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CUTICULAR HYDROCARBONS OF DAMPWOOD TERMITES, *Zootermopsis:* Intra- and Intercolony Variation and Potential as Taxonomic Characters[1]

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Abstract—Colonies of *Zootermopsis* were collected from the central Sierra Nevada and the Monterey Penninsula in California, and from southern Arizona. Cuticular hydrocarbons were identified by gas chromatography–mass spectrometry (GC-MS) and quantified by gas–liquid chromatography (GLC) for each caste of all colonies. Four consistent and distinct cuticular hydrocarbon patterns, or chemical phenotypes, were identified. Unique and abundant monomethyl- and dimethylalkanes, and an *n*-alkene provided easy separation of the various phenotypes. Significant differences in the proportions of the various components were found among castes within a colony and colonies within phenotypes from California. Differences in the hydrocarbon proportions for castes were not consistent between colonies. The current taxonomy of the genus *Zootermopsis* recognizes three species. Our identification of four consistent, unique cuticular hydrocarbon phenotypes from the three described species should alert systematists and others to a major concern. If there are truly only three extant species, then the hypothesis that cuticular hydrocarbon profiles in this genus are species specific is not acceptable. Conversely, if cuticular hydrocarbon profiles are truly species specific, then there is at least one new, undescribed species of *Zootermopsis*.

Key Words—Chemotaxonomy, Isoptera, Termopsidae, termites, methyl-branched hydrocarbons, lipids, agonisitic behavior, cuticular hydro-carbons.

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INTRODUCTION

The surface of all terrestrial insects is covered with a complex mixture of aliphatic material. This surface lipid plays a key role in survival of the insect by providing protection from desiccation, as well as serving as a barrier to abrasion, microorganisms, and chemicals. Hydrocarbons are ubiquitous components in insect cuticular lipids (Blomquist and Dillwith, 1985; Jackson and Blomquist, 1976; Hadley, 1980, 1985). They have been shown to be important in chemical communication as sex attractants and aphrodisiacs; as territory-marking, recruitment, and alarm pheromones; as defense secretions; and as kairomones, and they have been postulated as species and caste recognition cues (Howard and Blomquist, 1982).

Moore (1969) was the first to report the composition of cuticular hydrocarbons from a termite, *Nasutitermes exitiosus* (Hill). He found the majority of the hydrocarbons to be paraffins from C_{24} to C_{47} with the odd-carbon-numbered compounds predominating. Blomquist et al. (1979) and Howard et al. (1978, 1980, 1982) have completely characterized the cuticular hydrocarbons of a *Zootermposis* species and two *Reticulitermes* species. Because the mixtures of hydrocarbons of these three species were markedly different from one another and *N. exitiosus*, they postulated that these compounds might serve as semiochemical cues for caste and species recognition. Furthermore, Howard and Blomquist (1982) noted that there are extremely few reports of two insect species having identical hydrocarbon mixtures. Because of this, we were curious about their usefulness as taxonomic characters for separating morphologically similar species.

In preliminary work, we collected what we tentatively identified as colonies of *Z. angusticollis* (Hagen) from the vicinity of Burney, California, on the Lassen National Forest, and extracted their cuticular hydrocarbons. Our chemical analyses identified the same hydrocarbon components as published by Blomquist et al. (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.

Zootermopsis is a genus of primitive termites in the family Termopsidae, which is restricted to western North America. Three species are recognized: *Z. angusticollis*, *Z. nevadensis* (Hagen), and *Z. laticeps* (Banks). The former two species occur in the forested areas along the Pacific Coast, in the Cascade Mountains and Sierra Nevada, and in some of the inland ranges west of the Continental Divide. The latter species occurs east and southeast of the Sonoran Desert in trees along water courses and around ponds (Weesner, 1970). Present keys to the species, written decades ago, are based on a limited number of specimens and use morphological features and measurements of the head, pronotum, and/or wings of either soldiers or imagoes (Emerson, 1933; Sumner,

1933). A thorough morphological analysis of *Zootermopsis* has never been done. The material studied by these early taxonomists was collected from a narrow geographic range, and we now recognize that this genus may be in need of revision.

With only three extant species, we felt that this genus would be ideal to test the hypothesis that cuticular hydrocarbon profiles are species specific. To date, no one has satisfactorily examined the qualitative or quantitative variation in cuticular hydrocarbons of castes within a termite colony or species, among different colonies in a species, or, most important of all, among all species within a genus. In this paper we report: (1) identification of all cuticular hydrocarbons from each recognized species of *Zootermopsis*, and (2) intercaste, intracolony, and intercolony variation in hydrocarbon mixtures within two species.

METHODS AND MATERIALS

Zootermopsis colonies or portions of colonies were collected in spring and summer of 1985 from the following two locations: near Placerville, California, on the Eldorado National Forest (eight colonies), and from Cypress Point near Pacific Grove, California, on the Monterey Peninsula (seven colonies). In the spring of 1986 additional collections were made at Cypress Point (nine colonies) and on the Eldorado National Forest (10 colonies). Also in the spring of 1986 samples from five colonies of *Z. laticeps* were obtained from an area along the Santa Cruz River near Rio Rico, Santa Cruz County, Arizona.

Two sets of chromatographic analyses were completed. Termites were removed from their galleries and placed in plastic dishes containing moist host wood. In the first analysis, one to five individuals of each caste from each of the colonies collected in 1985 were placed in separate numbered vials (one termite/vial) and kept at −20°C until extracted for hydrocarbon analysis. Individual termites were coded by number; thus the species or location was unknown before gas chromatographic analysis. In the second analysis, groups of five individuals from each caste from each of the colonies collected in 1985 and 1986 were placed in numbered vials as were the individual termites. Termites used in both the first and second analysis were removed from their host wood and frozen within days of collection.

Individual termites or groups of individuals were removed from the freezer and allowed to come to ambient temperature. Cuticular lipids were extracted by immersion of termites in 10 ml of hexane for 10 min. After extraction, hydrocarbons were separated from other nonpolar components by pipetting the 10-ml extract through a minicolumn packed with 100–200 mesh BioSil A activated overnight at 70°C in a drying oven. This hydrocarbom extract was then evaporated to dryness under a stream of nitrogen and redissolved in 30 μl of hexane for GLC analysis. After extraction, the insects

were stored in 70% ethanol for later identification by morphological features and to serve as voucher specimens. All voucher specimens will be deposited in the U.S. National Museum, Smithsonian Institution, Washington, D.C.

The first analysis of cuticular hydrocarbons was performed on a Hewlett Packard 5790A gas chromatograph using a wide-bore capillary column (30 m × 0.75 mm ID SPB-1) with a flame ionization detector (FID). Peak resolution was adequate with this system to observe obvious qualitative differences in cuticular hydrocarbon mixtures and allowed us to separate the California *Zootermopsis* into three obvious chemical phenotypes (see Results and Discussion). Individual termites were used for these analyses, which permitted us to quantify each hydrocarbon with the exception of a few which coeluted in the same peak. Peaks were characterized by retention time, and areas under curves (= quantity of a particular component) were automatically quantified by a Hewlett Packard 3390A electronic integrator. The total hydrocarbon was calculated by summing the area for each component and proportion of each component then calculated. For each of the three California chemical phenotypes, proportions of each hydrocarbon were transformed to the arcsine of the square root of the proportion and subjected to analysis of variance to evaluate differences among castes and within and among colonies, and to provide summary statistics.

Better hydrocarbon separation was obtained with the acquisition of a Hewlett Packard 5890 gas chromatograph equipped with a fused silica capillary column (30 m × 0.32 mm ID, SPB-1), FID, and operated in the split mode (with a split ratio of 33:1). The temperature program in each case had a starting temperature of 200°C. Temperature was increased at 3°C/min to 300°C, with a final holding time of 12 min. Helium carrier gas flow was 25 cc/min on the HP 5790A and 3 cc/min on the HP 5890. Hydrocarbons were quantified as in the first analysis.

Routine identification of all hydrocarbons was based on external standards or retention times from hydrocarbons identified by GC-MS. Equivalent chain length (ECL) for each hydrocarbon was calculated by comparing the retention time of a given peak to known *n*-alkanes in external standards. GC-MS analyses were performed on a Finnigan 4023 mass spectrometer interfaced with an INCOS data system. Electron impact (EI) mass spectrometry was performed at 70 eV. Mass spectra of methylakanes were interpreted according to the criteria of Nelson et al. (1972), Nelson (1978), and Pomonis et al. (1978). *n*-Alkenes were isolated by silver nitrate chromatography (column and/or TLC), and the positions of double bonds were determined by GC-MS analysis of the monomethoxy and dimethoxy derivatives of the parent compounds after methoxymercuration–demercuration (Blomquist et al., 1980).

To assure correct species identification and to correlate cuticular hydrocarbon mixtures with species, preserved, extracted specimens (imagoes and soldiers, if both were present) were shipped in numbered vials, one individual per vial, to termite specialists for identification. The termites were identified by

these specialists solely on the basis of external morphology without knowledge of location or host. The only information provided was the association of individuals from the same colony. The keys used were from Banks and Snyder (1920), Snyder (1954), Sumner (1933), and Weesner (1965).

RESULTS AND DISCUSSION

All of the abundant hydrocarbon components (mean percent $\geq 0.5\%$ of the total hydrocarbon component) and most of the less abundant components were identified for each location and/or species (Tables 1 and 2, Figure 1). All col-

TABLE 1. HYDROCARBONS IDENTIFIED FOR PHENOTYPES OF *Zootermopsis*.[a]

Peak	Hydrocarbon	ECL[b]	CN[b]	Diagnostic MS ions
1	*n*-Nonadecane	19.00	19	268
2	*n*-Eicosane	20.00	20	282
3	5-Methyleicosane	20.50	21	84/85, 238/239, 296
4	2-Methyleicosane	20.62	21	280/281, 296
5	*n*-Heneicosane	21.00	21	296
6	9-; 11-Methylheneicosane	21.37	22	140/141, 196/197, 310; 168/169, 310
7	7-Methylheneicosane	21.41	22	112/113, 224/225, 310
8	5-Methylheneicosane	21.50	22	84/85, 252/253, 310
9	2- or 4-Methylheneicosane	21.62	22	266/267, 310
10	3-Methylheneicosane	21.71	22	252/253, 280/281, 310
11	*n*-Docosane	22.00	22	310
12	7,15-Dimethylheneicosane	22.00	23	112/113, 238/239
13	5,17-Dimethylheneicosane	22.04	23	84/85, 266/267
14	3,11-; 3,13-Dimethylheneicosane	22.11	23	168/169, 182/183, 196/197, 210/211, 294/295
15	7-Methyldocosane	22.37	23	112/113, 238/239, 324
16	2- or 4-Methyldocosane	22.63	23	252/253, 280/281, 294/295, 308/309, 324
17	*n*-Tricosene	22.70	23	322
18	Unknown 1	22.77		
19	*n*-Tricosane	23.00	23	324
20	9-; 11-Methyltricosane	23.36	24	140/141, 224/255, 338; 168/169, 196/197, 338
21	7-Methyltricosane	23.43	24	112/113, 252/253, 338
22	5-Methyltricosane	23.50	24	84/85, 280/281, 338
23	3-Methyltricosane	23.73	24	280/281, 308/309, 338
24	5,13-; 5,17-Dimethyltricosane	23.95	25	84/85, 168/169, 210/211, 294/295; 84/85, 112/113, 266/267, 294/295
25	*n*-Tetracosane	24.00	24	338
26	3,11-; 3,13-Dimethyltricosane	24.10	25	168/169, 182/183, 196/197, 210/211, 322/232, 352
27	2-Methyltetracosane	24.72	25	308/309, 336/337, 352

TABLE 1. Continued

Peak	Hydrocarbon	ECL[b]	CN[b]	Diagnostic MS ions
28	*n*-Pentacosane	25.00	25	352
29	9-; 11-; 13-Methylpentacosane	25.38	26	140/141, 252/253, 366; 168/169, 224/225, 366; 196/197, 366
30	5-Methylpentacosane	25.52	26	85/86, 308/309, 366
31	3-Methylpentacosane	25.73	26	336/337, 366
32	5,17-Dimethylpentacosane	25.90	27	84/85, 140/141, 266/267, 322/323
33	*n*-Hexacosane	26.00	26	366
34	2-or 4-Methylheptacosane	26.59	27	308/309, 336/337, 364/365
35	*n*-Heptacosane	27.00	27	380
36	11-Methylheptacosane	27.35	28	168/169, 252/253, 394
37	5-Methylheptacosane	27.53	28	84/85, 336/337, 394
38	5,17-Dimethylheptacosane	27.88	29	84/85, 168/169, 266/267, 350/351
39	*n*-Octacosane	28.00	28	394
40	2- or 4-Methyloctacosane	28.60	29	336/337, 364/365, 392/393, 408
41	*n*-Nonacosane	29.00	29	408
42	11-; 13-; 15-Methylnonacosane	29.32	30	168/169, 280/281, 422; 196/197, 252/253, 422; 196/197, 224/225, 422
43	7-Methylnonacosane	29.42	30	112/113, 336/337, 422
44	5-Methylnonacosane	29.51	30	84/85, 364/365, 422
45	5,17-Dimethylnonacosane	29.83	31	84/85, 196/197, 266/267, 378/379
46	*n*-Triacontane[c]	29.99	30	
47	Unknown 2	30.05		
48	3,7-Dimethylnonacosane	30.13	31	126/127, 336/337, 406/407
49	Unknown 3	30.45		
50	*n*-Hentriacotene	30.60	31	434
51	Unknown 4	30.70		
52	13-; 15-Methylhentriacontane	30.87	32	196/197, 280/281, 450; 224/225, 252/253, 450
53	Unknown 5	30.96		
54	7-; 9-Methylhentriacontane	31.37	32	112/113, 140/141, 336/337, 364/365, 392/393
55	5-Methylhentriacontane	31.46	32	84/85, 364/365, 392/393
56	2- or 4-Methylhentriacontane	31.75	32	406/407, 434/435, 450
57	*n*-Dotriacontane[c]	31.98		
58	Unknown 6	32.26		
59	Unknown 7	32.61		
60	5,13-; 5,15-; 5,17-Dimethyltritriacontane	33.60	35	84/85, 210/211, 308/309, 434/435; 238/239, 280/281, 434/435; 252/253, 266/267, 434/435
61	5,17-Dimethylpentatriacontane	35.70	37	84/85, 266/267, 280/281, 462/463
62	7,11-Dimethylpentatriacontane	35.70	37	112/113, 182/183, 364/365, 434/435
63	7,13-Dimethylheptatriacontane	38.53	39	112/113, 210/211, 364/365, 463/464

[a] Peak numbers refer to peaks identified in Figure 1.
[b] ECL = equivalent chain length; CN = carbon number.
[c] Hydrocarbon determination made by ECL, not GC-MS.

TABLE 2. HYDROCARBONS IDENTIFIED FROM FOUR PHENOTYPES OF *Zootermopsis*.[a]

Peak	Hydrocarbon	ECL[b]	Phenotype[c]			
			I	II	III	IV
1	*n*-Nonadecane	19.00	+	0	0	0
2	*n*-Eicosane	20.00	+	0	0	tr
3	5-Methyleicosane	20.50	tr	0	0	0
4	2-Methyleicosane	20.62	tr	0	0	tr
5	*n*-Heneicosane	21.00	+++	+++	+++	+++
6	9-; 11-Methylheneicosane	21.37	0	++	0	tr
7	7-Methylheneicosane	21.41	+	++	++	tr
8	5-Methylheneicosane	21.50	+++	++	0	0
9	2- or 4-Methylheneicosane	21.62	0	0	0	++
10	3-Methylheneicosane	21.71	++	++	++	++
11	*n*-Docosane	22.00	0	++	++	++
12	7,15-Dimethylheneicosane	22.00	++	0	0	0
13	5,17-Dimethylheneicosane	22.04	+++	0	0	0
14	3,11-; 3,13-Dimethylheneicosane	22.11	0	+++	0	0
15	7-Methyldocosane	22.37	0	+	0	0
16	2- or 4-Methyldocosane	22.63	0	++	+++	+++
17	*n*-Tricosene	22.70	0	0	++	++
18	Unknown 1	22.77	0	0	0	++
19	*n*-Tricosane	23.00	+++	+++	+++	+++
20	9-; 11-Methyltricosane	23.36	0	+	0	tr
21	7-Methyltricosane	23.43	+	++	0	tr
22	5-Methyltricosane	23.50	++	0	0	0
23	3-Methyltricosane	23.73	+	+	+++	++
24	5,13-; 5,17-Dimethyltricosane	23.95	tr	0	0	0
25	*n*-Tetracosane	24.00	+	0	++	+
26	3,11-; 3,13-Dimethyltricosane	24.10	0	++	0	0
27	2-Methyltetracosane	24.72	0	0	tr	+
28	*n*-Pentacosane	25.00	+++	++	+++	+++
29	9-; 11-; 13-Methylpentacosane	25.38	tr	tr	tr	0
30	5-Methylpentacosane	25.52	tr	0	0	0
31	3-Methylpentacosane	25.73	0	0	+	0
32	5,17-Dimethypentacosane	25.90	tr	0	0	0
33	*n*-Hexacosane	26.00	tr	tr	+	tr
34	2- or 4-Methylheptacosane	26.59	0	0	tr	0
35	*n*-Heptacosane	27.00	+	++	tr	++
36	11-Methylheptacosane	27.35	0	tr	0	0
37	5-Methylheptacosane	27.53	tr	tr	0	0
38	5,17-Dimethylheptacosane	27.88	tr	0	0	0
39	*n*-Octacosane	28.00	tr	+	0	0
40	2- or 4-Methyloctacosane	28.60	0	++	0	0
41	*n*-Nonacosane	29.00	++	++	0	tr
42	11-; 13-; 15-Methylnonacosane	29.32	0	+	0	0
43	7-Methylnonacosane	29.42	tr	0	0	tr
44	5-Methylnonacosane	29.51	++	++	0	tr
45	5,17-Dimethylnonacosane	29.83	tr	++	0	0

TABLE 2. Continued

Peak	Hydrocarbon	ECL[b]	Phenotype[c] I	II	III	IV
46	*n*-Triacontane	29.99	tr	0	0	0
47	Unknown 2	30.05	0	tr	0	0
48	3,7-Dimethylnonacosane	30.13	tr	+	tr	0
49	Unknown 3	30.45	tr	0	0	0
50	*n*-Hentriacontene	30.60	0	+	0	0
51	Unknown 4	30.70	0	0	tr	0
52	13-; 15-Methylhentriacontane	30.87	0	+	0	0
53	Unknown 5	30.96	tr	0	0	0
54	7-, 9-Methylhentriacontane	31.37	tr	tr	0	0
55	5-Methylhentriacontane	31.46	tr	++	0	0
56	2- or 4-Methylhentriacontane	31.75	0	++	0	0
57	*n*-Dotriacontane	31.98	0	tr	tr	0
58	Unknown 6	32.26	0	0	+	0
59	Unknown 7	32.61	0	0	tr	0
60	5,13-; 5-15-; 5,17-Dimethyltricontane	33.60	+	++	0	0
61	5,17-Dimethylpentatriacontane	35.70	++	++	0	0
62	7,11-Dimethylpentatriacontane	35.70	0	0	0	++
63	7,13-Dimethylpentatriacontane	38.53	0	0	0	++

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

[b] Hydrocarbon identifications by GC-MS (see Table 1 and Figure 1).

[c] Phenotypes and collection locations: I = Eldorado NF, California, *Z. nevadensis*; II and III = Pacific Grove, California, *Z. angusticollis* and *Z. nevadensis*, respectively; IV = Rio Rico, Santa Cruz County, Arizonia, *Z. laticeps*.

onies from the Eldorado National Forest possessed qualitatively identical mixtures of hydrocarbons, which we will call phenotype I. Ninety-one percent of the taxonomic determinations for this phenotype were *Z. nevadensis*; the remaining 9% were determined to be *Z. angusticollis*. Two different hydrocarbon phenotypes (II and III) were recognized from the Pacific Grove area; both were qualitatively different from phenotype I. All colonies in either phenotypes II or III had the same hydrocarbon components. All of the taxonomic determinations for phenotype II were *Z. angusticollis*; 84% of the determinations for phenotype III were *Z. nevadensis*. Colonies collected from Rio Rico in southern Arizona were identified as *Z. laticeps*. These termites all had qualitatively identical hydrocarbon profiles that were qualitatively different from phenotypes I, II, and III. *Zootermopsis laticeps* constitutes phenotype IV.

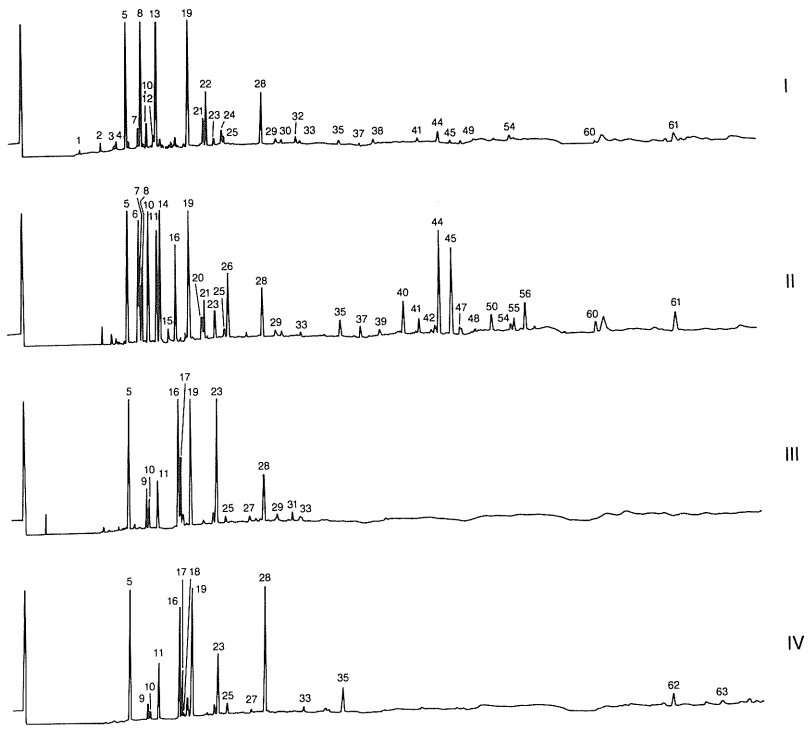

FIG. 1. Gas chromatograms of the surface hydrocarbons of four phenotypes of *Zootermopsis*. Numbers identify peaks whose compositions are listed in Table 1.

GC-MS was used to characterize each of the major hydrocarbon components of each phenotype, and the mass spectra of some of the components unique to certain phenotypes are presented in Figures 2–6. The mass spectrum of peak 13 (Figure 1), which elutes about one equivalent chain length (ECL = 22.04) in front of the corresponding *n*-alkane and is identified as the symetrical 5, 17-dimethylheneicosane, is presented in Figure 2. The strong *m/z* 84/85 ion (Figure 2) is interpreted as arising from cleavage internal to the methyl group on carbons 5 and 17 giving rise to a six-carbon fragment ion. Cleavage external to the methyl groups gives rise to ions at *m/z* 266/267, in which the much greater intensity of the odd-numbered ion is consistent with the fragment containing a second methyl group.

Peak 16 from phenotype IV is interpreted as 2-methyldocosane based on its retention time and mass spectrum (Figure 3). The ECL = 22.63 and strong M − 15 ion at *m/z* 309 and M − 43 ion at *m/z* 281 are consistent with a 2-methylalkane. In some spectra of components identified as 2- or 4-methylal-

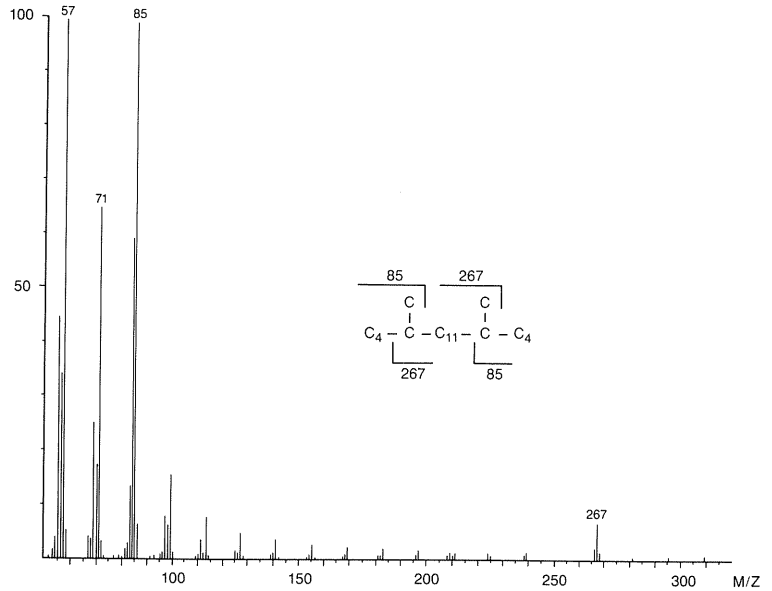

FIG. 2. EI mass spectrum of peak 13, Figure 1, identified as 5,17-dimethylheneicosane.

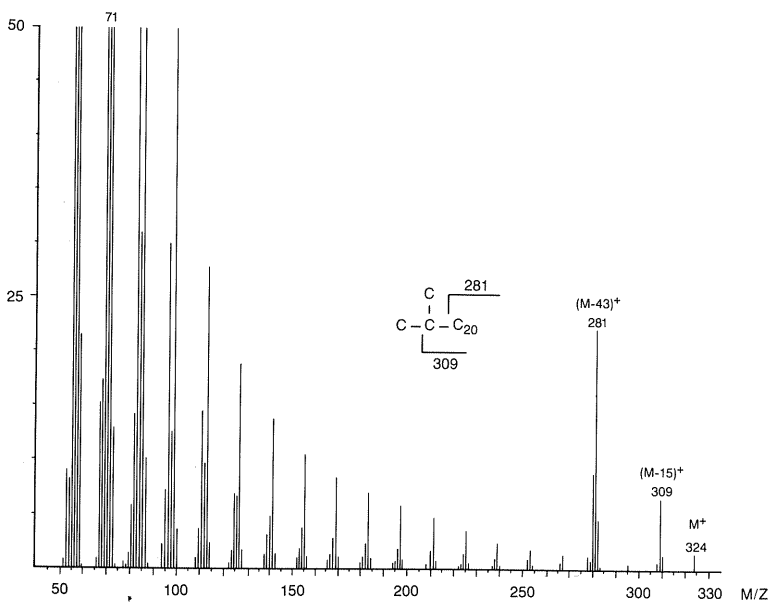

FIG. 3. EI mass spectrum of peak 16, Figure 1, identified as 2-methyldocosane.

kanes, there is a relatively strong ion pair at M − 71:M -72, indicating that 4-methylalkanes may also be present. The M − 71:M -72 ion from the alkanes identified as 2- or 4-methylalkanes varied in intensity among different spectra and therefore are designated as either 2- or 4-methylalkanes. Even when the M − 71:M − 72 ions are relatively strong, we cannot rule out the possibility that the peak also contains a 2-methylalkane.

Phenotype II also contains peaks identified as a mixture of 3,11- and 3,13-dimethylalkanes. In Figure 4 we show the mass spectrum of peak 26, which is identified as a mixture of 3,11- and 3,13-dimethyltetracosanes. The major isomer appears to be the 3,11- component, which gives rise to ions at *m/z* 182/183, 196/197, and 322/323. The 3,13- isomer gives rise to ions at *m/z* 168/169, 210/211, and 322/323. This phenotype appears to be the only one that contains 3,11- and 3,13-dimethylalkanes (peaks 14 and 26, Figure 1, Table 2).

Phenotypes III and IV contain an alkene (peak 17), which we identify as 9-tricosene from the mass spectra of its mono- and dimethyoxy derivatives. The spectra (Figures 5A and 5B) show the expected fragment ions (*m/z* 157, 171, 227, and 241 for the monomethoxy derivative and 157 and 227 for the dimethoxy derivative). Phenotype IV also contains two unique dimethylalkanes, 7,11-dimethylpentatriacontane and 7,13-dimethylheptatriacontane (peaks 62 and

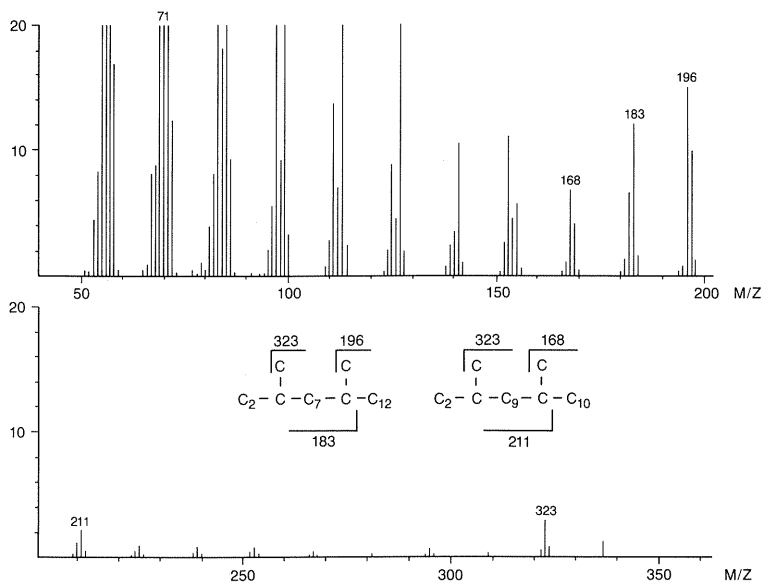

FIG. 4. EI mass spectrum of peak 26, Figure 1, identified as 3,11- and 3,13-dimethyltricosane.

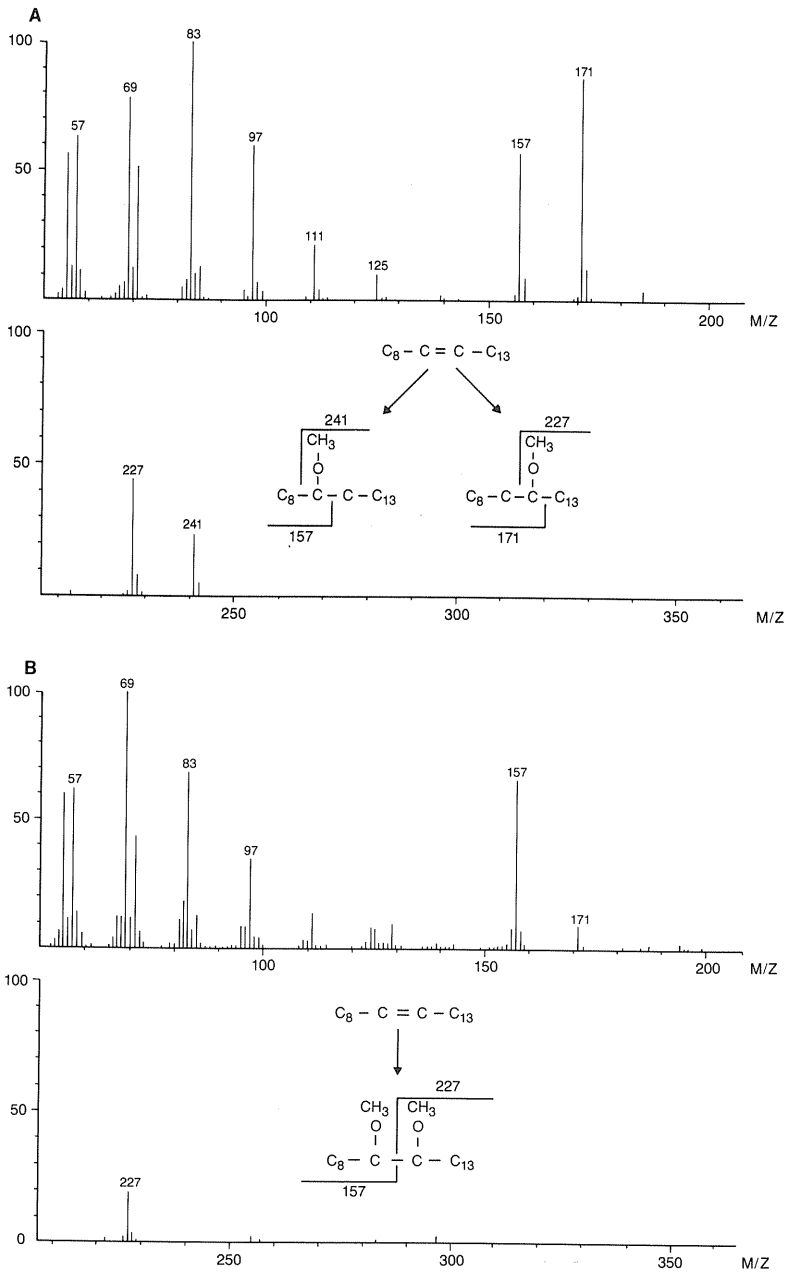

FIG. 5. EI mass spectra of (A) monomethoxy and (B) dimethoxy derivatives of 9-tricosene.

63). One of these (peak 63) elutes much later than any of the other components in any of the *Zootermopsis* phenotypes. The mass spectrum of 7,11-dimethylpentatriacontane is presented in Figure 6. Cleavage internal to one or the other of the branching methyl groups gives rise to fragments at *m/z* 112/113 and 364/365. Cleavage external to the branching methyl groups gives rise to fragments that contain a second methyl branch and are at *m/z* 183 and 435.

Mass spectra of each of the other major components for all four phenotypes were interpreted in a similar manner to that described above, and each component and its diagnostic ions are presented in Table 1.

Phenotype I contains major amounts (ca. 10% of the total hydrocarbon) of the symmetrical dimethylalkane 5,17-dimethylheneicosane (peak 13, Figure 1 and Table 3). This dimethylalkane is not shared with any other phenotype (Table 2). Phenotype I is rich in minor 5,17-dimethyl components including a homologous series of 5,17-dimethyl C_{25}, C_{27}, C_{29}, C_{31}, C_{35}, and C_{37}. With the exception of 5,17-dimethyl C_{31}, C_{35}, and C_{37}, these components of this homologous series are unique to phenotype I (Table 2).

Phenotype II is the most complex of the *Zootermopsis* chemical phenotypes, sharing many of the components eluting before *n*-nonacosane with at least one of the other phenotypes. This phenotype possesses many unique compounds in major quantities (>1.0%, Table 4): an isomeric mixture of 9- and

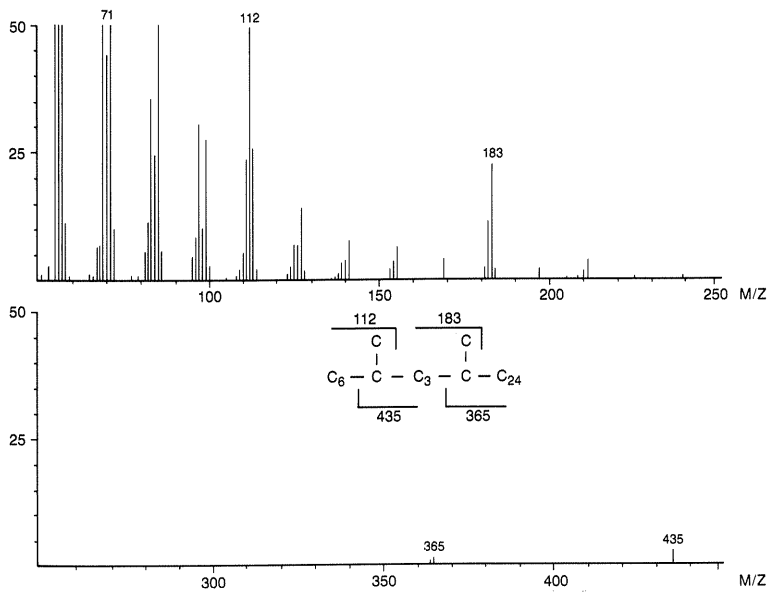

FIG. 6. EI mass spectrum of peak 62, Figure 1, identified as 7,11-dimethylpentatriacontane.

TABLE 3. STATISTICS FOR PERCENT HYDROCARBON FROM FOUR CASTES FROM EIGHTEEN COLONIES

		Overall (N = 53)				
Peak No.[a]	Hydrocarbon[a]	X	SD	Minimum value[b]	Maximum Value	Proportion ≤0.1% of total[c]
1	n-C_{19}	0.6	0.8	0.0	3.3	0.55
2	n-C_{20}	0.9	1.3	0.0	9.3	0.15
3	5-MeC_{21}	0.0	0.0	0.0	0.2	0.98
4	2-MeC_{21}	0.2	0.2	0.0	0.7	0.41
5	n-C_{21}	36.1	8.6	16.5	51.2	0.00
7	7-MeC_{22}	0.6	0.2	0.3	1.3	0.00
8	5-MeC_{22}	12.6	3.1	6.9	22.2	0.00
10	3-MeC_{22}	1.1	0.2	0.6	1.8	0.00
12	7,15-$DimeC_{23}$	1.9	0.6	0.3	3.1	0.00
13	5,17-$DimeC_{23}$	9.9	5.4	3.8	27.9	0.00
19	n-C_{23}	16.3	3.6	10.1	27.6	0.00
21	7-MeC_{24}	0.9	0.4	0.4	2.2	0.00
22	5-MeC_{24}	2.9	1.2	1.3	5.8	0.00
23	3-MeC_{24}	0.6	0.2	0.4	1.3	0.00
24	5,17-$DimeC_{25}$	0.2	0.3	0.0	1.0	0.43
25	n-C_{24}	0.7	0.2	0.4	1.5	0.00
28	n-C_{25}	6.3	3.5	2.4	19.7	0.00
29	9-; 11-; 13-MeC_{26}	0.2	0.2	0.0	0.9	0.47
30	5-MeC_{26}	0.3	0.2	0.0	0.8	0.23
32	5,17-$DimeC_{27}$	0.3	0.2	0.0	0.7	0.28
33	n-C_{26}	0.4	0.3	0.0	1.6	0.15
35	n-C_{27}	0.7	0.6	0.0	3.1	0.02
37	5-MeC_{28}	0.3	0.3	0.0	1.3	0.34
38	5,17-$DimeC_{29}$	0.4	0.6	0.0	3.0	0.30
39	n-C_{28}	0.1	0.1	0.0	0.7	0.75
41	n-C_{29}	1.0	1.4	0.0	6.5	0.11
43	7-MeC_{30}	0.2	0.4	0.0	2.2	0.71
44	5-MeC_{30}	1.3	0.9	0.0	5.8	0.06
45	5,17-$DimeC_{31}$	0.4	0.6	0.0	3.2	0.32
46	n-C_{30}	0.1	0.3	0.0	1.6	0.81
48	3,7-$DimeC_{31}$	0.3	0.4	0.0	1.2	0.30
49	Unknown 3	0.1	0.1	0.0	0.5	0.70
53	Unknown 5	0.3	0.3	0.0	1.2	0.34
54	7-,9-MeC_{32}	0.4	0.2	0.0	1.1	0.09
55	5-MeC_{32}	0.1	0.2	0.0	0.5	0.64
60	5,17-$DimeC_{35}$	0.5	1.0	0.0	7.4	0.42
61	5,17-$DimeC_{37}$	1.1	0.5	0.0	2.9	0.02

[a] Hydrocarbons and peak numbers are the same as those reported in Table 1 and Figure 1. Carbon number is the total number of carbons, including methyl groups.
[b] Values not necessarily zero but may have had a very low quantity (<0.1% of total hydrocarbon) or were simply not detectable for a given GLC analysis.
[c] Proportion of the 53 chromatograms where a given hydrocarbon was not detectable.
[d] Castes are SO = soldier, PS = pseudergate, NY = nymph, and AL = alate (imago).

OF *Zootermopsis* COLLECTED FROM ELDORADO NATIONAL FOREST, CALIFORNIA (PHENOTYPE I)

Castes[d]						
SO (N = 17)	PS (N = 18)	NY (N = 11)	AL (N = 7)	Statistical differences[e]		
X SD	X SD	X SD	X SD	Colony	Caste	Interaction
1.0 (1.0)	0.6 (0.7)	0.6 (0.9)	0.0 (0.1)			
1.5 (2.1)	0.8 (0.5)	0.6 (0.5)	0.2 (0.2)			
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)			
0.3 (0.2)	0.2 (0.2)	0.1 (0.2)	0.1 (0.2)			
37.9 (4.7)	39.5 (6.7)	37.4 (9.0)	21.0 (3.4)	*	*	*
0.5 (0.2)	0.5 (0.2)	0.6 (0.2)	1.0 (0.2)	*	*	*
12.7 (2.5)	12.9 (2.8)	11.4 (3.1)	13.0 (5.0)	*	*	*
1.2 (0.2)	1.0 (0.2)	0.9 (0.2)	1.1 (0.3)	*	*	NS
1.8 (0.2)	2.2 (0.4)	2.0 (0.8)	1.0 (0.4)	*	*	*
8.3 (1.7)	7.6 (2.1)	8.6 (3.6)	21.7 (4.5)	*	*	*
15.3 (2.6)	15.8 (2.9)	16.9 (4.1)	18.6 (5.7)	*	*	*
0.8 (0.3)	0.7 (0.2)	0.8 (0.3)	1.7 (0.3)	NS	*	*
3.0 (1.1)	2.3 (0.8)	2.6 (0.8)	4.7 (0.8)	*	*	*
0.6 (0.2)	0.5 (0.1)	0.6 (0.1)	1.0 (0.2)	NS	*	NS
0.3 (0.2)	0.1 (0.1)	0.1 (0.2)	0.7 (0.2)			
0.7 (0.1)	0.7 (0.2)	0.8 (0.3)	0.8 (0.2)	*	*	NS
5.5 (2.0)	5.9 (4.1)	7.6 (4.4)	7.1 (2.5)	*	*	NS
0.2 (0.2)	0.1 (0.2)	0.2 (0.2)	0.2 (0.1)	*	*	NS
0.3 (0.2)	0.2 (0.2)	0.3 (0.2)	0.4 (0.2)	*	*	NS
0.4 (0.2)	0.2 (0.2)	0.2 (0.2)	0.3 (0.2)	*	NS	NS
0.3 (0.1)	0.4 (0.4)	0.4 (0.4)	0.3 (0.2)	*	NS	NS
0.5 (0.3)	0.7 (0.7)	0.9 (0.9)	0.4 (0.2)	*	NS	NS
0.2 (0.3)	0.2 (0.3)	0.5 (0.4)	0.4 (0.4)	*	*	NS
0.5 (0.8)	0.4 (0.5)	0.4 (0.3)	0.5 (0.5)	*	*	*
0.0 (0.1)	0.1 (0.2)	0.0 (0.1)	0.0 (0.0)	*	*	*
1.0 (1.4)	1.3 (1.7)	0.9 (0.8)	0.3 (0.2)	*	*	NS
0.2 (0.5)	0.2 (0.4)	0.2 (0.3)	0.0 (0.0)	*	*	*
1.3 (0.5)	1.3 (1.2)	1.4 (0.8)	0.8 (0.6)	*	*	*
0.5 (0.8)	0.5 (0.7)	0.2 (0.3)	0.1 (0.2)			
0.1 (0.1)	0.2 (0.4)	0.1 (0.3)	0.0 (0.0)			
0.2 (0.2)	0.2 (0.2)	0.5 (0.4)	0.7 (0.5)			
0.1 (0.2)	0.1 (0.1)	0.1 (0.1)	0.1 (0.2)			
0.3 (0.3)	0.3 (0.3)	0.2 (0.2)	0.1 (0.1)			
0.5 (0.2)	0.4 (0.2)	0.5 (0.4)	0.3 (0.2)			
0.1 (0.2)	0.1 (0.1)	0.1 (0.2)	0.2 (0.1)			
0.4 (0.3)	0.7 (1.7)	0.3 (0.4)	0.3 (0.3)			
1.2 (0.5)	1.0 (0.3)	1.1 (0.7)	0.9 (0.5)			

[e] Proportions of each hydrocarbon were transformed to the arcsine of the square root of the proportion and subjected to analysis of variance. Asterisk indicates significant difference (α = 0.05) between colony, caste, or interaction term for a given hydrocarbon. Analysis of variance was done only for hydrocarbon components detectable from individual termites from collections made in 1985. The gas chromatograph with a 0.75-mm-ID capillary column was used.

TABLE 4. STATISTICS FOR PERCENT HYDROCARBON FROM FOUR CASTES FROM SEVEN COLONIES OF *Zootermopsis* COLLECTED FROM PACIFIC GROVE, CALIFORNIA (PHENOTYPE II)

		Overall ($N = 20$)					Castes[d]				Statistical differences[e]		
Peak No.[a]	Hydrocarbon[a]	X	SD	Minimum value[b]	Maximum value	Proportion ≤0.1% of total[c]	SO ($N = 7$) X SD	PS ($N = 6$) X SD	NY ($N = 6$) X SD	AL ($N = 1$) X SD	Colony	Caste	Interaction
5	n-C_{21}	23.9	5.4	12.8	32.2	0.00	20.7 (4.8)	27.7 (4.8)	25.4 (3.0)	14.2 —	*	*	NS
6	9-; 11-MeC_{22}	3.8	0.6	2.4	5.1	0.00	3.9 (0.6)	3.7 (0.3)	3.8 (0.9)	4.0 —	*	*	*
7	7-MeC_{22}	1.6	0.3	1.0	2.1	0.00	1.8 (0.2)	1.5 (0.2)	1.4 (0.3)	2.1 —	*	*	*
8	5-MeC_{22}	2.6	0.6	1.6	4.9	0.00	2.6 (0.3)	2.5 (0.3)	2.6 (1.2)	2.9 —	*	*	*
10	3-MeC_{22}	8.5	1.2	6.9	11.0	0.00	9.3 (1.3)	8.3 (0.9)	7.6 (0.8)	8.7 —	*	*	*
11	n-C_{22}	3.0	0.7	1.9	4.4	0.00	2.5 (0.3)	3.7 (0.6)	3.2 (0.5)	1.9 —	*	*	NS
14	3,11-; 3,13-$DimeC_{24}$	5.2	1.4	2.2	7.7	0.00	6.6 (0.7)	3.9 (0.5)	4.8 (1.5)	6.0 —	*	*	NS
15	7-MeC_{23}	0.5	0.4	0.2	2.2	0.00	0.5 (0.1)	0.7 (0.7)	0.4 (0.1)	0.5 —	NS	*	NS
16	2- or 4-MeC_{23}	2.3	0.6	0.5	3.6	0.00	2.7 (0.6)	2.0 (0.8)	2.2 (0.3)	2.5 —	*	*	*
19	n-C_{23}	17.3	2.6	14.1	23.0	0.00	16.5 (2.4)	18.0 (2.6)	18.0 (3.1)	15.3 —	*	*	*
20	9-; 11-MeC_{24}	0.6	0.3	0.3	1.3	0.00	0.7 (0.2)	0.5 (0.2)	0.5 (0.1)	1.3 —	*	*	*
21	7-MeC_{24}	1.2	1.1	0.5	5.2	0.00	1.1 (0.6)	0.9 (0.6)	0.9 (0.4)	5.2 —	*	*	*
23	3-MeC_{24}	0.8	0.2	0.5	1.0	0.00	0.9 (0.1)	0.8 (0.1)	0.7 (0.2)	0.6 —	NS	NS	NS
25	n-C_{24}	0.4	0.1	0.3	0.7	0.00	0.4 (0.2)	0.4 (0.1)	0.4 (0.1)	0.4 —	*	*	NS
26	3,11-; 3,13-$DimeC_{25}$	1.2	0.4	0.6	2.1	0.00	1.5 (0.3)	0.9 (0.2)	1.0 (0.2)	2.0 —	*	*	NS
28	n-C_{25}	3.3	1.2	1.9	6.1	0.00	3.4 (1.5)	3.0 (1.0)	3.6 (1.3)	3.8 —	*	*	*
29	9-; 11-MeC_{26}	0.2	0.1	0.0	0.5	0.25	0.2 (0.2)	0.1 (0.2)	0.1 (0.1)	0.5 —	*	*	NS
33	n-C_{26}	0.3	0.1	0.0	0.7	0.05	0.4 (0.2)	0.3 (0.2)	0.4 (0.1)	0.3 —	*	NS	NS

35	*n*-C_{27}	2.6	0.8	1.7	5.1	0.00	2.9 (1.1)	2.3 (0.5)	2.7 (0.5)	2.2 —	*	*	*
36	11-MeC_{28}	0.3	0.2	0.0	0.7	0.00	0.3 (0.2)	0.3 (0.2)	0.4 (0.1)	0.2 —	*	*	NS
37	5-MeC_{28}	0.2	0.2	0.0	1.0	0.10	0.2 (0.1)	0.3 (0.4)	0.2 (0.1)	0.3 —	*	NS	NS
39	*n*-C_{28}	0.5	0.4	0.3	2.4	0.00	0.4 (0.1)	0.7 (0.8)	0.4 (0.1)	0.3 —	*	NS	*
40	2- or 4-MeC_{29}	1.9	1.3	0.5	6.6	0.00	2.7 (1.9)	1.7 (0.8)	1.5 (0.4)	0.6 —	*	NS	NS
41	*n*-C_{29}	1.6	0.3	1.0	2.2	0.00	1.8 (0.4)	1.4 (0.4)	1.6 (0.2)	1.6 —	*	*	*
42	11-; 13-; 15-MeC_{30}	0.6	0.3	0.0	1.1	0.10	0.5 (0.4)	0.5 (0.4)	0.7 (0.2)	0.7 —	*	*	*
44	5-MeC_{30}	3.6	0.7	1.8	5.0	0.00	3.8 (0.7)	3.6 (0.5)	3.5 (0.6)	1.8 —	*	*	NS
45	5,17-DimeC_{31}	2.7	0.7	1.7	4.0	0.00	2.8 (0.7)	2.4 (0.8)	2.5 (0.5)	4.0 —	*	*	*
47	Unknown 2	0.2	0.3	0.0	1.1	0.60	0.3 (0.4)	0.1 (0.1)	0.1 (0.1)	0.0 —	*	NS	NS
48	3,7-DimeC_{31}	0.6	0.3	0.4	1.4	0.00	0.5 (0.1)	0.7 (0.5)	0.7 (0.3)	0.8 —	*	NS	NS
50	*n*-$C_{31:1}$	0.5	0.4	0.0	1.4	0.20	0.5 (0.5)	0.4 (0.4)	0.5 (0.3)	0.0 —	*	*	NS
52	13-; 15-MeC_{32}	0.6	0.3	0.0	1.4	0.05	0.8 (0.4)	0.4 (0.2)	0.5 (0.1)	0.4 —	*	*	*
54	7-; 9-MeC_{32}	0.2	0.1	0.0	0.4	0.30	0.3 (0.1)	0.1 (0.1)	0.2 (0.1)	0.0 —	*	*	NS
55	5-MeC_{32}	1.0	0.2	0.7	1.2	0.00	1.0 (0.2)	1.0 (0.2)	1.0 (0.1)	0.8 —	*	*	NS
56	2- or 4-MeC_{32}	1.5	0.6	1.0	3.5	0.00	1.7 (0.5)	1.3 (0.3)	1.3 (0.1)	3.5 —	*	*	*
57	*n*-C_{32}	0.1	0.2	0.0	0.6	0.55	0.2 (0.2)	0.1 (0.1)	0.1 (0.1)	0.0 —	*	*	*
60	5,13-; 5,17-DimeC_{35}	3.2	2.8	0.4	8.9	0.00	2.4 (2.8)	2.7 (2.2)	3.6 (2.7)	8.9 —	NS	NS	NS
61	5,17-DimeC_{37}	1.4	0.4	0.8	2.3	0.00	1.4 (0.4)	1.3 (0.5)	1.4 (0.3)	2.0 —	NS	*	NS

[a] Hydrocarbons and peak numbers are the same as those reported in Table 1 and Figure 1. Carbon number is the total number of carbons, including methyl groups.

[b] Values not necessarily zero but may have had a very low quantity (<0.1% of total hydrocarbon) or were simply not detectable for a given GLC analysis.

[c] Proportion of the 20 chromatograms where a given hydrocarbon was not detectable.

[d] Castes are SO = soldier, PS = pseudergate, NY = nymph, and AL = alate (imago).

[e] Proportions of each hydrocarbon were transformed to the arcsine of the square root of the proportion and subjected to analysis of variance. Asterisk indicates significant difference ($\alpha = 0.05$) between colony, caste, or interaction term for a given hydrocarbon. Analysis of variance was done only for hydrocarbon components detectable from individual termites from collections made in 1985. The gas chromatograph with a 0.75-mm-ID capillary column was used.

TABLE 5. STATISTICS FOR PERCENT HYDROCARBON FROM FOUR CASTES FROM EIGHT COLONIES

		Overall (N = 27)				
Peak No.[a]	Hydrocarbon[a]	$\bar{X}$	SD	Minimum value[b]	Maximum value	Proportion ≤0.1% of total[c]
5	n-C_{21}	12.7	3.6	5.5	19.4	0.00
9	2- or 4-MeC_{22}	1.0	0.3	0.4	1.7	0.00
10	3-MeC_{22}	1.0	0.4	0.3	1.9	0.00
11	n-C_{22}	2.6	0.6	1.8	3.6	0.00
16	2- or 4-MeC_{23}	15.1	5.5	6.1	25.2	0.00
17	n-$C_{23:1}$	3.0	2.0	0.8	8.2	0.00
19	n-C_{23}	33.3	6.2	21.6	43.3	0.00
23	3-MeC_{24}	8.5	2.4	3.8	14.2	0.00
25	n-C_{24}	1.4	0.4	0.1	2.4	0.00
27	2-MeC_{25}	0.3	0.3	0.0	0.7	0.41
28	n-C_{25}	18.7	8.2	10.3	42.7	0.00
29	9-; 11-MeC_{26}	0.2	0.3	0.0	1.0	0.67
31	3-MeC_{26}	0.8	0.2	0.6	1.2	0.00
33	n-C_{26}	0.5	0.5	0.0	2.0	0.30
34	2-MeC_{27}	0.0	0.2	0.0	0.1	0.96
35	n-C_{27}	0.1	0.2	0.0	0.9	0.70
48	3,7-DimeC_{31}	0.1	0.2	0.0	1.0	0.78
51	Unknown 4	0.1	0.2	0.0	0.7	0.70
57	n-C_{32}	0.1	0.1	0.0	0.4	0.78
58	Unknown 6	0.5	0.7	0.0	3.4	0.48
59	Unknown 7	0.0	0.1	0.0	0.5	0.85

[a] Hydrocarbons and peak numbers are the same as those reported in Table 1 and Figure 1. Carbon number is the total number of carbons, including methyl groups.
[b] Values not necessarily zero but may have had a very low quantity (<0.1% of total hydrocarbon) or were simply not detectable for a given GLC analysis.
[c] Proportion of the 27 chromatograms where a given hydrocarbon was not detectable.
[d] Castes are SO = soldier, PS = pseudergate, NY = nymph, and AL = alate (imago).

11-methylheneicosane (peak 6), 3,11- and 3,13-dimethylheneicosane (peak 14, which elutes at nearly the same time as the unique peak 13 found in phenotype I), 3,11- and 3,13-dimethyltricosane (peak 26), 2- or 4-methyloctacosane (peak 40), and 2- or 4-methyhentriacontane (peak 56).

Phenotype III clearly contains the simplest hydrocarbon profile of the phenotypes from California. Only 10 hydrocarbon components comprise greater than 1.0% of the total hydrocarbon fraction (Table 5). This is the only phenotype we collected from California with an olefin (peak 17). All of the other

OF *Zootermopsis* COLLECTED FROM PACIFIC GROVE, CALIFORNIA (PHENOTYPE III)

Castes[d]				*Statistical differences*[e]		
SO (N = 9)	*PS* (N = 8)	NY (N = 8)	AL (N = 2)			
$\bar{X}$ *SD*	$\bar{X}$ *SD*	$\bar{X}$ *SD*	$\bar{X}$ *SD*	Colony	Caste	Interaction
11.7 (2.5)	13.6 (4.6)	12.4 (4.1)	4.3 (2.0)	*	*	*
1.2 (0.2)	0.9 (0.2)	0.8 (0.3)	1.4 (0.1)	*	*	*
1.3 (0.3)	0.9 (0.3)	0.8 (0.5)	1.2 (0.3)	*	*	*
2.3 (0.4)	2.8 (0.5)	2.8 (0.7)	2.8 (0.3)	NS	*	NS
18.6 (3.0)	14.0 (3.5)	12.3 (7.0)	15.0 (9.6)	*	*	*
4.6 (1.9)	2.0 (0.7)	2.1 (1.9)	3.9 (1.4)	*	*	*
29.1 (4.3)	35.5 (5.0)	36.0 (7.5)	31.7 (4.5)	*	*	*
9.7 (2.4)	7.9 (1.7)	7.3 (2.6)	10.6 (0.4)	*	*	*
1.3 (0.2)	1.5 (0.3)	1.7 (0.4)	0.7 (0.9)	NS	*	*
0.4 (0.2)	0.3 (0.3)	0.3 (0.3)	0.0 (0.0)			
17.5 (5.0)	18.1 (9.5)	21.2 (10.8)	16.3 (4.3)	*	*	*
0.2 (0.2)	0.1 (0.2)	0.1 (0.2)	0.5 (0.7)			
0.9 (0.2)	0.7 (0.1)	0.8 (0.2)	0.8 (0.0)			
0.4 (0.4)	0.6 (0.5)	0.6 (0.7)	0.3 (0.4)			
0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)			
0.1 (0.1)	0.2 (0.3)	0.1 (0.2)	0.0 (0.0)			
0.1 (0.2)	0.0 (0.1)	0.0 (0.1)	0.5 (0.7)			
0.1 (0.2)	0.2 (0.3)	0.2 (0.2)	0.0 (0.0)			
0.1 (0.2)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)			
0.3 (0.5)	0.8 (1.1)	0.4 (0.4)	0.0 (0.0)			
0.0 (0.1)	0.0 (0.1)	0.1 (0.2)	0.0 (0.0)			

[e] Proportions of each hydrocarbon were transformed to the arcsine of the square root of the proportion and subjected to analysis of variance. Asterisk indicates significant difference (α = 0.05) between colony, caste, or interaction term for a given hydrocarbon. Analysis of variance was done only for hydrocarbon components detectable from individual termites from collections made in 1985. The gas chromatograph with a 0.75-mm-ID capillary column was used.

major (>5.0%) or significant (>1.0%) components of phenotype III are shared with at least one of the other phenotypes. Phenotype IV is also fairly simple, with only 13 significant components (Table 6), and is quite similar to phenotype III. The major differences are the four unique compounds: 2- or 4-methylheneicosane (peak 9), unknown 1 (peak 18) eluting between *n*-tricosene and *n*-tricosane, 7,11-dimethylpentatriacontane (peak 62), and 7,13-dimethylheptatriacontane (peak 63).

Variation in the proportions of hydrocarbons among individuals of each

TABLE 6. STATISTICS FOR PERCENT HYDROCARBON FROM FOUR CASTES FROM FIVE COLONIES OF *Zootermopsis laticeps* COLLECTED FROM RIO RICO, SANTA CRUZ COUNTY, ARIZONA (PHENOTYPE IV)

		Overall (n = 8)[b]					Castes[e]					
							SO (*N* = 2)		*PS* (*N* = 4)		*NY* (*N* = 1)	
Peak No.[a]	Hydrocarbon[a]	$\bar{X}$	SD	Minimum value[c]	Maximum value	Proportion ≤0.1% of total[d]	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*
5	*n*-C_{21}	16.3	7.9	2.5	31.2	0.00	8.6	(8.7)	20.3	(7.7)	15.4	—
9	2- or 4-MeC_{22}	1.9	0.5	1.3	2.5	0.00	2.2	(0.1)	1.7	(0.4)	2.5	—
10	3-MeC_{22}	1.6	0.4	1.1	2.2	0.00	1.8	(0.0)	1.4	(0.3)	2.2	—
11	*n*-C_{22}	2.9	0.4	2.3	3.5	0.00	3.1	(0.2)	2.9	(0.5)	3.2	—
16	2- or 4-MeC_{23}	30.3	4.9	19.3	35.7	0.00	33.4	(3.2)	29.0	(6.6)	30.2	—
17	*n*-$C_{23:1}$	3.0	1.3	1.0	5.2	0.00	3.8	(2.0)	2.4	(1.0)	2.5	—
18	Unknown 1	1.1	0.3	0.5	1.5	0.00	1.0	(0.2)	1.0	(0.5)	1.3	—
19	*n*-C_{23}	23.2	2.4	20.1	27.9	0.00	22.5	(1.1)	24.1	(2.8)	23.7	—
23	3-MeC_{24}	2.3	0.6	1.5	3.2	0.00	3.0	(0.3)	2.0	(0.4)	1.9	—
25	*n*-C_{24}	0.6	0.1	0.5	0.7	0.00	0.6	(0.0)	0.5	(0.0)	0.7	—
27	2-MeC_{25}	0.7	0.2	0.3	0.9	0.00	0.8	(0.1)	0.7	(0.3)	0.8	—
28	*n*-C_{25}	7.8	1.1	5.5	8.7	0.00	8.5	(0.2)	7.0	(1.2)	8.6	—
33	*n*-C_{26}	0.4	0.2	0.0	0.8	0.13	0.7	(0.2)	0.3	(0.2)	0.5	—
35	*n*-C_{27}	2.0	0.8	1.0	3.2	0.00	2.8	(0.6)	1.5	(0.4)	1.5	—
62	7,11-DimeC_{37}	1.5	0.7	1.0	3.2	0.00	2.3	(1.3)	1.2	(0.3)	1.2	—
63	7,13-DimeC_{39}	4.3	1.2	3.5	6.3	0.00	5.0	(1.9)	3.8	(0.3)	3.7	—

[a] Hydrocarbons and peak numbers are the same as those reported in Table 1 and Figure 1. Carbon number is the total number of carbons, including methyl groups.
[b] Includes one analysis of a group composed of four pseudergates and one soldier.
[c] Values not necessarily zero but may have had a very low quantity (<0.1% of total hydrocarbon) or were simply not detectable for a given GLC analysis.
[d] Proportion of the eight chromatograms where a given hydrocarbon was not detectable.
[e] Castes are SO = soldier, PS = pseudergate, and NY = nymph.

caste, among castes within a colony, and among colonies within phenotypes I, II, and III was examined during the first hydrocarbon analyses with individual termites. By using individual termites and the wide-bore (0.75 mm ID) capillary column, we were not able to separate and quantify all hydrocarbon components. Furthermore, in many of the individual chromatograms, one or more of the components were not detected, probably because of insufficient sample. In any case, we conducted analyses of variance of the transformed proportions of each hydrocarbon component (or coeluting components) for phenotypes I, II, and III and found numerous statistically significant differences (Table 3–5). For many of the hydrocarbon components, we observed statistically significant interactions among colonies and castes. This implies that caste-specific blends of hydrocarbons within a phenotype are not consistent from colony to colony. Furthermore, the minimum and maximum values for any given hydrocarbon component for colonies or castes were quite distant.

Much better hydrocarbon resolution and more consistent quantification were achieved with the 0.32-mm-ID capillary column and extraction of five individual termites (of the same caste and from the same colony). Quantification of the percentage of each hydrocarbon component for the four phenotypes of *Zootermopsis* are presented in Tables 3–6. The data for the California phenotypes are more precise because of the increased sample size: 18 colonies for phenotype I, seven colonies for II, and eight colonies for III. Although we did not statistically analyze these data, from the range of the colony and caste means it appears that colony-to-colony differences account for most of the variation. With the exception of the underrepresented alates, variation among the castes was small. Many more minor components ($<0.5\%$ of the total) were detected with the 0.32-mm-ID capillary column. The minimum value for nearly all of the minor components is zero because the component was very low in quantity or was simply not detected for a given GLC analysis (Tables 3–6).

These data suggest caution in assuming that cuticular hydrocarbons serve as semiochemical cues for caste recognition (Howard and Blomquist, 1982). None of the previous studies involved bioassays to test the caste-recognition hypothesis (Howard et al., 1978, 1982; Blomquist et al., 1979), and such bioassays will be very difficult to execute. The information content in these cuticular hydrocarbon mixtures is great. However, if members of a colony are, in fact, cognizant of the caste of each of the other members that they encounter, a chemical cue could involve many classes of compounds other than or in addition to hydrocarbons. This question remains to be resolved, and more data are needed to prove or disprove the hypothesis that hydrocarbons act as caste-recognition cues.

The results of this study clearly identify four distinct, consistent cuticular hydrocarbon phenotypes. Thus far, we (M.I.H., M.P., and L.J.N.) have collected 143 additional *Zootermopsis* spp. colonies throughout much of northern

California to study the geographic distribution of these phenotypes. We have also received live samples from Washington, Oregon, the San Francisco Bay area, and southern California, and have yet to see an additional hydrocarbon phenotype.

Agonistic bioassays pairing a soldier of one phenotype and three pseudergates or nymphs of the same phenotype or a different phenotype indicate that these cuticular hydrocarbon phenotypes are capable of recognizing one another (B.L. Thorne, personal communication). Preliminary results indicate that an encounter between a soldier from one colony and nymphs from a second colony of the same chemical phenotype very rarely produces a highly aggressive response (mandible snapping or biting). Similar encounters between chemical phenotypes II and III virtually always evoke an immediate soldier biting response, as do encounters between soldiers of phenotypes I, II, or III and nymphs of phenotype IV. Finally, encounters between soldiers of phenotype I and nymphs of either phenotype II or III or between soldiers of phenotype II or III and nymphs of phenotype I produce occasional highly aggressive responses that seem to depend on the individual soldier involved and perhaps on the nymphs' parental colony. Agonistic experiments never evoke intraphenotype aggression. All other pairwise interchemical phenotype interactions elicit highly aggressive responses all or part of the time. These results are potentially consistent with the hypothesis that individual termite recognition is based, at least in part, on cuticular hydrocarbon composition. Further evaluation of this behavior is obviously necessary and is the subject of continuing studies (Haverty and Thorne, unpublished).

To summarize the results of this study, identification of four consistent, unique cuticular hydrocarbon phenotypes from three extant species, and inconsistent identification of species using existing morphological keys, should alert insect biochemists, systematists interested in chemotaxonomy with cuticular hydrocarbons, and termite biologists to a major concern. If there are truly only three extant species, then we cannot accept the hypothesis that cuticular hydrocarbon profiles in this genus are species specific. Conversely, if cuticular hydrocarbon profiles are truly species specific, then there is at least one new, undescribed species of *Zootermopsis*. It would appear 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. We feel that it is extremely important to clarify the taxonomic status of this genus, as it has been frequently used as a model for behavioral and caste-determination studies. Determination of specific status of each phenotype may take many different approaches, including bioassays of aggressive behavior (in progress), breeding experiments (in progress), correlation of hydrocarbon chemistry and external morphology from an extensive geographic collection (in progress), enzyme analysis, DNA hybridization, etc. These studies will probably result in a revision of the genus.

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