Cuticular Hydrocarbons as Chemotaxonomic Characters for Bark Beetles: *Dendroctonus ponderosae*, *D. jeffreyi*, *D. brevicomis*, and *D. frontalis* (Coleoptera: Scolytidae)

MARION PAGE,[1] LORI J. NELSON,[1] MICHAEL I. HAVERTY,[1] AND GARY J. BLOMQUIST[2]

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ABSTRACT Cuticular hydrocarbons were extracted from adults of the sibling species *Dendroctonus ponderosae* Hopkins and *D. jeffreyi* Hopkins and from the closely related species *D. brevicomis* LeConte and *D. frontalis* Zimmermann. Four distinct chemical mixtures of cuticular hydrocarbons were identified by gas chromatography–mass spectrometry. Five classes of hydrocarbons were present: *n*-alkanes, alkenes, terminally branched methylalkanes, internally branched monomethylalkanes, and dimethylalkanes. Each of the two species pairs, *D. ponderosae–D. jeffreyi* and *D. brevicomis–D. frontalis*, had similar hydrocarbon profiles, yet unique hydrocarbon components were found. The best hydrocarbons for separating *D. ponderosae* and *D. jeffreyi* are the 3,7-dimethylalkanes. Obvious diagnostic hydrocarbon components are the 3,X-dimethylnonacosanes in *D. frontalis* and the homologous series of *n*-alkanes from *n*-hentriacontane to *n*-pentatriacontane in *D. brevicomis*. Host tree and geographic location did not qualitatively affect the cuticular hydrocarbon composition of *D. frontalis*. Hydrocarbon patterns of male and female *D. frontalis* were qualitatively and quantitatively similar. The species-specific nature of cuticular hydrocarbon mixtures in these four species and their potential for nondestructive species discrimination are discussed.

KEY WORDS Insecta, *Dendroctonus*, chemotaxonomy, cuticular lipids

HYDROCARBONS OCCUR in the cuticular lipids of all insects and are usually the predominant class of cuticular lipids (Blomquist & Dillwith 1985). In some insect species, hydrocarbons constitute >90% of the surface lipids. Cuticular hydrocarbon mixtures may be simple or exceedingly complex. The complex nature of insect cuticular hydrocarbon blends led some authors to suggest that hydrocarbon composition might be used to identify insect species (Jackson & Blomquist 1976, Lockey 1976, Howard & Blomquist 1982), which indeed was done (Carlson & Service 1980, Lockey 1981, Howard et al. 1982, Carlson & Bolten 1984, Haverty et al. 1988).

In the Coleoptera, the species-specificity of cuticular hydrocarbons has been studied most extensively at the family level in the Tenebrionidae. The species examined were found to have distinctive hydrocarbon compositions. Hydrocarbon mixtures are more similar within a genus than among genera (Lockey 1978; 1979a,b; 1980; 1981; 1982a,b,c; 1984a,b; 1985a,b). In an extensive survey of the Coleoptera, Jacob (1979) suggested that species in other families can be separated using this technique. Examination of the cuticular lipids of 13 species in eight superfamilies revealed significant differences in the composition of hydrocarbons that may be of "taxonomic importance" at the family level (Jacob 1979).

To date, there has been only one published study of the cuticular hydrocarbons of scolytid beetles (Page et al. 1990). In addition, there are no published reports correlating cuticular hydrocarbon mixtures of any Coleoptera with other measures of biological similarity or diversity; i.e., pheromone composition, cytogenetics, mating behavior, or morphometric analyses. For the four species of *Dendroctonus* characterized in this paper, an abundance of information is available on ecological classifications (i.e., location of larval galleries, patterns of adult galleries, and species of host attacked) (Mitton & Sturgeon 1982) and on taxonomic classifications based on host-finding and mating behavior, pheromone chemistry (D. L. Wood 1982), and classical morphological traits (S. L. Wood 1982).

In this investigation, we characterize the cuticular hydrocarbon mixtures of two sibling species and one pair of closely related species in the genus *Dendroctonus*. The mountain pine beetle, *D. ponderosae* Hopkins, and the Jeffrey pine beetle, *D. jeffreyi* Hopkins, are sibling species and are separated with difficulty on the basis of morphological characters (S. L. Wood 1982, Lanier & Wood 1968). The western pine beetle, *D. brevicomis* LeConte, can be distinguished from its close relative, the southern pine beetle, *Dendroctonus frontalis* Zimmermann, by several morphological characters such as larger body size, and on the basis of geographical distribution (S. L. Wood 1982, Lanier et al. 1988). The species-specific nature of the cuticular hydrocarbon mixtures in these four species and the potential of using hydrocarbons of museum speci-

[1] Pacific Southwest Forest & Range Experiment Station, USDA, Forest Service, P.O. Box 245, Berkeley, Calif. 94701.
[2] Department of Biochemistry, University of Nevada, Reno, Nev. 89557.

mens for nondestructive species discrimination are discussed.

Materials and Methods

Adult bark beetles were collected from naturally infested trees. Mountain pine beetles were collected from lodgepole pine (*Pinus contorta* Douglas) in the Hat Creek Ranger District, Lassen National Forest, near Burney, Calif., and from sugar pine (*P. lambertiana* Douglas) in the Minarets Ranger District, Sierra National Forest, near Bass Lake, Calif. Jeffrey pine beetles were collected from Jeffrey pine (*P. jeffreyi* Greville & Balfour) in the Hat Creek Ranger District, Lassen National Forest, near Manzanita Lake, Calif., and in the Minarets Ranger District near North Fork, Calif. Western pine beetles were collected from ponderosa pine (*P. ponderosae* Lawson) in the Placerville Ranger District, El Dorado National Forest, near Camino, Calif., and in the Bass Lake Ranger District, Sierra National Forest, near Oakhurst, Calif. Southern pine beetles were collected from loblolly pine (*P. taeda* L.) in the Catahoula Ranger District, Kisatchie National Forest, La., and near Camp Livingston in Rapids Parish, La. The last-named species also was collected from shortleaf pine (*P. echinata* Miller) in the Catahoula Ranger District, Kisatchie National Forest, La., and on longleaf pine (*P. palustris* Miller) in Rapids Parish, La. Trunks and bark of infested trees containing Jeffrey, mountain, and western pine beetles were removed from the field and stored in laboratory emergence cages (Browne 1972). As adults emerged from the bark, they were collected every 2–3 d, then were frozen at −20°C until hydrocarbons were extracted.

Beetles, both males and females, were removed from the freezer and allowed to warm to ambient temperature. Cuticular lipids were then extracted by immersing 15–50 beetles, as a group, in 10 ml hexane for 10 min. After extraction, hydrocarbons were separated from other components by pipetting the 10-ml extract and an additional 8 ml of hexane through 3 cm of activated BioSil-A (Bio-Rad Laboratories, Richmond, Calif.) in Pasteur pipette minicolumns (Page et al. 1990). All hydrocarbon extracts were evaporated to dryness under a stream of nitrogen and redissolved in 30 μl hexane for analysis. After extraction, beetles were stored in 70% ethanol as voucher specimens. All voucher specimens will be deposited in the Essig Museum, Department of Entomology, University of California, Berkeley.

Hydrocarbons were identified by gas chromatography-mass spectrometry (GC-MS). Analyses were performed on a Hewlett Packard 5890 gas chromatograph (Hewlett-Packard Company, Avondale, Pa.) equipped with a Hewlett Packard 5970B mass selective detector interfaced with a Hewlett Packard Chemstation computer. The GC-MS system was equipped with a fused silica capillary column (30 m by 0.2 mm ID, HP-1) and operated in the split mode (with a split ratio of 20:1). Each hydrocarbon mixture, for all *Dendroctonus* populations sampled, was analyzed by a temperature program from 200 to 320°C at 3°C/min with a final hold of 20 min.

Electron impact mass spectra were obtained at 70 eV. Retention times and mass spectra of extracted *n*-alkanes were compared with external standards. Alkenes and methyl-branched alkanes were tentatively identified by calculating their equivalent chain lengths (ECL) (Nelson & Sukkestad 1970, Jackson & Blomquist 1976). Mass spectra of alkenes and methylalkanes were interpreted as described by Nelson et al. (1972), Nelson (1978), Pomonis et al. (1978), and Blomquist et al. (1987). Alkenes were isolated by silver nitrate column chromatography, and the position of the double bonds was determined in selected alkenes by GC-MS analysis of the monomethoxy and dimethoxy derivatives of the parent compounds after methoxy mercuration–demercuration (Blomquist et al. 1980).

Results

Adults of each species pair, *D. ponderosae–D. jeffreyi* (Table 1; Fig. 1) and *D. brevicomis–D. frontalis* (Table 2; Fig. 2), had similar hydrocarbon components. They consisted of homologous series of *n*-alkanes; *n*-alkenes; 2- or 4-methylalkanes; 3-methylalkanes; internally branched monomethylalkanes with methyl branches on carbons 5, 7, 9, 11, 13, and 15; and isomeric mixtures of dimethylalkanes. No trimethylalkanes were identified.

In all four species, *n*-alkanes constitute the greatest percentage of the total hydrocarbon component. Homologous series ranging from *n*-docosane to *n*-pentatriacontane make up the *n*-alkane fraction. Odd-numbered alkanes (*n*-tricosane, *n*-pentacosane, *n*-heptacosane, and *n*-nonacosane) are the predominant compounds and account for approximately 60% of the total hydrocarbon. The most abundant *n*-alkanes are *n*-pentacosane and *n*-heptacosane.

The *n*-alkenes were identified by their diagnostic MS ions, which were two mass units less than the M^+ ion for the corresponding *n*-alkane and by their separation from alkanes by $AgNO_3$–Bio Sil A (Bio-Rad Laboratories, Richmond, Calif.) column chromatography. *n*-Pentacosene and *n*-heptacosene are present in the sibling species, whereas only *n*-pentacosene was detected in western and southern pine beetles. Mass spectra of the monomethoxy and dimethoxy derivatives of *n*-pentacosene and *n*-heptacosene indicate that the major isomer had a double bond at the 9 carbon; the 7 isomer was present in lesser amounts (Fig. 3).

It is difficult to distinguish 2- from 4-methylalkanes (Blomquist et al. 1987); therefore, all such peaks are presented as 2- or 4-methylalkanes. Of the four *Dendroctonus* species examined here, 2- or 4-methylalkanes were detected in all but the Jeffrey pine beetle. Peak 14 from *D. brevicomis*

Table 1. Cuticular hydrocarbons of adult *D. ponderosae* and *D. jeffreyi* (mixed sexes)

GC peak[a]	Hydrocarbon	Carbon no.	Equiv. chain length	Relative abundance[b]		Diagnostic MS ions
				D. ponderosae	*D. jeffreyi*	
1	*n*-Docosane	22	22.0	tr	tr	310
2	*n*-Tricosane	23	23.0	+++	+++	324
3	7-; 9-; 11-Methyltricosane[c]	24	23.3	tr	+	112/113, 252/253; 140/141, 224/225; 168/169, 196/197
4	5-Methyltricosane	24	23.5	+	+	84/85, 280/281
5	3-Methyltricosane	24	23.7	++	+	280/281, 308/309
6	*n*-Tetracosane	24	24.0	++	+	338
7	3,7-; 3,15-; 3,17-Dimethyltricosane[c]	25	24.1	++	o	127, 252/253, 323; 140/141, 239, 323; 112/112, 267, 323
8	6-Methyltetracosane +	25	24.6	++	o	98/99, 280/281
	2 or 4-Methyltetracosane[c]	25		++	o	308/309, 336/337
9	7-; 9-Pentacosene	25:1	24.8	++	++	350 (157, 171, 255, 269)[d]
10	*n*-Pentacosane	25	25.0	+++	+++	352
11	9-; 11-; 13-Methylpentacosane[c]	26	25.3	+	+	140/141, 252/253; 168/169, 224/225; 196/197
12	7-Methylpentacosane	26	25.4	+	+	112/113, 280/281
13	5-Methylpentacosane	26	25.5	++	+	84/85, 308/309
14	3-Methylpentacosane	26	25.7	++	++	308/309, 336/337
15	7,17-Dimethylpentacosane	27	25.8	o	+	112/113, 140/141, 267, 295
16	5,13-; 5,15-;	27	25.9	o	+	84/85, 168/169, 211, 323;
	5,15-; 5,17-Dimethylpentacosane[c]			+	o	84/85, 168/169, 239, 323; 84/85, 140/141, 267, 323
17	*n*-Hexacosane	26	26.0	++	++	366
18	3,7-Dimethylpentacosane	27	26.1	+++	o	127, 280/281, 351
19	7-; 8-Methyhexacosane[c]	27	26.2	+	o	112/113, 294/295; 126/127, 280/281
20	5-Methylhexacosane	27	26.5	+	o	84/85, 322/323
21	3-Methylhexacosane	27	26.7	+	o	322/323, 350/351
22	9-Heptacosene	27:1	26.8	tr	tr	378
23	*n*-Heptacosane	27	27.0	+++	+++	380
24	9-; 11-; 13-Methylheptacosane[c]	28	27.3	tr	+	140/141, 280/281; 168/169, 252/253, 196/197, 224/225
25	7-Methylheptacosane	28	27.4	o	tr	112/113, 308/309
26	5-Methylheptacosane	28	27.5	++	++	84/85, 336/337
27	3-Methylheptacosane	28	27.7	+	++	336/337, 364/365
28	5,15-; 5,17-Dimethylheptacosane[c]	29	27.9	+	+	84/85, 196/197, 239, 351; 84/85, 168/169, 267, 351
29	*n*-Octacosane	28	28.0	tr	+	384
30	3,7-Dimethylheptacosane	29	28.1	+	o	127, 308/309, 379
31	*n*-Nonacosane	29	29.0	tr	tr	408
32	5-Methylnonacosane	30	29.5	o	tr	84/85, 364/365
33	5,17-Dimethylnonacosane	31	29.8	o	tr	84/85, 196/197, 267, 379
34	11-Methylpentatriacontane	36	35.3	o	++	168/169, 364/365
35	11-Methylheptatriacontane	38	37.3	o	++	168/169, 392/393

[a] From Fig. 1.
[b] +, 0.5–1%; ++, 1–5%; +++, >5%; tr, trace (<0.5%); o, not detected.
[c] These components coelute.
[d] Diagnostic ions from methoxy derivatives.

(Table 2) illustrates this situation and is interpreted as 2- or 4-methylhexacosane. The high abundance of M-43 ion at m/z 337 is typical of 2-methylhexacosane; however, the mass spectrum showed varying intensities of the M-71–M-72 ion pair, indicating the possible presence of 4-methylhexacosane. Mountain pine beetle was the only one of the sibling species pair to produce 2- or 4-methyltetracosane, which occurs as a component in an isomeric mixture of another monomethylalkane (Table 1).

Peaks with equivalent chain lengths of about 0.3 carbon units less than corresponding *n*-alkanes and with spectra with strong $(M-29)^+$ ions and weaker $(M-57)^+$ ions were identified as 3-methylalkanes. Homologous series of 3-methylalkanes are present in all species. The two sibling species (*D. ponderosae* and *D. jeffreyi*) contain the homologous series 3-methyltricosane, 3-methylpentacosane, and 3-methylheptacosane. Western and southern pine beetles have a more complete series of 3-methylalkanes. Western pine beetle produces the series 3-methyltetracosane to 3-methyloctacosane, and southern pine beetle produces the series 3-methyltetracosane to 3-methylnonacosane.

Both species pairs appear to have nearly identical homologous series of the internally branched 7- and 5-methylalkanes. Mass spectra of 7-methylalkanes consistently show ion doublets at m/z 112/113, indicating a methyl branch on carbon 7. 7-methylalkanes in the sibling species are 7-methyltricosane, 7-methylpentacosane, and 7-methylheptaco-

Table 2. Cuticular hydrocarbons of adult *D. brevicomis* and *D. frontalis* (mixed sexes)

GC peak[a]	Hydrocarbon	Carbon no.	Equiv. chain length	Relative abundance[b]		Diagnostic MS ions
				D. brevicomis	*D. frontalis*	
1	*n*-Heneicosane	21	21.0	tr	tr	296
2	*n*-Docosane	22	22.0	+	tr	310
3	*n*-Tricosane	23	23.0	++	tr	324
4	*n*-Tetracosane	24	24.0	++	tr	338
5	9-; 7-Pentacosene	25:1	24.6	+	tr	350 (157, 171, 255, 269); (129, 143, 283, 297)[d]
6	3-Methyltetracosane	25	24.7	tr	tr	322/323, 336/337
7	*n*-Pentacosane	25	25.0	+++	+++	352
8	7-; 9-; 11-; 13-Methylpentacosane[c]	26	25.3	+	+	112/113, 280/281; 140/141, 252/253; 168/169, 224/225; 196/197
9	5-Methylpentacosane	26	25.5	+	+	84/85, 308/309
10	3-Methylpentacosane	26	25.7	+	+	308/309, 336/337
11	*n*-Hexacosane	26	26.0	++	++	366
12	13-; 15-Methylhexacosane[c]	27	26.3	+	+	196/197, 210/211; 182/183, 224/225
13	5-Methylhexacosane	27	26.5	tr	tr	84/85, 322/323
14	2- or 4-Methylhexacosane + 3-Methylhexacosane[c]	27	26.7	+	tr	336/337, 365/366; 322/323, 350/351
15	*n*-Heptacosane	27	27.0	+++	+++	380
16	9-; 11-; 13-Methylheptacosane[c]	28	27.3	+++	+++	140/141, 280/281; 168/169, 252/253; 196/197, 224/225
17	7-Methylheptacosane	28	27.4	++	++	112/113, 308/309
18	5-Methylheptacosane	28	27.5	++	++	84/85, 336/337
19	9,15-; 11,15-Dimethylheptacosane[c]	29	27.6	+	+	140/141, 196/197, 239, 295; 168/169, 196/197, 239, 267
20	3-Methylheptacosane	28	27.7	++	++	336/337, 364/365
21	5,13-; 5,15-; 5,17-Dimethylheptacosane[c]	29	27.8	++	o	84/85, 224/225, 211, 351; 84/85, 196/197, 239, 351; 84/85, 168/169, 267, 351;
	5,9-; 5,11-; 5,15-Dimethylheptacosane[c]	—		o	++	84/85, 280/281, 155, 351; 84/85, 252/253, 183, 351; 84/85, 196/197, 239, 351
22	*n*-Octocosane	28	28.0	++	+	394
23	3,13-; 3,15-; 3,17-; 3,7-Dimethylheptacosane[c]	29	28.05	+	+	224/225, 211, 379; 196/197, 239, 379; 168/169, 267, 379; 127, 308/309, 379
24	9-; 11-; 12-; 13-; 15-Methyloctacosane[c]	29	28.3	+	+	140/151, 294/295; 168/169, 266/267; 182/183, 252/253; 196/197, 238/239; 224/225, 210/211
25	7-Methyloctacosane	29	28.4	+	+	112/113, 322/323
26	5-Methyloctacosane	29	28.5	tr	tr	84/85, 350/351
27	2- or 4-Methyloctacosane	29	28.6	tr	+	365/366, 392/393
28	3-Methyloctacosane	29	28.72	tr	+	378/379, 350/351
29	*n*-Nonacosane	29	29.0	++	++	408
30	3,17-Dimethyloctacosane	30	29.1	o	tr	182/183, 267, 323
31	11-; 13-; 15-Methylnonacosane[c]	30	29.3	+	+	169/169, 280/281; 196/197, 252/253; 224/225
32	9-Methylnonacosane	30	29.35	o	++	140/141, 308/309
33	7-Methylnonacosane	30	29.4	+	++	112/113, 336/337
34	5-Methylnonacosane	30	29.5	+	++	84/85, 366/367
35	13,17-Dimethylnonacosane	31	29.6	+	tr	196/197, 267
36	9,13-; 9,15-; 9,17-Dimethylnonacosane[c]	31	29.65	+	tr	140/141, 252/253, 211, 323;
		31	29.65	+	tr	140/141, 224/225, 239, 323; 140/141, 196/197, 267, 323
37	3-Methylnonacosane	30	29.7	o	++	364/365, 392/393
38	7,17-Dimethylnonacosane	31	29.8	++	o	112/113, 196/197, 267, 351
39	5,17-Dimethylnonacosane	31	29.85	++	++	84/85, 196/197, 267, 351
40	*n*-Triacontane + 3,7-Dimethylnonacosane[c]	30	30.0	tr	o	422 127, 336/337, 407
41	3,17-Dimethylnonacosane + 3,7-Dimethylnonacosane[c]	31	30.1	o	++	196/197, 267, 407;
				o	+	127, 336/337, 407
42	*n*-Hentriacontane	31	31.0	+	o	436
43	3,7-Dimethyltriacontane	32	31.1	o	tr	127, 350/351, 421
44	11-Methylhentriacontane	32	31.3	tr	o	168/169, 308/309
45	*n*-Docotriacontane	32	32.0	+	o	450
46	*n*-Tritriacontane	33	33.0	+	o	464
47	*n*-Tetratriacontane	34	34.0	+	o	478
48	*n*-Pentatriacontane	35	35.0	+	o	492

[a] From Fig. 2.
[b] +, 0.5–1%; ++, 1–5%; +++, >5%; tr, trace (<0.5%); o, not detected.
[c] These components coelute.
[d] From mass spectrum of methyoxy derivatives.

sane (detected as trace amounts only in *D. jeffreyi*). 7-Methylpentacosane, 7-methylheptacosane, 7-methyloctacosane, and 7-methylnonacosane were identified in western and southern pine beetles. Mass spectra of 5-methylalkanes consistently show ion doublets at m/z 84/85, indicating a methyl branch on carbon 5. The sibling species produce the complete homologous series 5-methyltricosane, 5-methylpentacosane, and 5-methylheptacosane. The other species pair produces the series 5-methylpentacosane to 5-methylnonacosane. *D. jeffreyi* also produces the latter compound, but this is detected only in trace amounts.

Almost all the other internally branched monomethylalkanes elute as complex, isomeric mixtures with methyl branches on odd-numbered carbons 9 through 15. Their mass spectra have strong m/z ions at 140/141, 168/169, 196/197, and 224/225, indicating cleavage at the branch points at 9, 11, 13, and 15 carbons, respectively. The sibling species produce mixtures of 9-, and 11-methyltricosane; 9-, 11-, and 13-methylpentacosane; and 9-, 11-, and 13-methylheptacosane. Jeffrey pine beetle also appears to produce significant amounts ($\geq$1.0%) of a homologous series of 11-methylalkanes: 11-methylpentatriacontane and 11-methylheptatriacontane. *D. brevicomis* and *D. frontalis* have the same three-component, isomeric mixtures of hydrocarbons with 25, 26, 27, and 28 carbons in the parent chain. The 15-methyl isomers are present in the components containing 28 and 29 carbons in the parent chain.

All the dimethylalkanes in the four species of *Dendroctonus* have methyl branches on odd-numbered carbons separated by an odd number of carbons, which is common in insects (Blomquist et al. 1987). Most of the dimethylalkanes have methyl branches separated by three carbons. They include 3,7-dimethylalkanes (*D. ponderosae*, *D. brevicomis*, *D. frontalis*), 9,13-, 11,15-, and 13,17-dimethylalkanes (*D. brevicomis*, *D. frontalis*), and 5,9-dimethylheptacosane (*D. frontalis*). The mass spectrum of peak 18 (3,7-dimethylpentacosane) from *D. ponderosae* is representative of the spectra of this branching pattern (Fig. 4A). Major fragment ions at m/z 127, 280/281, and 351, along with the observed odd/even mass ratios, support this interpretation. The $(M\text{-}29)^+$ and $(M\text{-}57)^+$ ions at m/z 351 and 323 localize one methyl branch on the 3 carbon. The ions at m/z 280/281 and 127 indicate that the other methyl branch is on carbon 7. The remainder of the dimethylalkanes have methyl branches separated by 5, 7, 9, 11, and 13 carbons.

The hydrocarbon mixtures of *D. brevicomis* and *D. frontalis* contain significant amounts (>0.5%) of 9,15-dimethylheptacosane (peak 19) and 9,15-dimethylnonacosane (peak 36), which have internal methyl branches separated by five carbons. Dimethylalkanes with both methyl branches positioned internally elute about 1.4 ECL in front of the corresponding n-alkane (Tables 1 and 2;

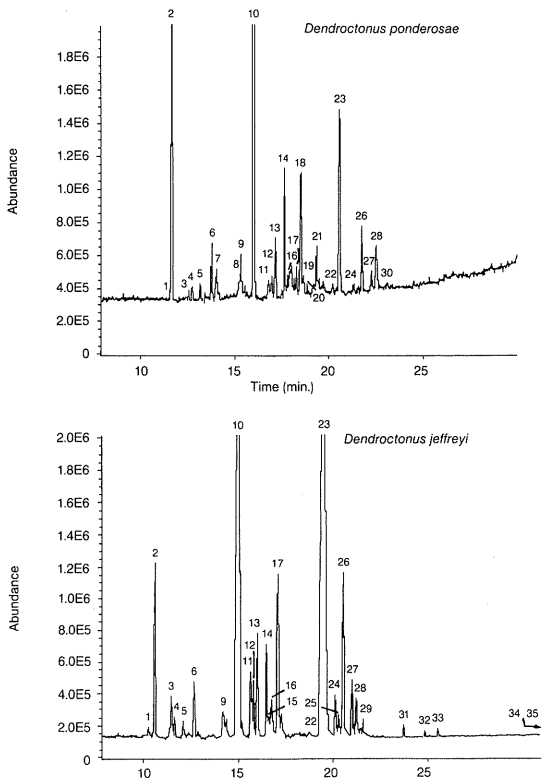

Fig. 1. Total ion chromatograms of cuticular hydrocarbons from *D. ponderosae* and *D. jefferyi*.

Blomquist et al. 1987). The fragments at m/z 140/141 and 196/197 of 9,15-dimethylheptacosane, where the even/odd ratio is greater than one, arise from cleavage internal to each of the methyl groups. The ion fragments at m/z 239 and 295, in which the odd-numbered ions predominate, arise from cleavage external to the two methyl groups. The mass spectra of the other internally branched dimethylalkanes were interpreted in a similar manner (Tables 1 and 2).

In all four species of *Dendroctonus*, all dimethylalkanes separated by 7, 9, 11, and 13 carbons are the 3,X isomers and 5,X isomers, which often occur together in hydrocarbon mixtures (Blomquist et al. 1987). The only exceptions are the 7,17-dimethylalkanes. The 3,X-dimethylalkanes elute about 0.9 carbon units in front of the corresponding n-alkane. The only homologous series of 3,X-dimethylalkanes is that of *D. ponderosae*: 3,7-dimethyltricosane, 3,7-dimethylpentacosane, and 3,7-dimethylheptacosane. 3,7-Dimethyltricosane elutes in an isomeric mixture of 3,15- and 3,17-dimethyltricosane (Fig. 4B).

The 5,X-dimethylalkanes have ECL values of 0.2 less than the n-alkane one carbon shorter (Lockey 1984a). Representatives of these dimethylalkanes with 7 carbon spacing are the 5,13-dimethylalkanes in *D. jeffreyi* and *D. brevicomis*. The

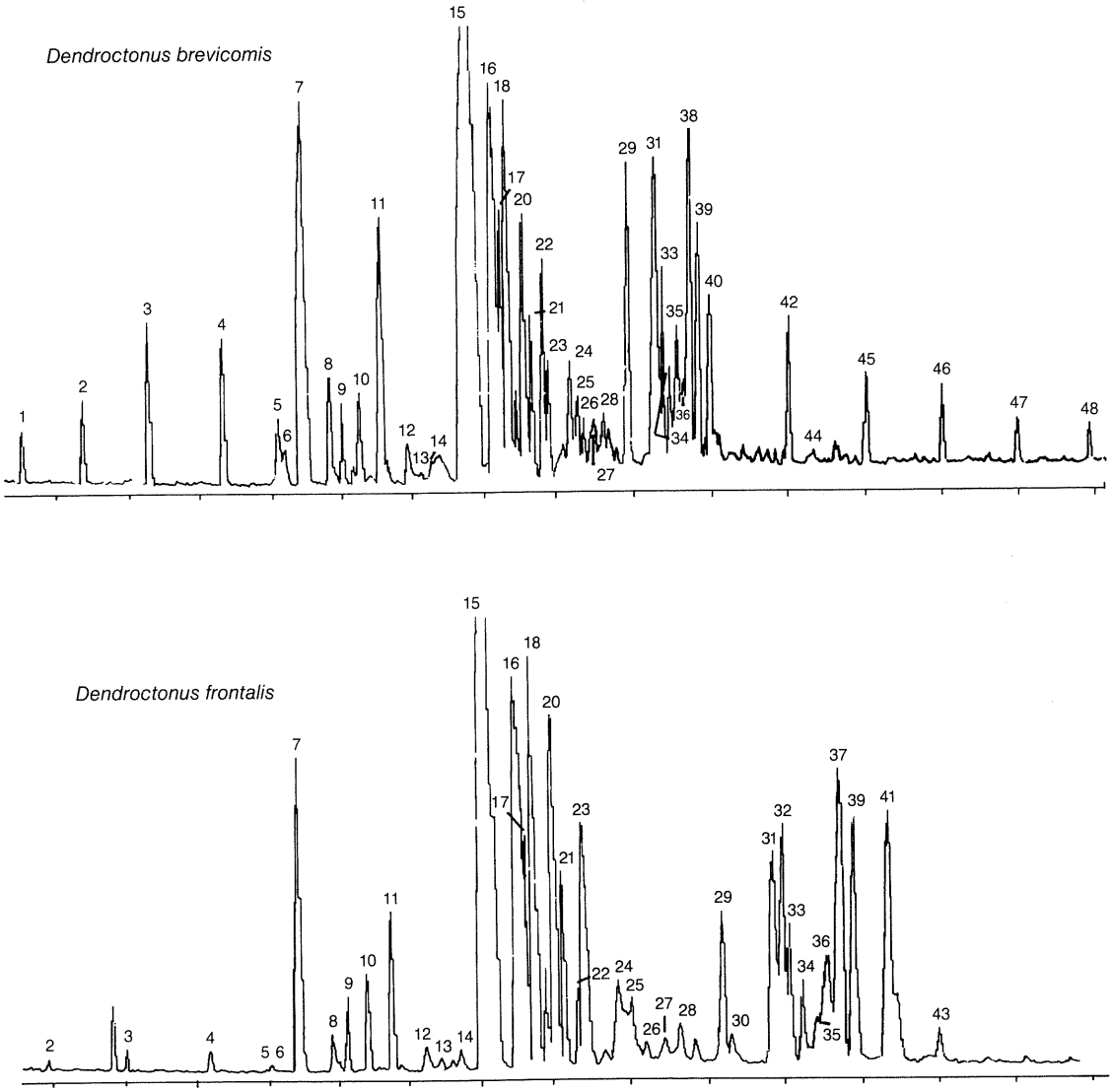

Fig. 2. Total ion chromatograms of cuticular hydrocarbons from *D. brevicomis* and *D. frontalis*.

components with methyl branches separated by 9 methylene groups include the 5,15-dimethylalkanes in all species. The dimethylalkanes with 11 carbons separating methyl branches were 5,17-dimethylheptacosane in *D. ponderosae*, *D. jeffreyi*, and *D. brevicomis* and 5,17-dimethylnonacosane in *D. jeffreyi*, *D. brevicomis*, and *D. frontalis*. The 7,X-dimethylalkanes elute before the 5,X isomers. 7,17-Dimethylpentacosane (peak 15) in *D. jeffreyi* (Fig. 5A) and 7,17-dimethylnonacosane (peak 38) in *D. brevicomis* (Fig. 5B) are representatives of dimethylalkanes with nine methylene units between methyl branches.

Stock & Amman (1985), using isoenzymes, concluded that host tree species were significantly correlated with genotype among mountain pine beetles. However, our characterization of the cuticular hydrocarbons of southern pine beetles from three hosts (longleaf, shortleaf pine, and loblolly pine in three different geographical areas) strongly suggests that host does not influence cuticular hydrocarbon composition (Table 3). There are no qualitative differences in hydrocarbon profiles; only quantitative differences exist. For example, the *n*-heptacosane represents 13% of the total hydrocarbons in beetles from longleaf pine, 17% and 25% in specimens from loblolly, and 36% in a population from shortleaf pine. Unlike the results of Jacob (1978) with the beetle *Rhagonychia fulva* Scopoli, cuticular hydrocarbon composition of male and female southern pine beetles from loblolly pine were quantitatively similar (Table 4).

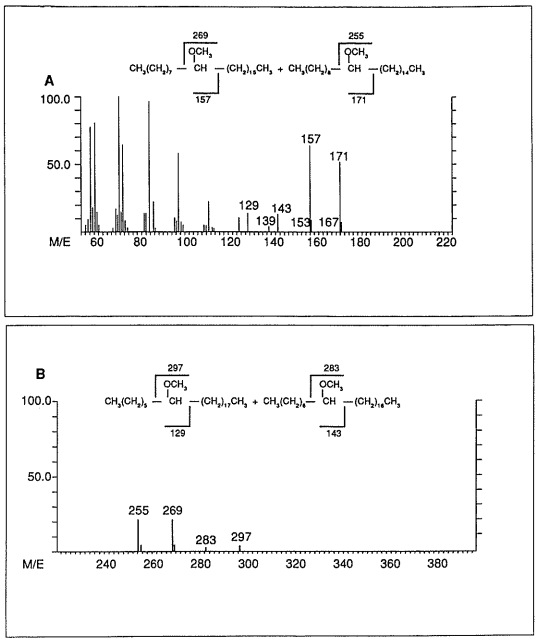

Fig. 3. EI mass spectra of (A) monomethoxy and (B) dimethoxy derivations of 9- and 7-pentacosene from *D. ponderosae*.

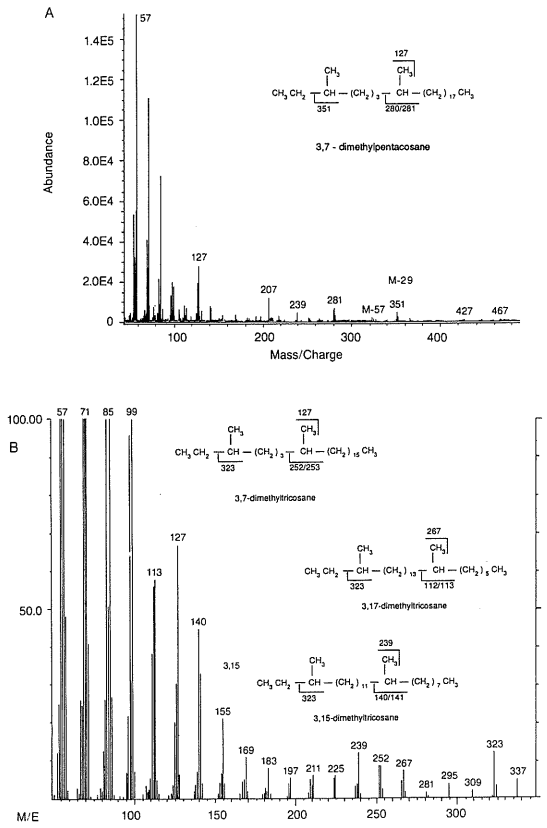

Fig. 4. EI mass spectra. (A) Peak 18 in Fig. 1, identified as 3,7-dimethylpentacosane. (B) Peak 7 in Fig. 1, identified as 3,7-; 3,15-; and 3,17-dimethyltricosane from *D. ponderosae*.

Discussion

The results presented here demonstrate that cuticular hydrocarbon mixtures of adults are useful as taxonomic characters for separation of the species pairs of *Dendroctonus* we examined in this study. Because it is difficult to separate adults in these pairs solely on the basis of external morphological characters, their host specificity and geographical range often are used to delineate species. The hydrocarbon profiles of the sibling species and the two closely related species of *Dendroctonus* we discuss in this paper are quite similar among pairs, yet different enough to separate species. Our results corroborate results from genetic analyses (Higby & Stock 1982), karyotype differences (Lanier & Wood 1968), and identification of pheromones for the two species pairs (D. L. Wood 1982). Our comparisons of cuticular extracts from adults suggest that internally branched monomethylalkanes and dimethylalkanes are meaningful diagnostic characters.

Dendroctonus ponderosae and *D. jeffreyi* are separated on the basis of morphology with great difficulty and were at one time synonymized (Wood 1963). However, the work of Lanier & Wood (1968) established them as separate species, although because of their morphological similarity, they are still considered sibling species. Their hydrocarbon patterns corroborate their similarity, yet a few hydrocarbon components are unique enough to allow separation. In our examination of the taxonomy of the scolytid cone beetles *Conophthorus* Hopkins, we established criteria for useful chemotaxonomic characters (Haverty et al. 1989, Page et al. 1990). Of these criteria, diagnostic hydrocarbons are abundant and unique and have unique elution times in *D. ponderosae* and *D. jeffreyi*. The most useful hydrocarbons for separating *D. ponderosae* and *D. jeffreyi* are the 3,7-dimethylalkanes (peaks 7, 18, 30). The elution times for these hydrocarbons are, unfortunately, close to the preceding *n*-alkane. However, they clearly elute separately with our capillary gas-liquid chromatography (Fig. 1).

The closely related species western pine beetle and southern pine beetle have hydrocarbon mixtures that are not qualitatively identical. However, they are similar enough to each other to confirm that these species are closely related, as suggested by electrophoretic analyses (Bentz & Stock 1986). We have identified single-component and isomeric mixtures of dimethylalkanes and even *n*-alkanes that meet the diagnostic criteria used for the sibling species. Obvious diagnostic hydrocarbon components are the 3,X-dimethylnonacosanes (peak 41) in *D. frontalis* and the homologous series of *n*-alkanes from *n*-hentriacontane to *n*-pentatriacontane in *D. brevicomis*. We found many unique

Table 3. Percentage of total hydrocarbons identified from four populations of southern pine beetle

Hydrocarbon	Populations of southern pine beetle[a]			
	I	II	III	IV
n-C21	<0.2	<0.2	<0.2	<0.2
n-C22	<0.2	<0.2	<0.2	<0.2
n-C23	0.23	<0.2	<0.2	0.3
n-C24	0.23	<0.2	<0.2	0.4
9-C25:1	<0.2	<0.2	<0.2	0.3
n-C25	4.8	3.5	4.1	9.8
7-; 9-; 11-; 13-MeC25	0.6	0.9	0.9	0.7
5-MeC25	0.8	1.6	2.0	0.5
3-MeC25	1.2	2.1	2.6	0.8
n-C26	1.9	0.9	0.8	2.9
9-; 11-; 13-; 15-C26	0.6	0.8	0.3	0.5
5-MeC26	0.2	0.3	0.4	0.2
2- or 4- + 3-MeC26	0.5	0.5	<0.2	<0.2
n-C27	25	17	13	36
9-; 11-; 13-MeC27	13	16	12	11
7-MeC27	—[b]	2.3	2.1	1.4
5-MeC27	6.2	6.2	5.4	4.5
9,15-; 11,15-DimeC27	1.5	1.6	1.8	1.3
3-MeC27	5.8	6.7	7.6	4.3
5,9-; 5,11-; 5,15-DimeC27	2.2	3.3	4.4	1.9
n-C28	1.0	0.5	<0.2	1.4
3,13-; 3,15-; 3,17-; 3,7-DimeC28	5.1	7.3	9.2	4.0
9-; 11-; 13-; 15-MeC28	1.4	1.2	1.3	0.8
7-MeC28	0.7	0.3	1.7	0.6
5-MeC28	0.3	<0.2	0.7	0.4
2- or 4-MeC28 + 3-MeC28	0.3	0.3	0.7	0.3
n-C29	1.3	0.9	0.7	2.1
3,17-DimeC29	0.3	0.5	<0.2	<0.2
11-; 13-; 15-MeC29	2.0	2.7	2.2	2.0
9-MeC29	2.2	2.1	1.5	1.8
7-MeC29	1.0	1.0	0.8	0.6
5-MeC29	0.7	0.4	1.0	0.3
13,17-DimeC29 + 9,13-; 9,15-; 9,17-DimeC29	2.3	2.5	3.0	1.2
3-MeC29	3.4	4.2	5.4	2.8
5,17-DimeC29	2.3	3.4	3.7	1.6
3,17-; 3,7-DimeC29	3.3	5.6	6.9	2.3
3,7-DimeC30	0.3	0.7	1.2	0.2

[a] Populations collected from following hosts and locations (all Louisiana): I, loblolly pine, Catahoula Ranger District, Kisatahie National Forest, Grant Parish (adults collected July 1985); II, loblolly pine, near Camp Livingston, Rapids Parish (adults collected July 1986); III, longleaf pine, Rapids Parish (adults collected December 1986); IV, shortleaf pine, Catahoula Ranger District, Kisatahie National Forest, Grant Parish (adults collected July 1985).

[b] 7-MeC27 from population I eluted as a shoulder of the 9-; 11-; 13-MeC27 peak and did not integrate separately.

Table 4. Percentage of total hydrocarbons identified from males and females of southern pine beetle from loblolly pine

Hydrocarbon	♂♂	♀♀
n-C21	<0.2	<0.2
n-C22	<0.2	<0.2
n-C23	<0.2	0.4
n-C24	<0.2	0.4
9-C25:1	<0.2	<0.2
n-C25	6.0	10.1
7-; 9-; 11-; 13-MeC25	0.8	0.7
5-MeC25	0.6	0.7
3-MeC25	0.8	1.1
n-C26	2.1	3.1
9-; 11-; 13-; 15-C26	<0.2	<0.2
5-MeC26	<0.2	<0.2
2- or 4- + 3-MeC26	<0.2	<0.2
n-C27	27	43
9-; 11-; 13-MeC27	7.6	8.2
7-MeC27	—[a]	1.1
5-MeC27	3.8	4.6
9,15-; 11,15-DimeC27	0.8	0.9
3-MeC27	4.3	5.2
5,9-; 5,11-; 5,15-DimeC27	1.2	1.4
n-C28	1.1	1.3
3,13-; 3,15-; 3,17-; 3,7-DimeC28	3.5	3.2
9-; 11-; 13-; 15-MeC28	0.7	0.6
7-MeC28	<0.2	<0.2
5-MeC28	<0.2	<0.2
2- or 4-MeC28 + 3-MeC28	<0.2	<0.2
n-C29	1.4	1.7
3,17-DimeC29	<0.2	<0.2
11-; 13-; 15-MeC29	1.4	1.4
9-MeC29	1.9	1.5
7-MeC29	<0.2	0.6
5-MeC29	0.4	0.5
13,17-DimeC29 + 9,13-; 9,15-; 9,17-DimeC29	1.2	1.1
3-MeC29	2.8	3.3
5,17-DimeC29	1.1	1.3
3,17-; 3,7-DimeC29	1.7	0.5
3,7-DimeC30	<0.2	<0.2

[a] 7-MeC27 eluted as a shoulder of the 9-; 11-; 13-MeC27 peak and did not integrate separately.

unique dimethylalkanes (for example, in peaks 20, 38, and 39); however, they are isomers and have the same retention times (ECL) as the isomers from the other closely related species. Because they require GC-MS for identification, we feel they are not very useful for taxonomic determination.

Cuticular hydrocarbons are relatively stable metabolic end products that appear to be genetically fixed. Because the insects studied so far synthesize most or all of their hydrocarbon components (Jackson & Blomquist 1976, Blomquist & Dillwith 1985), hydrocarbon composition and taxonomic grouping should be related. We can assume that synthesis is genetically controlled and is affected only slightly by environmental factors. The four species in our test group have an established data base on genetic variation, chromosome structure, pheromone chemistry, host selection behavior, and morphological structures. This first examination of two pairs of closely related *Dendroctonus* species corroborates other biosystematic information and strengthens the argument for species-specificity of cuticular hydrocarbon mixtures (Howard & Blomquist 1982).

Analysis of cuticular hydrocarbons is a "nondestructive" procedure for species determination; the specimens are not damaged. We have extracted the cuticular waxes from pinned museum specimens of western pine beetle (collected in 1914), other scolytids, and also from *Dioryctria* species. All specimens structurally unaltered, were returned to museum drawers.

We suspect that the characterization of cuticular hydrocarbon mixtures may be useful in determining systematic relationships. Our research on the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, suggests that canonical discriminate analyses of hydrocarbon profiles may be used

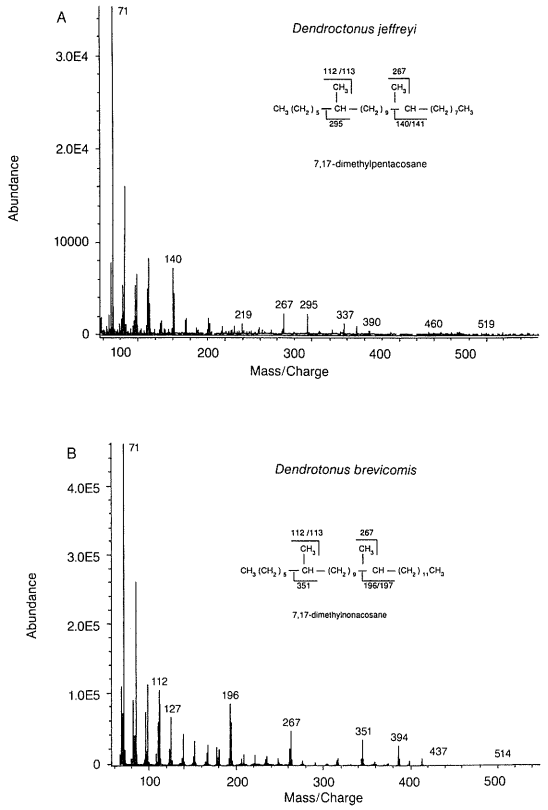

Fig. 5. EI mass spectra. (A) Peak 15 of Fig. 1, identified as 7,17-dimethylpentacosane from *D. jeffreyi*. (B) Peak 38 of Fig. 2, identified as 7,17-dimethylnonacosane from *D. brevicomis*.

to determine relatedness among populations of the same species (Haverty et al. 1990). Characterization and quantification of cuticular hydrocarbons of museum specimens, coupled with cladistic analyses, would be a next logical step in comparing phylogenetic relationships based on other biosystematic data with relationships determined by the analysis of cuticular hydrocarbons.

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