States numerous times (Haverty et al., 1990a). Populations from two geographically proximate locations, New Orleans and Lake Charles, displayed quantitatively different hydrocarbon mixtures, which strongly supports the hypothesis that at least two separate introductions were made into the state of Louisiana (Haverty et al., 1990a; La Fage, 1987). Since 1980, indications of established infestations in inland areas such as Auburn, Alabama; Meridian, Mississippi; Orlando, Florida; Atlanta, Georgia; and La Mesa, California, suggest this species may now be transported in infested wood products or potted plants via domestic commerce (Atkinson et al., 1993; Chambers et al., 1988; La Fage, 1987; Sponsler et al., 1988).

Allozyme electrophoresis has not yet proven to be a useful technique for investigating the distribution of *C. formosanus* populations in the United States. Korman and Pashley (1991) used standard starch gel electrophoresis to examine allozymic differentiation in colonies of *C. formosanus* from four populations (New Orleans, Lake Charles, Hallandale, and Honolulu). Very little variation was found. They found only limited differentiation in allele frequencies between populations, but concluded that mainland populations did not result from a single introduction.

Strong and Grace (1993) surveyed 13 colonies of *C. formosanus* in Hawaii and resolved 29 protein loci using cellulose acetate gel electrophoresis. Eight of these loci proved to be stable, but were found to be monomorphic across all colonies. Subsequently, preliminary results from starch gel electrophoresis confirmed the observation by Korman and Pashley (1991) of a low level of polymorphism at the glucose phosphate isomerase (Gpi) locus (Strong and Grace, 1993). Neither Korman and Pashley (1991) nor Strong and Grace (1993) were able to separate colonies or populations (several colonies from the same area) of *C. formosanus* by analysis of allozymes.

Similar conclusions were reached by Broughton and Grace (1994) when they surveyed mitochondrial DNA restriction sites from 10 colonies of *C. formosanus* from Hawaii. They discovered that there was no variation in mitochondrial DNA in any of the colonies they examined. Their results corroborate the findings of Korman and Pashley (1991) and Strong and Grace (1993) that *C. formosanus* populations in Hawaii, and on the U.S. mainland, have a very low level of genetic polymorphism, and suggest that this species was depauperate of genetic variation in comparison to the approximate 40% polymorphic loci for the Class Insecta as a whole (Nevo 1978). The survey of these geographically distinct colonies of *C. formosanus* on the Hawaiian islands of Oahu and Maui by Strong and Grace (1993) and Broughton and Grace (1994) raise

the possibility that the extremely low level of allozyme and mitochondrial DNA variation among these colonies was indicative of a "founder" event.

While the possibility of a single founder event in Hawaii is intriguing, resolution of this question by genetic techniques has thus far proven difficult. Since quantitative differences in cuticular hydrocarbons have been detected among geographically disparate populations of *C. formosanus* (Haverty et al., 1990a; Su and Haverty, 1991), we proposed to test whether resolution of quantitative differences in cuticular hydrocarbon mixtures of different colonies of *C. formosanus* within the same population or geographic area was possible. We report here the results of a study to characterize the variation of cuticular hydrocarbons of different colonies of *C. formosanus* from the same population (all from the island of Oahu). We report differences between castes, between colonies, and within the population over time to assess seasonality. This examination of the intrinsic variation in cuticular hydrocarbons will serve as the basis for developing optimal sampling regimes for further examination of variation within the species and assessment of potential founder events around the world.

METHODS AND MATERIALS

Collection of Termites. Samples of *C. formosanus* were collected monthly for 25 months (September 1990 through September 1992) from seven colonies from four separate locations on the island of Oahu, Hawaii. Four colonies were on the campus of the University of Hawaii: two adjacent to Gilmore Hall (Upper Gilmore and Lower Gilmore), and one each adjacent to Pope Hall and the former location of the Publication Building. One colony each was located in Kaneohe, on the Waipio Peninsula, and at the Poamoho Experiment Station of the University of Hawaii. The colony at the Poamoho Experiment Station is not a natural colony; it was derived from an incipient colony of unknown laboratory stock and was placed on the site in 1979 by Dr. Minoru Tamashiro [see Strong and Grace (1993) for details]. Samples were collected from each colony using the trapping technique developed by Tamashiro et al. (1973).

Each month termites from each colony were separated from wood, carton, soil, and other debris and one sample each of 200 workers, 50 soldiers, and up to 50 of nymphs or alates were placed in Petri dishes and dried in a desiccator with Drierite®. Termites were dried while alive, until they died, and then for an additional five days. We found that killing termites before drying them in ambient, laboratory air often resulted in decomposition of the termites. Concurrently, each month two voucher samples from each colony consisting of two or three individuals from each caste present were preserved in 80% ethanol; one sample was kept at the University of Hawaii and one kept at the Pacific

Southwest Research Station in Albany, California. About every three to four months, accumulated dried samples were shipped to California for characterization of the cuticular hydrocarbons. Dried samples were held at ambient temperature in Hawaii and California until they were extracted.

Hydrocarbons of C. formosanus. Detailed descriptions of extraction procedures for specific termite species have been previously published elsewhere (Blomquist et al., 1979; Howard et al., 1978, 1982, 1988; Haverty et al., 1988, 1990a,b). In our laboratory in California, dried termite samples were transferred to clean 20-ml vials and immersed in 10 ml of *n*-hexane for ten min. The resulting extract of cuticular lipids was pipetted through four cm of activated Biosil A in a Pasteur pipette minicolumn to isolate the hydrocarbon components. The hydrocarbon extract was then evaporated to dryness under nitrogen and redissolved in 60 μl of hexane.

Gas chromatography-mass spectrometry (GC-MS) analyses of the hydrocarbons were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a capillary column (25 m $\times$ 0.2 mm ID, HP-1) and a Hewlett-Packard 5970B Mass Selective Detector. The system was interfaced with HP Chemstation software for data acquisition and analysis. Helium was the carrier gas, column flow was 2 ml/min, and the GC-MS was operated in the split mode with a ratio of 7:1. The GC was temperature programmed from 200° to 320°C at 3°C/min with a final isothermal hold of 16 min. Electron impact (EI) mass spectra were obtained at 70 eV, and scanning was done from 55 to 640 mass units every 1.0 second.

Quantification of individual hydrocarbon peaks was done by integrating the total ion chromatogram resulting from summation of mass ion abundances. Because of the large number of peaks representing co-eluting compounds and the necessity of checking these peaks for the presence of all compounds, all samples were analyzed and quantified this way. Past analyses using a flame ionization detector, rather than a mass selective detector, gave poor resolution of later-eluting peaks (see Haverty et al., 1990a) and important information may have been lost regarding high molecular weight hydrocarbons (C37 and higher).

n-Alkanes and methyl-branched alkanes were identified by their mass spectral fragmentation patterns (Blomquist et al., 1987; Nelson et al., 1980; Pomonis et al., 1980) and corroborated by calculating equivalent chain lengths (ECL) (Nelson and Sukkestad, 1970; Jackson and Blomquist, 1976). Mass spectra of dimethylalkanes were interpreted as described in Page et al. (1990b) and Nelson et al. (1980). Mass spectra of a trimethylalkane was interpreted as described by Page et al. (1990a).

In the text and tables, shorthand nomenclature has been used to identify individual hydrocarbons or mixtures of hydrocarbons. For example, heptacosane becomes *n*-C27; 3-methylheptacosane becomes 3-MeC27; 3,15-dimethyl-

heptacosane becomes 3,15-DimeC27, and 13,15,17-trimethylnonacosane becomes 13,15,17-TrimeC29. Hydrocarbons are presented in the table in the order of elution on our GC/MS system.

GC-MS peak areas were expressed as percentages of the total hydrocarbon fraction. The response variable for all statistical tests was the percentage of each individual peak, which may represent two or more co-eluting compounds (see Table 1). Summary statistics (mean and standard deviation of the percent of each peak or hydrocarbon component) were calculated for each caste combining all colonies and collection dates.

Intercaste, Intercolony, and Seasonal Variation in Hydrocarbons. We examined different transformations of the percentages of the hydrocarbons; none provided uniform improvement in skewness or kurtosis. As a result, all statistical treatments of data to assess intercaste, intercolony, and seasonal variation were conducted with actual percentages.

Differences between workers and soldiers were determined by a *t*-test of the percentage of each peak (or hydrocarbon component) with colonies (summed over all 25 collection periods) representing the replicates (i.e., $N = 7$ for each caste). Differences between colonies or months of the year were determined by analysis of variance of the percentage of each peak, separately for workers and soldiers. Statistical differences were tested to maintain an overall $\alpha = 0.05$ for the analysis of each of the 39 hydrocarbon peaks. Thus for each *t*-test or analysis of variance, the significance of the *t* or F statistic was tested at the $\alpha = 0.001$ level ($\alpha = 0.05/39$, where 39 is the number of statistical analyses conducted, one for each of the 39 hydrocarbon peaks).

Differences of hydrocarbon patterns between castes and between colonies were revealed by canonical discriminant analysis to provide two-dimensional displays of the chosen variables. Canonical discriminant analysis is a dimension-reduction technique related to principal component analysis and canonical correlation that derives a linear combination of quantitative variables (in this case, the percentage of hydrocarbon components) that summarizes between-class variation. The procedure that we used computes and tests Mahalanobis distances for pairwise comparisons of the colonies. Statistical analyses were accomplished with the TTEST, ANOVA, and CANDISC procedures of SAS System Release 6.08 (SAS 1990).

RESULTS AND DISCUSSION

Hydrocarbons of C. formosanus. Improvements in chromatographic equipment (replacing a flame ionization detector with a mass selective detector) and extraction technique (drying specimens before extraction with hexane and increasing the sample size to 200 individuals) allowed us to improve earlier

TABLE 1. HYDROCARBON MIXTURES (MEAN AND STANDARD DEVIATION) OF WORKERS, *SOLDIERS*, NYMPHS, AND ALATES OF *Coptotermes formosanus* FROM SEVEN COLONIES FROM OAHU, HAWAII, COLLECTED OVER 25 CONSECUTIVE MONTHS

Peak	Hydrocarbon[a]	Worker[d,e]	Soldier[d,e]	Nymph[d]	Alate[d]
1	*n*-C25	0.79(0.69)[#]	1.02(1.35)	0.55(0.48)	0.62(0.36)
2	9-; 11-; 13-MeC25[b]	2.19(0.67)*[†]	1.24(0.41)*[†]	1.38(0.50)	1.36(0.41)
3	2-MeC25 + 3-MeC25[c]	9.97(2.23)[#]	8.48(1.81)[†]	6.86(1.71)	8.55(1.85)
4	*n*-C26	0.86(0.65)[#]	1.20(0.38)[#]	0.79(0.51)	0.91(0.35)
5	9-; 10-; 11-; 12-; 13-MeC26[b]	2.71(0.73)*	1.77(0.39)*	2.11(0.69)	2.40(0.69)
6	2-MeC26 + 3-MeC26[c]	3.68(0.86)*[#]	2.95(0.62)*	3.16(1.03)	3.72(0.90)
7	*n*-C27	2.42(0.89)*[#]	5.66(2.07)*[#†]	2.59(1.01)	3.23(0.65)
8	9-; 11-; 13-MeC27[b]	30.32(4.11)[†]	30.05(3.24)[#]	38.03(4.75)	37.54(6.26)
9	2-MeC27 + 9,13-DimeC27[c]	13.16(1.81)[#†]	15.25(2.61)[#]	15.79(2.27)	16.16(1.26)
10	3-MeC27	3.53(0.67)*	6.17(0.90)*[#]	3.54(0.77)	4.69(0.94)
11	*n*-C28	0.45(0.54)[#]	0.63(0.33)[#]	0.43(0.31)	0.41(0.16)
12	3,15-; 3,17-DimeC27[b]	0.45(0.19)*[#]	0.07(0.13)*	0.30(0.16)	0.40(0.12)
13	9-; 10-; 11-; 12-; 13-; 14-MeC28 + 12,14-DimeC28[b,c]	3.84(0.59)*[†]	3.21(0.52)*	4.53(0.61)	4.36(0.83)
14	2-MeC28 + 9,13-DimeC28[c]	0.47(0.14)[†]	0.29(0.21)[#]	0.59(0.21)	0.52(0.18)
15	3-MeC28	0.14(0.09)*[#]	0.03(0.08)*[#]	0.19(0.11)	0.16(0.12)
16	*n*-C29	0.23(0.38)[#]	0.21(0.24)[#]	0.19(0.22)	0.13(0.09)
17	9-; 11-; 13-; 15-MeC29[b] + 13,15-DimeC29[c]	13.32(1.72)[#]	13.30(1.67)[†]	14.47(3.24)	12.19(1.89)
18	2-MeC29 + 9,13-DimeC29[c]	0.56(0.22)*	0.23(0.26)*[#]	0.49(0.21)	0.29(0.13)
19	3-MeC29	0.47(0.16)[†]	0.33(0.26)[#]	0.61(0.22)	0.48(0.19)
20	13,15,17-TrimeC29	1.02(0.28)[#†]	0.85(0.35)	0.51(0.22)	0.50(0.14)
21	3,15-; 3,17-DimeC29 + 3,7-DimeC29[b]	0.24(0.10)*	0.01(0.03)*	0.16(0.10)	0.07(0.08)
22	14-; 15-MeC30[b] + 13,15-DimeC30[c]	0.37(0.11)*	0.15(0.18)*	0.33(0.15)	0.23(0.14)
23	13,15-; 15,17-DimeC31[b]	0.52(0.12)*[†]	0.27(0.18)*	0.28(0.14)	0.19(0.11)
24	11-; 13-; 15-; 17-MeC33[b]	0.20(0.10)*[#]	0.01(0.08)*	0.13(0.12)	0.02(0.03)
25	11-; 13-; 15-; 17-MeC35[b]	0.17(0.10)*	0.00(0.02)*	0.05(0.07)	0.00(0.00)
26	13-; 15-MeC37 + 15,17-DimeC37[c]	0.38(0.17)*[#]	0.11(0.16)*	0.04(0.06)	0.01(0.04)
27	13,17-; 15,19-DimeC37[b]	0.13(0.09)*[†]	0.01(0.06)*	0.00(0.00)	0.00(0.00)

TABLE 1. CONTINUED

Peak	Hydrocarbon[a]	Worker[d,e]	Soldier[d,e]	Nymph[d]	Alate[d]
28	14-; 15-; 16-MeC38[b]	0.09(0.09)*#	0.00(0.03)*	0.00(0.00)	0.00(0.00)
29	14,18-DimeC38	0.06(0.07)*#	0.00(0.03)*	0.00(0.00)	0.00(0.00)
30	13-; 15-MeC39[b] + 15,17-DimeC39[c]	0.96(0.45)#†	0.67(0.53)	0.16(0.12)	0.08(0.12)
31	13,17-; 15,19-DimeC39[b]	1.03(0.45)#†	0.66(0.55)#	0.17(0.12)	0.07(0.11)
32	13-; 14-; 15-; 16-; 17-; 18-MeC40[b]	0.23(0.18)†	0.12(0.21)	0.02(0.03)	0.00(0.00)
33	14,18-; 16,20-DimeC40[b]	0.22(0.22)*	0.08(0.24)*	0.02(0.03)	0.00(0.00)
34	13-;15-MeC41[b] + 15,17-DimeC41[c]	1.78(1.33)	1.90(1.33)	0.53(0.45)	0.25(0.23)
35	13,17-; 15,19-DimeC41[b]	1.82(1.27)	1.90(1.42)	0.60(1.01)	0.25(0.22)
36	15-; 16-; 17-MeC42[b]	0.22(0.30)	0.15(0.34)	0.05(0.05)	0.00(0.00)
37	14,18-DimeC42	0.09(0.20)	0.04(0.11)	0.02(0.03)	0.00(0.00)
38	13-; 15-MeC43[b] + 15,17-DimeC43[c]	0.70(0.81)	0.75(0.80)	0.25(0.18)	0.19(0.16)
39	13,17-DimeC43	0.22(0.36)	0.22(0.32)	0.06(0.07)	0.02(0.04)

[a] Carbon number is the total number of carbons in the parent chain, excluding methyl groups, e.g., 3-MeC25 = 3-methylpentacosane.
[b] An isomeric mixture. These components co-elute in this peak.
[c] Because of incomplete separation of these hydrocarbons both peaks are included as one value.
[d] Sample size = 175 for workers, 172 for soldiers, 42 for nymphs, and 9 for alates.
[e] Means (SD) for workers and soliders followed by "*" are significantly different at the $\alpha = 0.001$ level by *t*-test. Means followed by "#" represent significant differences among colonies for workers or soldiers, and means followed by "†" represent significant differences among collection months for workers or soldiers at the $\alpha = 0.001$ level by analysis of variance.

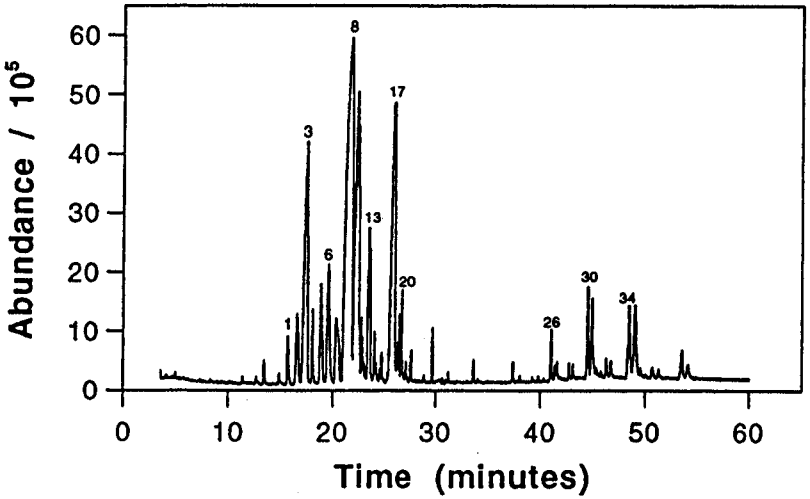

FIG. 1. Total ion chromatogram from 200 workers of *C. formosanus* from Pope Hall, University of Hawaii campus, Honolulu, Hawaii, in May 1992.

characterizations of the cuticular hydrocarbons of *C. formosanus* (Haverty et al., 1990a, 1991, 1992; McDaniel, 1990). We were able to resolve 39 individual peaks and characterize 52 individual or isomeric mixtures of hydrocarbons (Table 1 and Figure 1) from this species. Only *n*-alkanes and methyl-branched alkanes were found. *C. formosanus* does not make any olefins.

Normal alkanes present were *n*-C25, *n*-C26, *n*-C27, *n*-C28, and *n*-C29. Of the *n*-alkanes, *n*-C27 was the most abundant, comprising 2.4 and 5.7% of the total hydrocarbon for workers and soldiers, respectively, and intermediate amounts for nymphs and alates. The other *n*-alkanes generally represented no more than 1.2% each of the total hydrocarbons (Table 1).

Internally branched monomethylalkanes with methyl groups positioned at the nine carbon or greater elute about 0.7 carbon units in front of the *n*-alkane with the same number of total carbons. Their mass spectra have strong *m/z* ions representing cleavage internal to the methyl branch for which the ratio of even to odd is close to one (Blomquist et al., 1987). We identified isomeric mixtures of internally branched monomethylalkanes with parent carbon chains ranging from C25 to C43, except for C31, C32, C34, and C36. Positions of methyl branches ranged from carbon 9 to 18 (Table 1). Methyl branches located on even-numbered carbons were found only when the parent chain of the hydrocarbon had an even number of carbons, while branches on odd-numbered carbons were found to occur on hydrocarbons with either odd or even number of carbons in the parent chain. Internally branched monomethylalkanes were the

most abundant class of hydrocarbons produced by *C. formosanus*, representing roughly 45 to 50% of the total hydrocarbon. One isomeric mixture, 9-;11-;13-MeC27, accounted for over 30% of the total hydrocarbon for all castes surveyed (Table 1 and Figure 1).

2-Methylalkanes have an ECL of approximately 0.4 carbon units less than the corresponding *n*-alkane with the same number of total carbons and give a spectrum with a strong $(M-43)^+$ ion and a weaker $(M-15)^+$ ion. 3-Methylalkanes have an ECL of approximately 0.3 carbon units less than the corresponding *n*-alkane with the same number of total carbons and give a spectrum with a strong $(M-29)^+$ ion and a weaker $(M-57)^+$ ion. 2-Methyl- and 3-methylalkanes were identified for C25 to C29. In many cases the two peaks were not completely resolved on our system. These terminally branched monomethylalkanes comprise approximately 30% of the total hydrocarbons (Table 1 and Figure 1).

Internally branched dimethylalkanes constitute 15 to 20% of the total cuticular hydrocarbon fraction of *C. formosanus* (Table 1). There were two types of internally branched dimethylalkanes, those with either three methylene groups or those with one methylene group separating the methyl branches, representing roughly equal proportions of this class of compounds. Dimethylalkanes, such as 13,17-DimeC37 and 15,19-DimeC37, eluted about 1.4 carbon units before the corresponding *n*-alkane with the same number of total carbons, as did 14,18 and 16,20-dimethylalkanes. 9,13-Dimethylalkanes were present at C27, C28, and C29 and co-eluted with the 2-methylalkane of the same parent chain length.

Dimethylalkanes with one methylene group between methyl branches are not common in insects (Blomquist et al., 1987; Lockey, 1988), however, these compounds are fairly abundant in *C. formosanus* (Table 1). These alkanes have fragmentation patterns similar to other dimethylalkanes, except that an additional strong ion pair represents the daughter fragment formed by cleavage internal to the second methyl branch (Nelson et al., 1980). This ion pair has an even to odd ratio close to one. Another characteristic of this fragmentation is the relatively high intensity of the ion pairs representing the fragment ions formed by cleavage external to the second methyl branch, which have even to odd ratios of less than one (Nelson et al., 1980). The elution times for these compounds are 0.2 to 0.3 carbon units less than those of the other internally branched dimethylalkanes seen in this insect (Haverty et al., 1990a; Pomonis et al., 1980). Dimethylalkanes with one methylene spacing eluted slightly later than the monomethylalkane with the same parent chain length. Usually the two peaks did not fully resolve and thus were integrated as one peak. They occur together at C28, C29, C30, C37, C39, C41, and C43. The exception is the isomeric mixture 13,15-; 15,17-DimeC31, as no monomethyl alkane was identified at C31.

Terminally branched dimethylalkanes with the first branch on the 3-carbon had a strong $M-29^+$ ion and eluted about 1.0 carbon unit in front of the corresponding *n*-alkane with the same number of total carbons. These compounds

were not very abundant (less than 0.5 % of the total hydrocarbons) and occurred only at C27 and C29 (peaks 12 and 21, Table 1 and Figure 2). All were found to have the first methyl branch on the 3 carbon and the second on the 7, 15, or 17 carbon.

Only one trimethylalkane was identified: 13,15,17-TrimeC29. This compound was previously identified by McDaniel (1990). As in his study of *C. formosanus*, 13,15,17-TrimeC29 represented less than one percent of the total hydrocarbon. This is a symmetrical molecule with one methylene group separating each methyl branch, and it is not common in insects (Blomquist et al., 1987; Lockey, 1988). The ion patterns diagnostic for unusual, monomethylene interrupted spacing (1,1) were extrapolated from similar spectra for dimethylalkanes. The spectrum for 13,15,17-TrimeC29 is shown in Figure 2. The ion pair at 210/211 indicates a daughter fragment ion as described in the dimethyl section. The abundant ions at 238/239 and 280/281 represent fragment ions formed by cleavage external to the second and third methyl branches, respectively.

There were a few miscellaneous alkanes present in trace amounts that were inconsistently detected (i.e., 2-MeC24; 3,7-DimeC31) or that were in such small quantities as to be difficult to characterize and/or quantify. These compounds were disregarded for the quantitative purposes of this study.

Comparisons with Earlier Studies of Hydrocarbons of C. formosanus. The cuticular hydrocarbons of *C. formosanus* in the United States were previously characterized concurrently by Haverty et al. (1990a, 1992) and McDaniel (1990). McDaniel (1990) identified numerous mono- and dimethylalkanes, in trace amounts, in *C. formosanus* [see Table 1, McDaniel (1990)] that were not detected by Haverty et al. (1990a). McDaniel (1990) also identified the unique trimethylalkane, 13,15,17-TrimeC29, in trace amounts. In the study reported in this paper, we found and identified most of these compounds as well as numerous other hydrocarbons not seen by McDaniel (1990) or Haverty et al. (1990a, 1992). These include: *n*-C28, *n*-C29, 2-MeC28, 2-MeC29, 3-MeC28, 3-MeC29, 14-;15-MeC30, 14-;15-;16-MeC38, 13-;14-;15-;16-;17-;18-MeC40, 15-;16-;17-MeC42, 3,15-;3,17-DimeC27, 3,15-;3,17-DimeC29, 9,13-DimeC27, 9,13-DimeC28, 9,13-DimeC29, 12,14-;13,15-DimeC28, 13,15-DimeC29, 13,15-DimeC30, 13,15-;15,17-DimeC31, 15,17-DimeC37, 15,17-DimeC39, 15,17-DimeC41, 15,17-DimeC43, 14,18-DimeC38, 14,18-; 16,20-DimeC40, 14,18-DimeC42.

The possible reasons that these hydrocarbons were not detected in previous studies may be that sample sizes were inadequate [Haverty et al. (1990a) used only 50 to 100 workers or soldiers], or that the termites were processed when fresh or frozen rather than dried. Haverty et al. (1996) recently found that the extraction of hydrocarbons is more efficient if the specimens are dry rather than fresh or frozen. In our earliest investigations of hydrocarbons of *C. formosanus* (Haverty et al., 1990a), quantification was done using a flame ionization detec-

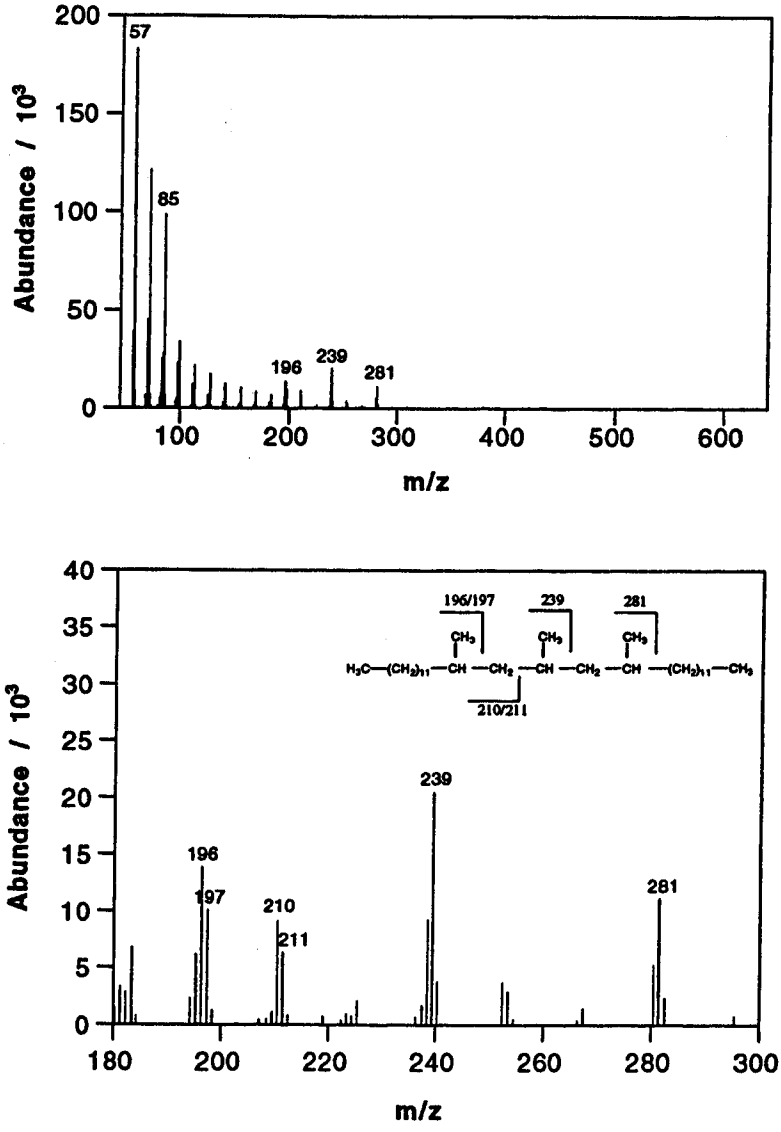

FIG. 2. Mass spectrum of 13,15,17-trimethylnonacosane from 200 workers of *C. formosanus* from Pope Hall, University of Hawaii campus, Honolulu, Hawaii, in September 1992.

tor; many of the smaller peaks, especially those at higher molecular weights (>C31), were not resolved. In addition, samples used for GS-MS analyses by Haverty et al. (1990a) were apparently not of sufficient concentration to detect and identify all of these peaks.

Intercaste Variation in Hydrocarbons. The cuticular hydrocarbon patterns of all four castes examined appear to be qualitatively quite similar. When we examined quantitative differences between workers and soldiers we found 19 of the 39 peaks to be statistically different at the $\alpha = 0.001$ level (Table 1). A few of these differences involve hydrocarbons that rarely were seen in the soldier samples, such as peaks 12, 15, 21, 24, 25, 27, 28, and 29 (Table 1). These hydrocarbons occur in workers in trace to slight amounts ($\leq 0.24\%$); in many worker samples these peaks were not resolved either. We suggest the absence of these peaks in soldier samples might be the result of a smaller sample size, resulting in less absolute hydrocarbon in the sample, i.e., the soldier samples were likely more dilute than those of the workers. Soldier samples included only 50 individuals whereas worker samples included 200 individuals. We did not, however, calculate differences in absolute quantities of hydrocarbons of any caste.

Nymphs and alates were collected on very few occasions compared to workers and soldiers. Because of the sporadic nature of these collections we did not compare them statistically to either workers or soldiers; nymphs and alates occurred seasonally and were not collected equally over the 25 collection periods. Many peaks in nymphs (27–29, 32, and 33) and alates (25–29, 31–33, 36, and 37) were seldom resolved and usually resulted in trace amounts. In some cases (peaks 27–29, 32–33, and 36–37) the hydrocarbons were never detected for alates and only occasionally for nymphs. These peaks that occur in trace amounts or are absent in nymphs and alates occurred in minute amounts ($<0.38\%$) in workers, with the exception of 13,17-; 15,19-DimeC39, which represented an average of 1.03% of the total hydrocarbon in workers. As was the case with soldiers, we attribute the absence of these peaks in nymph and alate samples to a smaller sample size, resulting in less absolute hydrocarbon in the sample. Nymph and alate samples included, at the most, 50 individuals whereas worker samples included 200 individuals.

Our sampling regime for this study was so extensive that the differences in hydrocarbon mixtures among the castes were easily displayed using canonical discriminant analysis (Figure 3). Soldiers and workers are statistically different from one another and from nymphs and alates at the $\alpha = 0.001$ level (Table 2). Nymphs and alates were not significantly different from one another at $\alpha = 0.001$.

Brown et al. (1996) found similar intercaste differences in *Drepanotermes perniger* (Froggatt) in Australia. All castes and stages were qualitatively similar

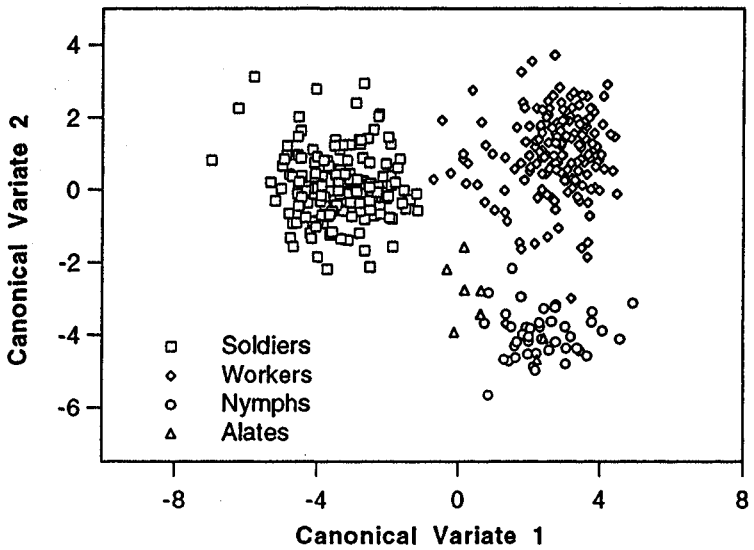

FIG. 3. Plot of four castes of *C. formosanus* from seven colonies from four locations in Oahu, Hawaii, along two axes of canonical discriminant space.

and quantitative differences were judged to be minor. The main differences were in amounts of 3-MeC27, I-MeC27, 3,X-DimeC25, and 3,X-DimeC27. Castes were easily separated by canonical variate analysis; the differences appear to diminish with age of the individuals.

Intercolony Variation in Hydrocarbons. We assessed differences between colonies separately for hydrocarbons of workers and soldiers by analysis of variance for each peak. We detected statistically significant differences (α = 0.001) between colonies in 18 peaks of workers and 12 peaks of soldiers. One of the goals of this research project was to separate or identify colonies on the

TABLE 2. MAHALANOBIS DISTANCE BETWEEN CASTES OF *Coptotermes formosanus* SHIRAKI FROM SEVEN COLONIES FROM FOUR LOCATIONS ON OAHU, HAWAII[a]

Caste	Worker	Nymph	Alate
Soldier	37.82†	50.07†	32.24†
Worker		25.11†	26.29†
Nymph			9.74

[a]Probabilities of Mahalanobis distance test the different between each value and zero. Distances with an '†' are statistically greater than zero at the α = 0.001 level.

basis of their hydrocarbon mixtures. Because there were so many statistically significant differences and they seldom involved the same hydrocarbons, we used all hydrocarbons in canonical discriminant analysis.

We were able to display differences between colonies with plots of hydrocarbon proportions along two axes of canonical discriminant space (Figure 4) and to test the similarity between colonies. Statistical analyses of Mahalanobis distances for all possible comparisons of workers or soldiers from these seven colonies indicate that all distances are statistically different from zero (Table 3). In other words, each of these seven colonies of *C. formosanus* can be separated from all the others on the basis of the concentrations of their cuticular hydrocarbon components.

Brown et al. (1996) used canonical variate analysis to separate geographic localities of *D. perniger*. Intercolony differences (from the same geographic location) were much smaller than geographic differences. As with intercaste differences, the absolute differences between geographic areas were small.

Seasonal Variation in Hydrocarbons. When we examined each of the 39 hydrocarbon values for the 25 collection periods, we obtained 30 statistically significant differences for workers and 24 for soldiers. Such a large number of statistically significant differences may simply be a result of differences between the extreme values (high and low) for the quantities of hydrocarbons over the 25-month period. It is quite possible that so many significant differences resulted from artifacts of technique. These problems with technique could involve slight differences in the way the termites were dried and/or held in our laboratories in Hawaii or California over the 25 months of this study. Also, the chromatographic sensitivity could very well have changed over that period of time. Both of these effects could have been operating concurrently. A more likely explanation is that there was, in fact, a seasonal effect.

To test the hypothesis of a seasonal effect, we combined our samples so that we were evaluating differences between months of the year rather than 25 separate, sequential collection periods. Thus, for each month of the year, we characterized the seven colonies twice, except for September; this month we characterized the seven colonies three times. In this way, we felt that we could determine any seasonal differences, not just chance statistically significant differences among the 25 collection periods, that were repeatable over the 2-year period.

We detected statistically significant differences ($\alpha = 0.001$) between months of the year for 12 hydrocarbon peaks of workers and 4 peaks from soldiers. We then visually examined the monthly trends of each of these statistically significant hydrocarbon peaks to determine whether the differences were due to a seasonal trend or simply due to extreme differences between the months with the most and least amount of the particular hydrocarbon peak. We found that two different hydrocarbon peaks each for workers and soldiers showed distinct

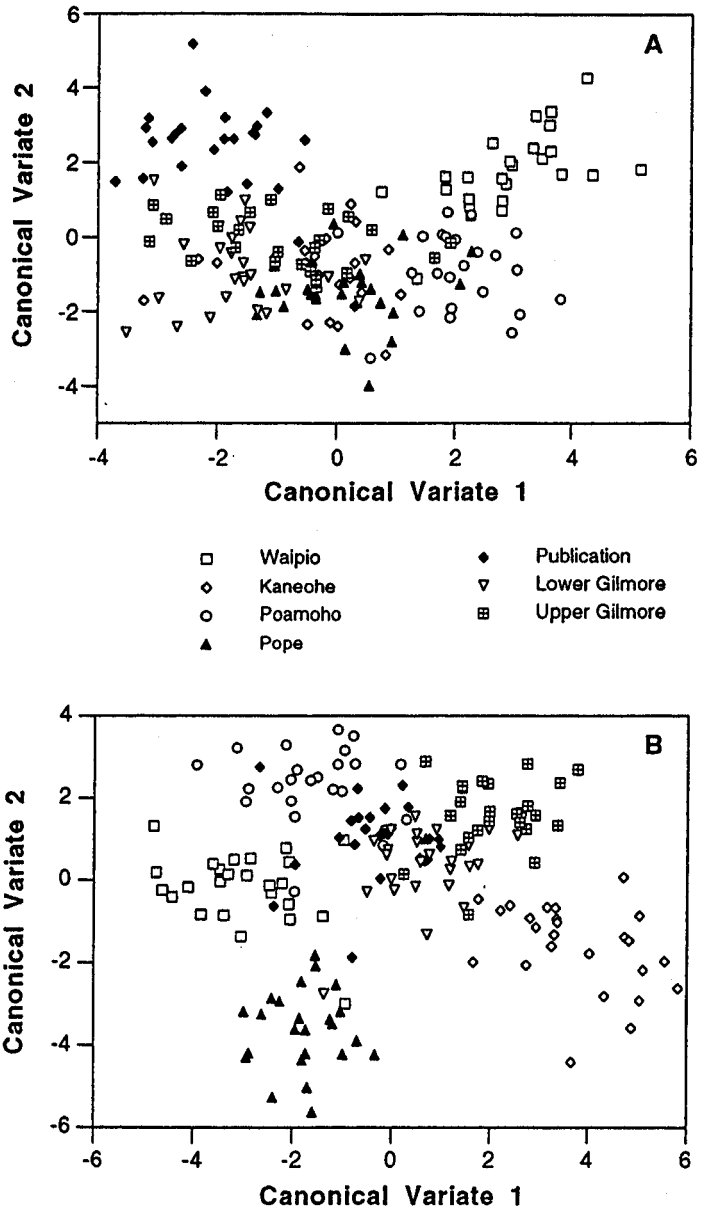

FIG. 4. Plot of seven colonies of *C. formosanus* from four locations in Oahu, Hawaii, along two axes of canonical discriminant space; A = workers, B = soldiers.

TABLE 3. MAHALANOBIS DISTANCE BETWEEN COLONIES, FOR BOTH WORKERS AND SOLDIERS, OF *Coptotermes formosanus* SHIRAKI FROM FOUR LOCATIONS ON OAHU, HAWAII[a]

Colony[b]	2	3	4	5	6	7
			Soldiers			
1	22.58	18.63	23.53	29.95	31.52	21.98
2		20.31	10.70	21.40	13.79	10.56
3			19.91	32.97	19.84	19.51
4				25.42	15.97	14.19
5					17.56	15.50
6						7.81†
			Workers			
1	54.91	25.93	26.64	33.78	24.50	36.73
2		47.09	41.19	38.39	25.52	27.47
3			38.86	23.08	23.06	30.77
4				33.64	25.18	45.57
5					18.28	31.15
6						10.96

[a] Probabilities of Mahalanobis distance test the difference between each value and zero. Distances with an '†' are **not** statistically greater than zero at the $\alpha = 0.001$ level.
[b] Colonies are as follows: 1 = Waipio, 2 = Kaneohe, 3 = Poamoho, 4 = Pope Hall, 5 = Publication City, 6 = Lower Gilmore, and 7 = Upper Gilmore.

seasonal trends (Figure 5). These trends were also obvious when colonies were examined separately for each of these hydrocarbon peaks. For these particular peaks, the trends were not an artifact of one or two colonies determining the average value for the month nor were they an artifact of technique. The differences were truly seasonal and were repeated from year to year. The statistically significant differences for the other peaks (10 for workers and 2 for soldiers) were due to differences between the extreme values; no seasonal trend was evident.

The biological significance of these trends is not obvious, but the major shifts in proportions for these hydrocarbon peaks correlate with the timing of the production of alates of *C. formosanus* on Oahu. Although alates of *C. formosanus* can be collected throughout the year, the major flights occur in May through July (Bess, 1970). This is almost exactly the same period (April through June) that alates were found in the colonies we sampled.

It seems reasonable to assume that these statistically significant shifts in amounts of hydrocarbon components are associated with the flight of the alates.

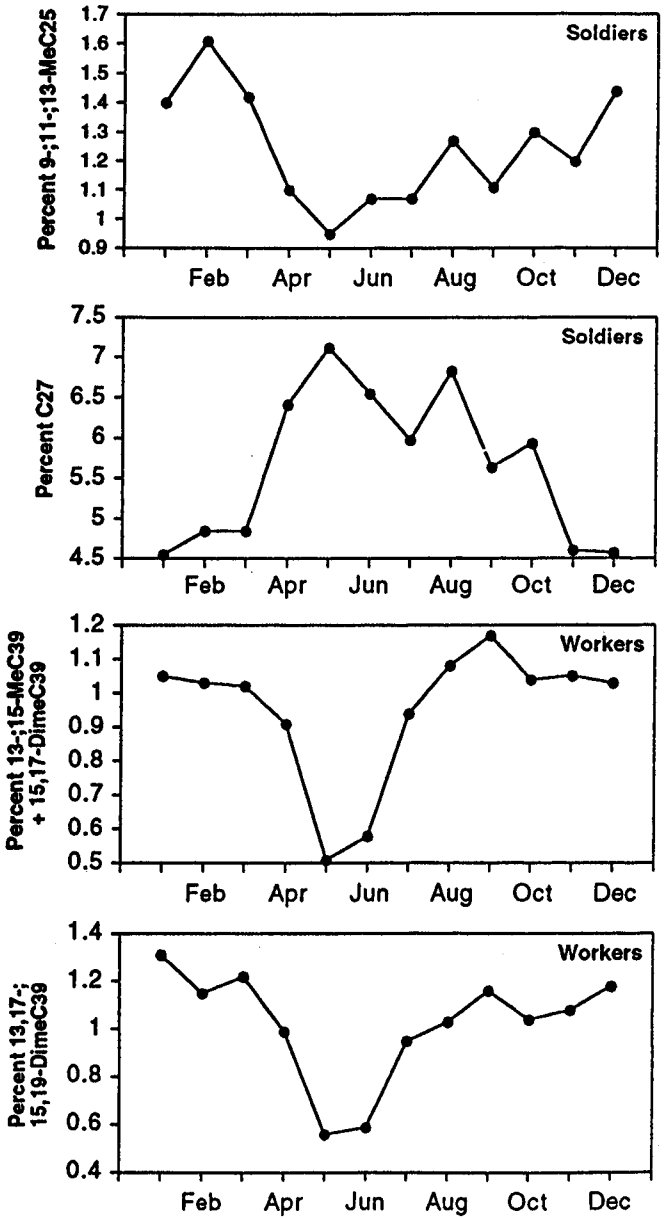

FIG. 5. Seasonal differences in hydrocarbons of *C. formosanus* soldiers (9-; 11-; 13-MeC25 and C27) and workers (13-; 15-MeC39 + 15,17-DimeC39 and 13,17-; 15,19-DimeC39) as a percentage of total hydrocarbons detected.

With the production of imagoes, the mature nymphs have metamorphosed and some individuals in the "worker" category will then differentiate to immature nymphs during this period. This would likely change the composition of the individuals remaining in the "worker" classification. Similarly, as the flight season approaches, termite colonies usually increase their investment in soldiers (Haverty and Howard, 1981; Howard and Haverty, 1981). Thus, a significant proportion of the individuals in the "worker" category are also differentiating to soldiers in April and May. After each flight many soldiers leave the nest to provide protection for the departing alates. Many of these soldiers are sealed out of the nest and lost to the colony (Bess, 1970; Nutting, 1969). The events surrounding the flight of the alates will result in a mix of young and old soldiers which could possess slightly different hydrocarbon mixtures.

Our survey of hydrocarbon mixtures of the same colonies for 25 months does not yet confirm that these colonies were derived from a single introduction to the Hawaiian Islands, or at least introductions of very closely related individuals. Given that *C. vastator* Light is suspected to have been introduced into Hawaii, and apparently was established at one time (Bess, 1970), it is quite possible that *C. formosanus* was introduced (and established) numerous times into Hawaii. The volume of movement of civilian and military materiel from the Far East through the Port of Honolulu and Pearl Harbor could easily have resulted in multiple introductions. However, confirmation of one or more founder events will require a broader sampling of *C. formosanus* populations within Hawaii, and comparison with populations from distant regions, such as Japan, Taiwan, and North America.

From the results reported here, we know that intercolony variation is greater than intracolony variation in cuticular hydrocarbon composition. We also know that we can characterize the colonies by quantifying hydrocarbons from either workers or soldiers; we need not collect data from both castes. Therefore, we propose that subsequent research on the biogeography of *C. formosanus* emphasize sampling hydrocarbons only from workers from as many separate colonies as possible from a given location, similar to the study of *D. perniger* by Brown et al. (1996). To minimize seasonal variation it is advisable to sample all colonies at times other than during the flight season. This latter criterion has the disadvantage of not providing imagoes for voucher specimens, but does provide a greater "window" for collecting.

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