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Source: Annals of the Entomological Society of America, 105(6):817-831.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/AN12026

Biochemical Evidence that *Dendroctonus frontalis* Consists of Two Sibling Species in Belize and Chiapas, Mexico

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Ann. Entomol. Soc. Am. 105(6): 817–831 (2012); DOI: http://dx.doi.org/10.1603/AN12026

ABSTRACT *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae: Scolytinae) is a major economic pest of pines in the United States, Mexico, and Central America. We report biochemical investigations relevant to the taxonomic status and semiochemistry of two distinct morphotypes of *D. frontalis* recently detected in the Central American region. Morphotype A beetles (pre-episternal area of prothorax of both sexes smooth, bulging callus on anterolateral margin of prothorax of females) and morphotype B beetles (pre-episternal area of prothorax of both sexes with fine ridges, reduced female callus) collected in infestations in Chiapas, Mexico differed significantly in their production of 10 behaviorally-active compounds occurring in the genus *Dendroctonus*, including the major pheromone components for *D. frontalis*. Notably, host-attacking morphotype B females produced hundreds of nanograms of both *endo*-brevicomin and frontalin, whereas morphotype A females produced similar amounts of frontalin but subnanogram quantities of *endo*-brevicomin. Reanalysis of a published *D. frontalis* trapping study in Chiapas indicated that both morphotypes responded in greatest numbers when frontalin and *endo*-brevicomin baits were both present. In addition, we quantified 18 different cuticular hydrocarbons (the methyl-branched alkane components) from both morphotypes collected in Belize and Chiapas as well as morphotype A beetles from the southeastern United States, and principal component analysis revealed nonoverlapping clusters associated with either morphotype. This evidence of two distinct, complex phenotypes coexisting in the same sites and host trees supports the hypothesis that the *D. frontalis* morphotypes represent separate species and consequently indicates that the taxonomy of *D. frontalis* should be re-evaluated in the Central American region.

KEY WORDS *Dendroctonus frontalis*, southern pine beetle, *Pinus*, systematics, chemical ecology

Bark beetles in the genus *Dendroctonus* Erichson (Coleoptera: Curculionidae: Scolytinae) are likely the most destructive pests of pine forests worldwide, and in the Central American region they are associated with periodic, devastating outbreaks that can rapidly destroy forest cover over vast areas (Billings et al. 2004). Seven different species of *Dendroctonus* are known to attack pines in Central America and southern Mexico, including *D. adjunctus* Blandford, *D. approximatus* Dietz, *D. frontalis Zimmermann*, *D. mexicanus* Hopkins, *D. parallelocollis* Chapuis, *D. valens* LeConte, and *D. vitei* Wood (Wood 1982, Salinas-Moreno et al. 2004). Several species (e.g., *D. frontalis*, *D. vitei*, and *D. mexicanus*) have overlapping geographic, elevational, and host ranges and are exceedingly difficult to distinguish with external morphological and behavioral traits alone (Wood 1982, Zúñiga et al. 1999). However, they can be identified reliably by karyotype and morphological characters of the male genitalia, particularly the shape of the seminal rod (Wood 1963, Vité et al. 1975, Lanier et al. 1988, Zúñiga et al. 2002). Since their discovery, genitalic characters have been an essential resource for entomologists desiring to identify species of *Dendroctonus* for studies of distribution, morphology, population genetics, and chemical ecology (Moser et al. 2005, Anducho-Reyes et al. 2008, Moreno et al. 2008, Rios-Reyes et al. 2008).

Timber losses to bark beetles in the Central American region have been attributed predominantly to the southern pine beetle *D. frontalis* (Beal et al. 1964, Haack and PaizSchwartz 1997, Billings et al. 2004). Between 2000 and 2002, a bark beetle outbreak of unusual severity occurred in the Mountain Pine Ridge

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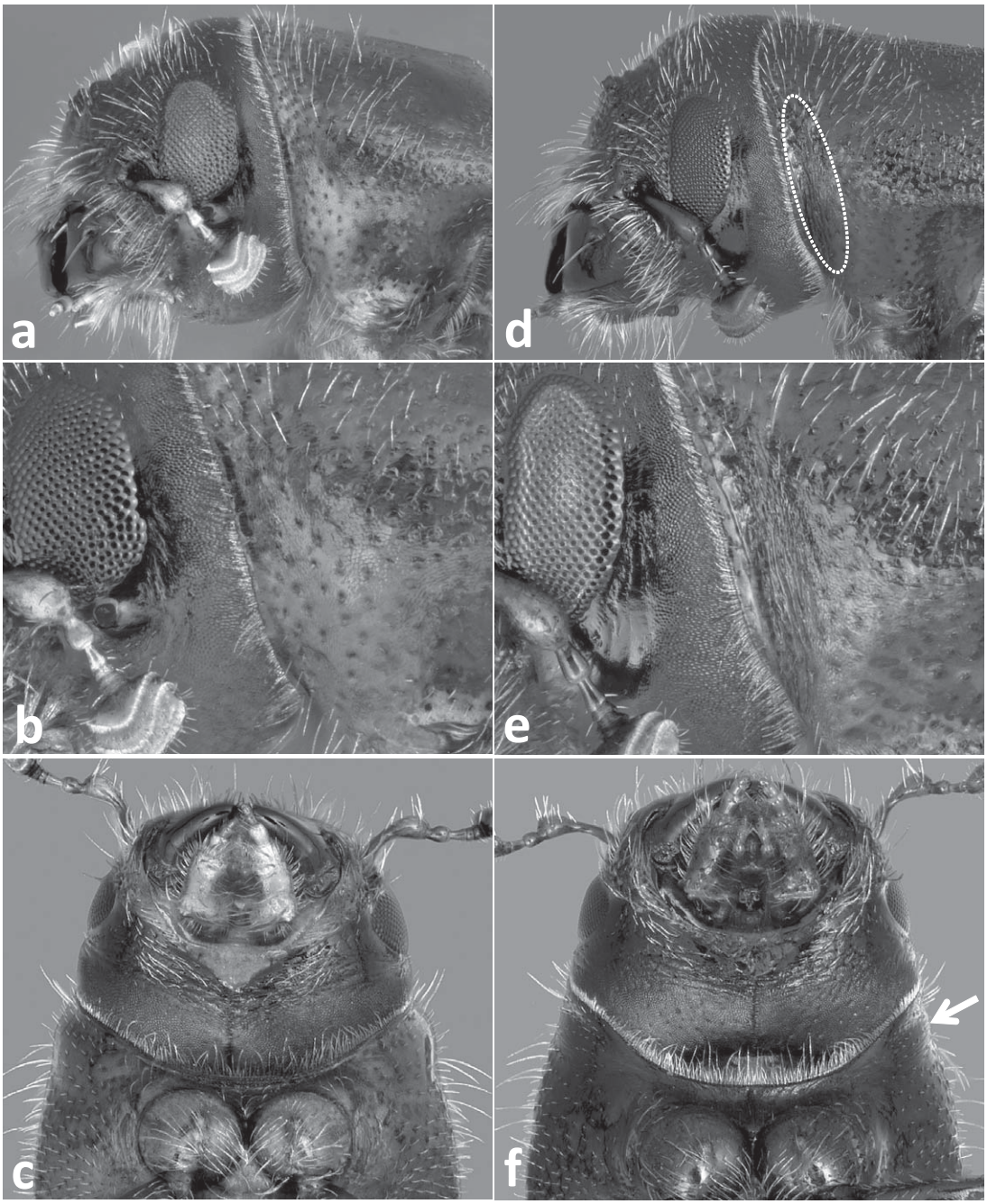

Fig. 1. Characters used for distinguishing *D. frontalis* morphotype A (a-c) and morphotype B (d-f). Fine parallel ridges on the pre-episternal area of the prothorax of both sexes occur in morphotype B (d and e, male) but are absent in morphotype A (a and b, male). Female morphotype A possess a conspicuous, bulging callus along the anterolateral margin of prothorax (c) that is absent in female morphotype B (f).

Forest Reserve of Belize, which killed nearly all mature pines across 25,000 ha, or roughly 80% of pine forest on the reserve (Haack et al. 2000, Macías-Sámano et al. 2001, Billings et al. 2004). Initial reports identified *D. frontalis* as the primary mortality agent (Haack et al. 2000). However, subsequent investigation by Midtgaard and Thunes (2002) indicated that two distinct, morphological variants of *D. frontalis* were present (Fig. 1), implying that an undescribed species possibly contributed to the extensive mortality in Belize. The variants were distinguished by characters of the prothorax (described below in the Meth-

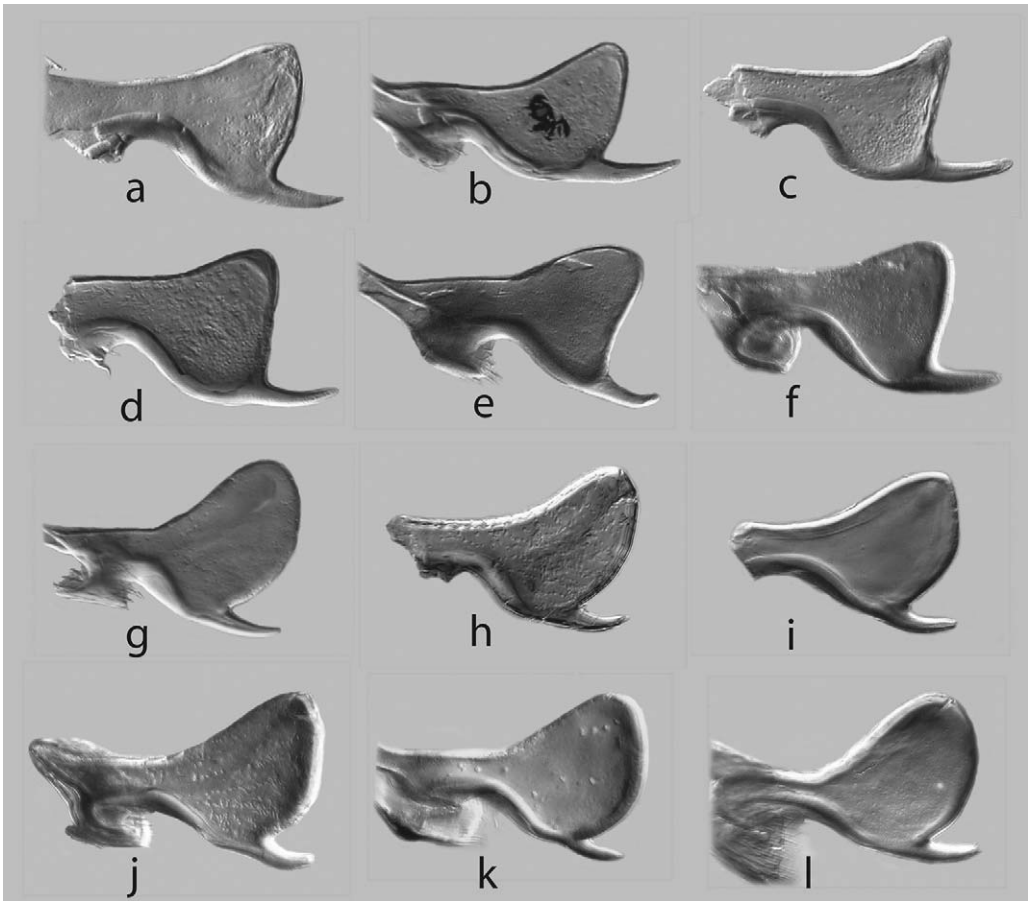

Fig. 2. Seminal rods of *D. frontalis*, lateral view: (a-f) morphotype B from (a-e) Chiapas and (f) Belize; (g-l) morphotype A from (g-i) Chiapas, (j) Belize, and (k and l) Georgia. Seminal rods are oriented with venter down and posterior right. Scale is not uniform among images.

ods) and to some extent by size, and these variants subsequently were discovered in collections made more broadly within the range of *D. frontalis* within Central America and in Chiapas, Mexico. A formal description of a new species or other revision of the taxonomy of *D. frontalis* has not yet been published, in part because of insufficient biological and other support for the separation of the morphotypes into two species. Notably, the seminal rods of the two morphotypes (Fig. 2) lack differences that are visibly conspicuous or of a similar magnitude as those that separate the described species of this genus (Wood 1963, Vité et al. 1975, Lanier et al. 1988).

To clarify the taxonomic status of the two *D. frontalis* morphotypes, we analyzed the composition of 1) known *Dendroctonus* pheromone components produced by either morphotype collected in Chiapas, Mexico; and 2) cuticular hydrocarbons extracted from either morphotype collected in Chiapas, Mexico and Belize and from one morphotype collected in the southeastern United States. In addition, we reanalyzed a previously-published trapping study of *D. frontalis* in Chiapas, Mexico to determine whether the morphotypes responded differently to two major components of the *D. frontalis* aggregation pheromone. Species of *Dendroctonus* commonly use pheromones to mediate their colonization of host trees, and, although pheromones of different *Dendroctonus* species share many of the same constituent compounds, the particular combination of components released by each sex is apparently unique to each species and may produce species-specific responses (Pitman et al. 1969, Lanier and Burkholder 1974, Borden 1982, Seybold et al. 2000). In addition, the complex blends of long-chain hydrocarbons that compose the outer wax layer of the cuticle of insects are also typically species-specific and can serve as chemotaxonomic characters with utility in distinguishing among closely-related species (Lucas et al. 2002, Francisco et al. 2008). Cuticular hydrocarbons have been used to clarify the taxonomic status of morphologically indistinct bark beetle species including some species of *Dendroctonus* (Page et al. 1990a,b; Page et al. 1997). If the two morphotypes represent a single species, we anticipated that individuals collected in the same locations would not differ signifi-

cantly in the composition of either their volatile pheromones or cuticular waxes.

Materials and Methods

Handling and Classification of Insects. Logs or bark infested by *Dendroctonus* brood were retrieved from naturally-infested *Pinus* spp. in the field and enclosed in screen boxes or zippered pillow covers. Emerged beetles found crawling on the interior surfaces of the enclosures were collected multiple times each day and held in ventilated plastic enclosures lined with damp paper towel. All of the beetles collected in Chiapas, Mexico and Belize and used in chemical analyses possessed external morphological characters diagnostic for *D. frontalis* (Wood 1982), and male seminal rod shape was likewise diagnostic for *D. frontalis* (Vité et al. 1975, Lanier et al. 1988). For the experiments, beetles were classified as "morphotype A" if the pre-episternal area of the prothorax was shiny and smooth (Fig. 1a and b) and the anterolateral margins of the female prothorax were raised conspicuously and bulging (Fig. 1c). This latter feature is the "transverse elevated callus" described in female *D. frontalis* by Wood (1982). Beetles were classified as "morphotype B" if either sex possessed a series of fine ridges on the pre-episternal area of the prothorax oriented roughly parallel to the anterior margin (Fig. 1d and e), and the anterolateral margins of the female prothorax were not conspicuously raised (Fig. 1f). The beetles were sorted under fluorescent lighting, which greatly improves visualization of cuticular characters such as the prothoracic ridges of morphotype B (Kirkendall et al. 2008). Presence of a pair of tubercles separated by a deep median groove on the male frons was used for distinguishing the sexes (Wood 1982). The reliability of this method was confirmed subsequently through dissection of genitalia. The seminal rods of males used in the biochemical studies were mounted in Berlese's medium and photographed with a Nikon Eclipse 90i microscope (Nikon, Tokyo, Japan) and Auto-Montage software (Syncroscopy, Frederick, MD). The pronota of all sampled insects were measured at their widest point when viewed from above with a binocular microscope fitted with an ocular micrometer.

Collection and Quantitation of *Dendroctonus* Semiochemicals. Adults of both morphotypes were reared from infested *P. oocarpa* Schiede ex Schltdl. bark and bolts obtained from active infestations in Lagunas de Montebello National Park, Chiapas Mexico (16° 06′46″ N, 91° 43′57″ W, 1,494 m elevation). Volatiles were collected from individual beetles by using a technique detailed elsewhere (Sullivan 2005; Pureswaran et al. 2007) and summarized here. Single, live beetles were confined within vertically-oriented, capped 100-μl conical vials containing a 2-mm depth (≈0.3 mg) of conditioned Super Q adsorbent (80–100 mesh; Alltech, Deerfield, IL) in their tip. The septa caps of each vial were each punctured with a ≈0.5-mm-diameter hole to permit unhindered beetle respiration, and during incubation the caps were covered by several layers of activated charcoal mesh (Universal Replacement Prefilter, no. 38002, Southborough, MA) to prevent incursion of outside volatiles through the ventilation openings. The beetle-enclosing vials were incubated at ambient temperature (≈20–30°C) for 20–30 h. For any given treatment category, both morphotypes were sampled simultaneously under identical conditions. After incubation, each beetle was removed and then 50-μl redistilled hexane (spiked with 180-ng heptyl acetate) together with the excised hindgut of the beetle were placed into the vial. The hexane was permitted to extract the hindgut/adsorbent passively for 1–2 d at ambient temperature in the sealed vial. The extract was transferred to a GC autosampler vial and then the conical vial contents were rinsed once with 50-μl nonspiked hexane that was subsequently added to the autosampler vial.

On 10–12 May 2006, female beetles were forced to attack freshly-cut logs from a healthy *P. oocarpa* (50 cm in length, 13 cm in diameter) by confining them within gelatin capsule halves secured over 1-mm-diameter holes drilled into the bark and spaced >5 cm apart. After 1 d, a portion of those females that had entered the bark and were expelling frass from the gallery entrance were excised carefully and sampled immediately as described above ("solitary" treatment group). A male of the same morphotype was added to the gelatin capsule of the remaining successful female entrances. After a further 1 d, the successfully-established beetle pairs were excised and each male and female sampled separately ("paired" treatment group). On 27–28 October 2007, beetles collected in the previous 24 h from emergence enclosures were sampled without having had any contact with a fresh host ("newly-emerged" treatment groups). Each treatment was replicated 2–12 times for each sex and morphotype.

Two microliters of each sample were analyzed by GC-MS (either a Hewlett–Packard 6890 GC – 5973 MSD or a Hewlett–Packard GCD) in splitless mode with a polyethylene glycol capillary column (HP-INNOWax; 60-m by 0.25-mm by 0.25-μm film). The temperature program was 40°C for 1 min, 16°C/min to 80°C, then 7°C per min to 240°C and held for 10 min. We quantified beetle production of 13 semiochemicals reported for the genus *Dendroctonus*, including 12 compounds that have been shown to be both produced by and behaviorally active with *D. frontalis* (Skillen et al. 1997, Sullivan 2005). Semiochemicals in the extracts were identified both by retention time and mass spectral matches to commercially-obtained standards; each compound was quantified relative to the internal standard heptyl acetate and response curves calculated from a dilution series of each compound. Within each sampled treatment category and sex, quantities produced by the two morphotypes were compared by using Wilcoxon two-sample test with a t-approximation. *P* values were subjected to a Bonferroni correction according to the number of different compounds detected within a treatment category (i.e., maximum 10–13).

Reanalysis of Trapping Experiment. Catches of *D. frontalis* in a bait evaluation trial performed in Las

Lagunas de Montebello National Park, Chiapas, Mexico (Moreno et al. 2008), were sorted subsequently by morphotype, and subjected to a new statistical analysis. The full details of the field procedures are given in the aforementioned publication but summarized here. Beetle responses to five trap bait treatments: 1) *P. oocarpa* turpentine alone, 2) turpentine and frontalin, 3) turpentine and *endo*-brevicomin, 4) all three bait components, 5) all three bait components but with *endo*-brevicomin displaced 4 m horizontally from the trap) were compared within six separate Latin squares in which columns of each individual square were represented by each of five different trap locations and rows by each of five consecutive trapping days. Turpentine (Pinosa S. de R.L. de C.V., Morelia, Mexico) was released from a wick bait (5 g/d at 26°C), frontalin (racemic) from a capped LDPE microcentrifuge tube (Chemtica, San Jose Costa Rica; 2.5 mg/d at 20°C), and *endo*-brevicomin (racemic) from a bubble-cap bait (Chemtica, 0.5–0.8 mg/d at 24°C). Traps (12-unit multiple-funnel type; Chemtica) were spaced at least 100 m apart both within and between squares and were assumed to have no interaction with each other. Within each square, these trap positions were adjacent, that is, traps of any single square were arranged in a single line. Assignment of treatments was performed at random and without replacement within both columns and rows of each square, according to the demands of the Latin square design. Thus initial assignment of treatments to the five traps of each square was performed at random without duplication, and, likewise, trap treatments were reassigned randomly without duplication to each trap on each day when the catches were collected. Four squares were run simultaneously on 3–8 July 2007 and two more run simultaneously on 10–15 August 2007. Two of the four squares run in July had been omitted from the original analysis (Moreno et al. 2008) to balance the replication between the two months. Traps were always positioned >100 m outside of active infestations.

A mixed model analysis of variance (ANOVA) was carried out on cube-root transformed trap catches for both morphotypes and sexes, using Proc Mixed (SAS 9.1 for Windows, SAS Institute, Inc.). For this and subsequent statistical analyses, we determined whether the data met assumptions of parametric statistics and identified the most appropriate transformation by examining the residuals plots. Preliminary analysis indicated no effect of trapping period (i.e., August versus July) so treatment was viewed as a whole plot factor in multiple Latin squares with traps (or columns) nested within squares and dates (or rows) common across squares within periods. Morphotype and sex were viewed as subplot factors. Thus main effects and interactions for treatment, morphotype, and sex were considered fixed, whereas square, trap within square, and date within period, as well as the interactions treatment*square, treatment*trap(square) and morphotype*square, were considered random factors. Catches were consistently zero for one treatment (turpentine plus *endo*-brevicomin), and thus inclusion of this treatment in the analyses likely would have resulted in underestimated error variance for the other treatments and in overly-liberal tests. Therefore this treatment was omitted from the ANOVAs, and the standard error for pairwise comparisons among all five treatments was calculated from the ANOVA on remaining treatments. Because of strong morphotype*treatment and morphotype*sex interactions, additional analyses were carried out separately for each morphotype to assess effects of treatment and sex. The cube root transformation was used for the analysis of catch for morphotype A and a square-root transformation for the less variable counts for morphotype B to better meet the homogeneity of variance assumption. The mixed model fixed effects were treatment, sex and treatment*sex, and random effects were square, trap(square), date(period), treatment*square and treatment*trap(square). Pairwise comparisons among treatment means were carried out separately at each level of sex by using a Bonferroni adjustment for the number of comparisons (10) in each subset.

Extraction and Analysis of Cuticular Hydrocarbons. Both morphotypes were reared from brood-infested bark of *P. oocarpa*, *P. maximinoi* H. E. Moore, and *P. caribaea* Morelet collected in locations in Chiapas, Mexico, and Belize (Table 1). *D. frontalis* also were reared from infested bark of *P. taeda* L. collected in both Georgia and Mississippi (Table 1), and these insects all possessed the external features of morphotype A. Infested bark was held inside zippered pillow cases or screen enclosures, and emerged beetles were collected every 1–3 d. Beetles were maintained in groups inside vented plastic enclosures containing moistened paper towel either at room temperature or under refrigeration. Live beetles were steeped individually for 15–30 min inside glass vials (2 ml capacity) in hexane sufficient to completely submerge the insect (150–400 μl), then recovered from the extract with a hexane-cleaned wire hook and transferred to a vial of 70% ethanol for later examination. The extract was transferred with a glass Pasteur pipette to a glass, conical-interior microvial, evaporated to dryness in a fume hood, redissolved in 10 μl hexane, and reconcentrated to 3 μl before injection manually into the splitless inlet of a GC-MS (Hewlett–Packard model GCD). The column was a Hewlett–Packard HP-1 (polydimethylsiloxane; 50 m by 0.2 mm in diameter, 0.11-μm film thickness); the temperature program was 170°C for 0.7 min, then 3°C/min to 320°C, then isothermal for 9 min. The relative proportions among 18 mono- and dimethylalkanes identified previously as constituents of the cuticular waxes of *D. frontalis* (Page et al. 1990b) were calculated as follows: First, peaks of these alkanes were identified within the total ion chromatogram (TIC) by both their Kovats indices and mass spectra consistent with specific methyl branch positions (Blomquist et al. 1987, Carlson et al. 1998). Overlap of the TIC peaks for many alkanes prevented accurate abundance calculations based on TIC integrations alone, therefore we estimated TIC integration areas based on integration of single ions.

Table 1. Collection data for specimens examined in cuticular hydrocarbon analyses of two morphotypes of the southern pine beetle, *D. frontalis*

Site	Coordinates	Elevation	Tree no.	Tree species	Sample size		Collection date
					Morph A	Morph B	
1. Mountain Pine Ridge Forest Preserve, Belize	16° 59.8′ N, 88° 46.4′ W	955 m	1	*P. oocarpa*	2	7	14–19 July 2009
	17° 00.3′ N, 88° 51.0′ W	909 m	2	"	10	1	"
	16° 58.6′ N, 88° 50.4′ W	780 m	3	*P. caribaea*	0	1	"
2. Lagunas de Montebello National Park, Chiapas, Mexico	16° 07′ N, 91° 44′ W	1,494 m	4	*P. maximinoi*	0	6	19 Aug.-3 Sept. 2007
	"	"	5	"	0	6	"
	"	"	6	*P. oocarpa*	1	2	"
	"	"	7	"	30	22	"
3. Homochitto National Forest, MS	31° 22.8′ N, 90° 55.2′ W	80 m	-	*P. taeda*	25	-	1–30 Sept. 2007
	31° 29.7′ N, 90° 48.3′ W	100 m	-	"	31	-	"
	31° 31.9′ N, 90° 44.9′ W	150 m	-	"	34	-	"
4. Oconee National Forest, GA	33° 47.2′ N, 83° 14.6′ W	170 m	-	"	19	-	16 Oct. 2008

For each alkane, we selected a single diagnostic ion that was both 1) associated with fragmentation at the alkane's methyl branch position (or one of the two positions in the case of dimethyl alkanes), and 2) superabundant in the mass spectrum of the target alkane relative to coeluting or closely-eluting alkanes. The integration area of the diagnostic ion peak of the target alkane was calculated, and the relative contribution of diagnostic ion abundance to the total ion abundance of the mass spectrum of the compound was used to estimate the area of the alkane's TIC peak. We calculated the relative abundance of each of the methylalkanes by dividing each estimated TIC peak area by the sum of all 18 peak areas, and used these relative abundances in statistical analyses. n-Alkanes are major components of the cuticular hydrocarbon blend of *D. frontalis* and other *Dendroctonus* sp. (Page et al. 1990b) but were excluded from our analysis because of their lack of detectable diagnostic ions and evidence of n-alkane contamination in some samples. Both a multivariate analysis of variance (MANOVA) and univariate ANOVAs were performed on the untransformed abundances (SAS 9.0) based on the factors morphotype (two levels: A and B), site (four levels: Chiapas, Belize, Mississippi, and Georgia) and sex. Effects included in the model were morphotype (which compared morphotype means across all locations), sex, site nested within morphotype, and sex nested within site and morphotype. All effects in the model were regarded as fixed. Tests for the overall effect of each factor in the MANOVA model were performed with Wilks' Lambda statistic ($\alpha = 0.05$). Within the univariate ANOVAs, an ESTIMATE statement was used to test for both a mean difference between morphotypes and for a site-by-morphotype interaction based on just the sites where both morphotypes were present (i.e., Chiapas and Belize; Bonferroni adjustment for two contrasts; $\alpha = 0.05$). In addition, the relative abundances for all 18 compounds from 197 samples were subjected to principal components analysis and the scores for the first two principal components were plotted on x-y axes for visual inspection of clustering. Percentage of joint nonoverlap of the distributions (sensu Mayr et al. 1953, p. 145–147) of the two morphotypes was estimated for each of the 18 compounds as well as the first two principal components. Calculation of nonoverlap assumed that for each morphotype the hydrocarbon relative abundances were normally distributed with mean and standard deviation equal to the corresponding sample estimates. Using the methods of Mayr et al. (1953), nonoverlap was the sum of the areas beneath the two normal curves, one area to the left, the other to the right of the point of intersection of the curves. Variances were allowed to differ between morphotypes, so for each cuticular-hydrocarbon this intersection point was obtained as the solution to a quadratic equation (obtained by equating the two normal densities), rather than using the approximation suggested in Mayr et al. (1953). For some hydrocarbons, the point of intersection did not fall between the means for the two morphotypes, and nonoverlap was not calculated. Calculation of nonoverlap for individual hydrocarbons was carried out both using all of the sampled locations, and also using just the Chiapas and Belize locations. To identify biochemical markers for the two morphotypes within the cuticular hydrocarbon blends, we calculated the mean and variance (within morphotypes) for the abundance ratio between every two hydrocarbons for all possible hydrocarbon pairs (153 possible), and then calculated the percentage of joint nonoverlap for the distributions of these ratios between the two morphotypes, both for the Chiapas and Belize specimens alone as well as for all samples. The nonoverlap percentages were ranked, and distributions of the hydrocarbon ratios that provided the greatest nonoverlap between morphotypes were plotted as histograms.

Results

Collection and Quantitation of *Dendroctonus* Semiochemicals. *Dendroctonus frontalis* morphotype A and B beetles from Chiapas, Mexico differed significantly in the quantities, timing of production, and sex association of specific semiochemicals reported from *D. frontalis* or other *Dendroctonus* spp. (Fig. 3; Table 2). The bicyclic acetals frontalin and brevicomin were

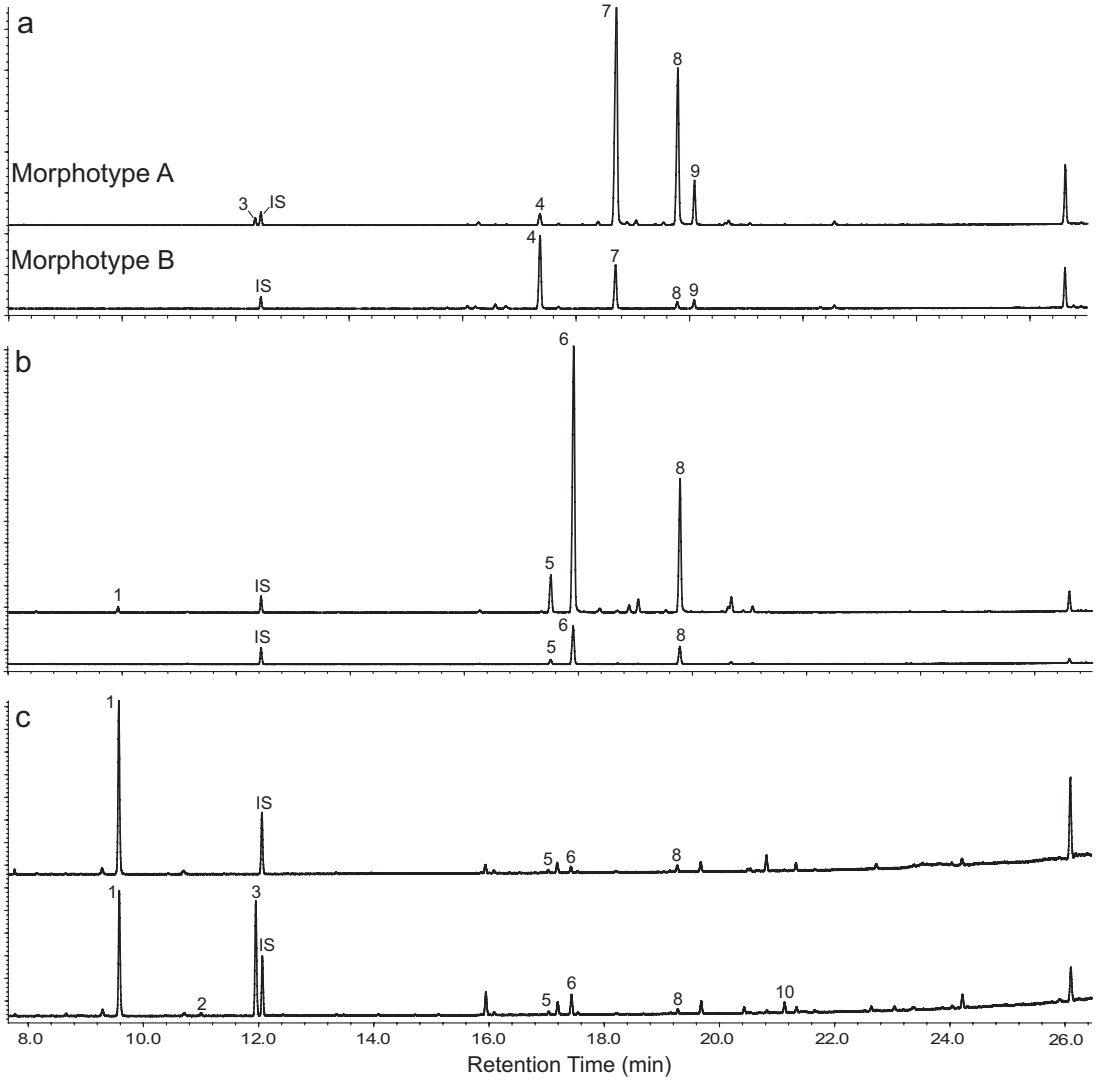

Fig. 3. Total ion chromatograms of GC-MS analyses of volatiles collected from individual *D. frontalis* adults: (a) male and (b) female newly-emerged from infested bark, (c) female mining alone for <1d in a *P.oocarpa* log in the laboratory. Identified peaks are (1) frontalin, (2) *exo*-brevicomin, (3) *endo*-brevicomin, (4) myrtenal, (5) *cis*-verbenol, (6) *trans*-verbenol, (7) verbenone, (8) myrtenol, (9) 1-phenylethanol, (10) 2-phenylethanol. The upper chromatogram is from morphotype A, the lower chromatogram is from morphotype B. "IS" denotes the internal standard (heptyl acetate) which was included in uniform concentration for all samples.

isolated consistently in nanogram or greater quantities from newly-emerged morphotype A females and males, respectively, but these generally were undetectable in newly-emerged morphotype B individuals. However, solitary mining and paired morphotype B females produced both frontalin and *endo*-brevicomin in large quantities (hundreds of nanograms) along with lesser amounts of *exo*-brevicomin; in contrast, morphotype A females produced large amounts of frontalin but merely subnanogram quantities of brevicomin. Newly-emerged morphotype A beetles produced significantly higher (average 3–10 fold) quantities of oxygenated monoterpene semiochemicals than newly-emerged morphotype B, including *cis*-verbenol and *trans*-verbenol (in females), verbenone (in males), and myrtenol (in both sexes). However, small amounts of ipsdienol were detected from most solitary and paired morphotype B females but not from morphotype A females. Production of both 1- and 2-phenylethanol was significantly greater in newly-emerged morphotype A males than in newly-emerged morphotype B males; by contrast production of 2-phenylethanol was significantly greater in both newly-emerged and solitary mining morphotype B females than morphotype A females in these same categories.

Reanalysis of Trapping Experiment. Examination of catches indicated that 1864 morphotype A and 56 morphotype B individuals (a 33:1 ratio) had been

Table 2. Quantities (ng per beetle) of *Dendroctonus* semiochemicals isolated from two morphotypes of the southern pine beetle, *D. frontalis*, in Chiapas, Mexico

Compound	Origin	Newly emerged[a]				Solitary in log 1 d[b]		Paired in log 1 d[c]			
		Female		Male		Female		Female		Male	
		$\bar{x} \pm$ SE	n[d]	$\bar{x} \pm$ SE	n	$\bar{x} \pm$ SE	n	$\bar{x} \pm$ SE	n	$\bar{x} \pm$ SE	n
Frontalin	Morph A	47.7 ± 9.4	12/12	-	0/12	640 ± 132	11/11	19.8 ± 9.2	3/3	-	0/2
	Morph B	- **[f]	0/9	-	0/11	799 ± 158	10/10	108 ± 40	11/11	0.2 ± 0.2[e]	1/10
exo-Brevicomin	Morph A	0.4 ± 0.4	1/12	7.1 ± 2.4	12/12	<0.1[e]	1/11	0.3 ± 0.3	1/3	14.0 ± 8.5	2/2
	Morph B	-	0/9	1.6 ± 1.4 *	2/11	16.6 ± 2.8 **	10/10	11.9 ± 2.1	11/11	10.4 ± 1.6	10/10
endo-Brevicomin	Morph A	-	0/12	208 ± 53	12/12	<0.1[e]	1/11	-	0/3	233 ± 9	2/2
	Morph B	-	0/9	0.7 ± 0.4 **	3/11	702 ± 126 **	10/10	710 ± 120	11/11	388 ± 55	10/10
Myrtenal	Morph A	13.4 ± 3.4	12/12	301 ± 57	12/12	0.5 ± 0.2	4/11	0.8 ± 0.4[e]	2/3	35.3 ± 8.8	2/2
	Morph B	3.1 ± 0.9	9/9	807 ± 162	11/11	1.2 ± 0.7	3/10	3.2 ± 2.4	6/11	185 ± 24	10/10
cis-Verbenol	Morph A	611 ± 82	12/12	-	0/12	35.5 ± 9.2	11/11	40.8 ± 22.0	3/3	2.8 ± 1.8	2/2
	Morph B	95.3 ± 8.4 *	9/9	-	0/11	141 ± 64	10/10	129 ± 27	11/11	0.3 ± 0.2	2/10
Acetophenone	Morph A	2.0 ± 0.4	12/12	46.7 ± 13.1	12/12	7.3 ± 2.9	11/11	17.8 ± 3.0	3/3	3.8 ± 1.8	2/2
	Morph B	3.2 ± 1.3	8/9	29.6 ± 5.7	11/11	3.7 ± 0.7	10/10	7.7 ± 2.8	11/11	5.1 ± 1.9	7/10
Ipsdienol	Morph A	-	0/12	-	0/12	-	0/11	-	0/3	7.3 ± 1.3	2/2
	Morph B	-	0/9	-	0/11	8.8 ± 4.4 *	8/10	9.3 ± 2.1	10/11	0.2 ± 0.1[e]	3/10
trans-Verbenol	Morph A	4.19 ± 0.68 by 10^3	12/12	0.3 ± 0.2[e]	5/12	100 ± 33	11/11	370 ± 232	3/3	159 ± 75	2/2
	Morph B	575 ± 82 *	9/9	-	0/11	662 ± 376	10/10	538 ± 153	11/11	108 ± 66	8/10
Verbenone	Morph A	30.7 ± 7.6	12/12	4.88 ± 0.53 by 10^3	12/12	3.8 ± 0.9	10/11	20.5 ± 11.1	3/3	368 ± 234	2/2
	Morph B	27.7 ± 5.9	9/9	1.39 ± 0.29 by 10^3 *	11/11	31.3 ± 7.6	10/10	42.4 ± 10.5	11/11	193 ± 44	10/10
Myrtenol	Morph A	2.32 ± 0.42 by 10^3	12/12	2.98 ± 0.41 by 10^3	12/12	53.0 ± 13.4	11/11	81.0 ± 18.8	3/3	1.33 ± 0.82 by 10^3	2/2
	Morph B	350 ± 79 *	9/9	248 ± 69 **	11/11	91.6 ± 42.0	10/10	87.8 ± 20.0	11/11	651 ± 170	10/10
1-Phenylethanol	Morph A	-	0/12	610 ± 127	12/12	0.6 ± 0.5[e]	2/11	1.3 ± 0.9[e]	2/3	27.3 ± 3.8	2/2
	Morph B	-	0/9	172 ± 31 *	11/11	0.3 ± 0.2[e]	2/10	0.9 ± 0.6	2/11	10.0 ± 3.1	10/10
cis-Myrtanol	Morph A	45.3 ± 9.9	12/12	53.3 ± 11.0	12/12	8.1 ± 3.4	9/11	-	0/3	22.3 ± 22.3	1/2
	Morph B	16.7 ± 5.2	9/9	28.8 ± 9.0	11/11	0.8 ± 0.8[e]	1/10	-	0/11	-	0/10
2-Phenylethanol	Morph A	1.6 ± 0.4	11/12	9.1 ± 1.3	12/12	11.4 ± 3.5	11/11	10.7 ± 3.0	3/3	14.8 ± 2.8	2/2
	Morph B	9.2 ± 1.8 *	9/9	3.1 ± 0.2 **	11/11	118 ± 25 *	10/10	95.5 ± 25.1	11/11	6.8 ± 0.8	10/10

Semiochemicals were sampled by confining live beetles individually for 20–30 h inside 100-μl-capacity tapered glass vials containing 0.3-mg chemical adsorbent. Afterward, each beetle was removed and its hindgut was excised and extracted simultaneously with the adsorbent (Sullivan 2005).

[a] Sampled beetles had emerged from bark of naturally-infested *Pinus oocarpa* in the previous 24 h (27–28 Oct. 2007).

[b] Brood beetles had emerged from logs or bark of naturally-infested *P. oocarpa* and were forced to attack freshly-cut logs from a healthy *P. oocarpa;* on the following day beetles were excised from the logs and sampled (10–12 May 2006).

[c] Females established on *P. oocarpa* logs for 1 d (as b above), then a single male of the same morphotype was placed into each gallery entrance; on the following day both beetles were excised from the logs and sampled separately (10–12 May 2006).

[d] The number of individuals in which the compound was detected/the no. of beetles sampled.

[e] Identified by retention time and presence of ≥3 diagnostic ions of the compound's mass spectrum.

[f] Asterisks indicate a significant difference between morphotypes in the quantity of the semiochemical isolated (*$P < 0.05$, **$P < 0.01$; Wilcoxon test with Bonferroni correction for the number of compounds examined).

Table 3. Responses (mean ± SEM catch per trap per day) of two morphotypes of *D. frontalis* to baited funnel traps at Lagunas de Montebello National Park, Chiapas, Mexico (adapted from Moreno et al. [2008])

Bait treatment	Morphotype A		Morphotype B	
	Males	Females	Males	Females
1) Turpentine alone	0.07 ± 0.07a (2)	0a (0)	0a (0)	0.03 ± 0.03a (1)
2) Turpentine and frontalin	3.23 ± 0.90b (97)	0.13 ± 0.07a (4)	0.07 ± 0.04a (2)	0.10 ± 0.07a (3)
3) Turpentine and *endo*-brevicomin	0a (0)	0a (0)	0a (0)	0a (0)
4) Turpentine, frontalin and *endo*-brevicomin	22.07 ± 5.40c (662)	5.03 ± 1.50b (151)	0.23 ± 0.16a (7)	0.20 ± 0.10a (6)
5) Treatment four above with *endo*-brevicomin displaced 4 m from trap	27.53 ± 3.47c (826)	4.07 ± 0.46b (122)	0.90 ± 0.31b (27)	0.33 ± 0.14a (10)

Total catches are given in parentheses. Reported means are of untransformed insect counts, however the ANOVA was performed on transformed counts. Within sex and morphotype, means associated with the same letter were not significantly different (Bonferroni-adjusted pairwise comparisons, $P < 0.05$).

caught during the trapping study of Moreno et al. (2008). Low catches limited the capacity of the statistical tests to detect bait preferences of morphotype B. However, the two morphotypes differed significantly in their responses to the five bait treatments: with morphotype included as a fixed subplot factor in the ANOVA, strong morphotype*treatment ($F = 58.55$; df = 3, 343; $P < 0.001$) and morphotype*treatment*sex ($F = 6.39$; df = 3, 343; $P < 0.001$) interactions were detected. With ANOVAs performed on the two morphotypes separately, a significant sex*treatment interaction was detected for morphotype A ($F = 24.28$; df = 3, 116; $P < 0.001$) and morphotype B ($F = 2.69$; df = 3, 116; $P = 0.049$), and thus all pairwise comparisons of treatment effects were carried out on each sex separately (Table 3). The three-component bait treatments (i.e., those with turpentine, frontalin, and *endo*-brevicomin) trapped significantly more male and female morphotype A than the other treatments. Furthermore, morphotype A were attracted similarly to the three-component bait whether the *endo*-brevicomin release device was attached directly to the trap or displaced 4 m away. By contrast, male morphotype B were significantly more attracted to the treatment consisting of turpentine, frontalin, and displaced *endo*-brevicomin than to any other treatment, and this was the only treatment that was significantly more attractive than turpentine alone. No significant preference for the baits was detected in female morphotype B, likely because of the exceedingly low numbers trapped.

Analysis of Cuticular Hydrocarbons. No cuticular hydrocarbons were discovered that qualitatively distinguished the two morphotypes of *D. frontalis;* however, we found significant differences between morphotypes in the relative quantities of methyl-branched alkanes (Table 4). A MANOVA revealed a significant effect of morphotype ($F = 553.45$; df = 17, 169; $P < 0.001$), site nested within morphotype ($F = 47.05$; df = 68, 665; $P < 0.001$), sex ($F = 3.40$; df = 17, 169; $P < 0.001$), and sex nested within site and morphotype ($F = 1.90$; df = 85, 821; $P < 0.001$) on the proportions among 18 quantified methylalkanes. The morphotype main effect (which compared morphotypes based on all sites) was significant in the univariate ANOVAs for 13 of the 18 hydrocarbons (3-; 7-; 9-; 11-methylheptacosane; 3,7-dimethylheptacosane; 5-;7- methyloctacosane; 5-; 11-; 13-methylnonacosane; 3,7-; 3,17-; 5,17-dimethylnonacosane; $F = 15.22–2208$; df = 1, 185; $P < 0.001$) and site nested within morphotype was significant for all 18 compounds ($F = 16.46–815.2$; df = 4, 185; $P < 0.001$). Sex was a significant factor in the ANOVAs for five of the 18 compounds (7-; 11-methylheptacosane; 7-methyloctacosane; 11-;13-methylnonacosane; $F = 6.29–13.72$; df = 1, 185; $P < 0.013$). The two morphotypes differed significantly in the relative quantities of 14 hydrocarbons when the comparison included only those sites where both morphotypes were collected (i.e., Chiapas and Belize) (Table 4). When the interaction between morphotype and site was tested using just Chiapas and Belize, this interaction was significant for 10 hydrocarbons (Table 4). In a plot of the first and second principal components (68% of total variation) of a principal component analysis of all 197 samples (Fig. 4), morphotype B formed a cluster distinct from morphotype A, which itself formed two clusters corresponding to sampling site (i.e., Chiapas and Belize versus Mississippi and Georgia). The hydrocarbon pairs whose ratios possessed distributions with the highest degree of nonoverlap between the two morphotypes were 5- and 9-methylheptacosane (all sites; nonoverlap = 99.95%; Fig. 5a) and 13- and 15- methylnonacosane (Chiapas and Belize only; nonoverlap >99.99%; Fig. 5b).

Insect Measurements. For the Chiapas and Belize insects used in the biochemical analyses, average (± SD) pronotal widths for morphotypes A and B were 1.17 ± 0.11 and 1.44 ± 0.13, respectively. For insects collected in the southeastern United States (all morphotype A), pronota were 1.10 ± 0.10 mm wide. The smallest pronotal width for a morphotype B was 1.12 mm (Chiapas) whereas the largest pronotal width for morphotype A was 1.40 mm (Chiapas).

Discussion

Our data indicate that the two morphotypes of *D. frontalis* collected in the Chiapas, Mexico and Belize possess distinct biochemical phenotypes, both with regard to the composition of their cuticular hydrocarbons and production of volatile compounds that function as pheromones for *D. frontalis* and other *Dendroctonus.* The simultaneous occurrence of these two

Table 4. Relative proportions (mean ± SEM) among 18 methyl-branched hydrocarbons present in the cuticular waxes of two morphotypes of the southern pine beetle, *D. frontalis*

#	Cuticular hydrocarbon	Retention time	Kovats index	Target Ion[a]	Additional diagnostic ions	Morphotype A		Morphotype B	Contrast of morphotypes			
									All sampled locations		Chiapas and Belize only	
						All locations (n = 152)	Chiapas and Belize (n = 43)	Chiapas and Belize (n = 45)	P value[b]	percentage distribution non-overlap	P value[c]	Percentage distribution nonoverlap
1	11-Methylheptacosane	30.53	2735	168	169, 252/253	0.168 ± 0.002	0.173 ± 0.003	0.0537 ± 0.0022	<0.001	99.8	<0.001*	>99.9
2	9-Methylheptacosane	30.60	2738	140	141, 280/281	0.0790 ± 0.0009	0.0886 ± 0.0014	0.0223 ± 0.0008	<0.001	>99.9	<0.001	>99.9
3	7-Methylheptacosane	30.73	2744	112	113, 308/309	0.0689 ± 0.0040	0.146 ± 0.003	0.109 ± 0.003	<0.001	76.9	<0.001	85.1
4	5-Methylheptacosane	30.93	2753	84	85, 336/337	0.135 ± 0.002	0.152 ± 0.004	0.146 ± 0.005	0.119	–[d]	0.003*	–
5	3-Methylheptacosane	31.43	2775	365	364, 336/337	0.155 ± 0.003	0.123 ± 0.003	0.0570 ± 0.0026	<0.001	97.9	<0.001*	95.5
6	5,X-Dimethylheptacosane	31.63	2784	84	85, 351	0.0410 ± 0.0010	0.0399 ± 0.0019	0.0559 ± 0.0030	0.053	70.2	0.006*	70.4
7	3,7-Dimethylheptacosane	32.22	2811	309	308, 127, 308/309	0.0248 ± 0.0012	0.0398 ± 0.0015	0.0425 ± 0.0014	<0.001	78.0	0.663	–
8	7-Methyloctacosane	32.92	2843	112	113, 322/323	0.0177 ± 0.0005	0.0263 ± 0.0007	0.0246 ± 0.0006	<0.001	75.5	0.052	58.0
9	5-Methyloctacosane	33.12	2852	84	85, 350/351	0.0048 ± 0.0001	0.0065 ± 0.0003	0.0097 ± 0.0002	<0.001	95.6	<0.001*	86.2
10	15-Methylnonacosane	34.87	2933	224	225	0.0162 ± 0.0004	0.0194 ± 0.0006	0.0132 ± 0.0006	0.112	64.5	<0.001*	77.4
11	13-Methylnonacosane	34.88	2933	196	197, 252/253	0.0227 ± 0.0005	0.0177 ± 0.0007	0.0814 ± 0.0029	<0.001	99.2	<0.001	99.7
12	11-Methylnonacosane	34.89	2934	168	169, 280/281	0.0120 ± 0.0004	0.0168 ± 0.0010	0.0269 ± 0.001	<0.001	90.2	<0.001*	78.3
13	7-Methylnonacosane	35.08	2943	112	113, 336/337	0.0211 ± 0.0006	0.0253 ± 0.0017	0.0194 ± 0.0017	0.124	–	0.232*	60.5
14	5-Methylnonacosane	35.29	2953	84	85, 364/365	0.0152 ± 0.0004	0.0145 ± 0.0007	0.0665 ± 0.0031	<0.001	98.4	<0.001	98.3
15	3-Methylnonacosane	35.78	2976	393	392, 56/57	0.0647 ± 0.0017	0.0419 ± 0.0020	0.0489 ± 0.0028	0.903	65.6	<0.001*	–
16	5,17-Dimethylnonacosane	35.97	2985	196	197, 84/85, 267, 379	0.0758 ± 0.0020	0.0490 ± 0.0027	0.178 ± 0.007	<0.001	93.3	<0.001	98.2
17	3,17-Dimethylnonacosane	36.42	3006	196	197, 267, 407	0.0662 ± 0.0031	0.0113 ± 0.0007	0.0213 ± 0.0011	<0.001	87.9	0.067	81.1
18	3,7-Dimethylnonacosane	36.52	3011	127	126, 336/337, 407	0.0123 ± 0.0003	0.0094 ± 0.0005	0.0232 ± 0.0006	<0.001	90.3	<0.001*	96.7
	Principal component 1					−1.476 ± 0.090	0.017 ± 0.104	4.985 ± 0.086	–	>99.9	–	>99.9
	Principal component 2					0.255 ± 0.167	3.239 ± 0.087	−0.860 ± 0.176	–	–	–	99.1

Estimated total ion chromatogram (TIC) peak area for each compound divided by sum of peak areas for all 18 compounds.

[a] Molecular weight (amu) of fragment ion utilized for peak area integration and estimation of TIC area of each alkane peak.

[b] ANOVA F Test for morphotype main effect.

[c] Contrast of the morphotype means at Chiapas and Belize performed using the ESTIMATE statement of PROC GLM (SAS) with Bonferroni correction. Asterisk indicates a significant site by morphotype interaction ($P < 0.05$) based on Chiapas and Belize only.

[d] Overlap was not calculated when intersection of the estimated normal probability distributions did not lie between the morphotype means.

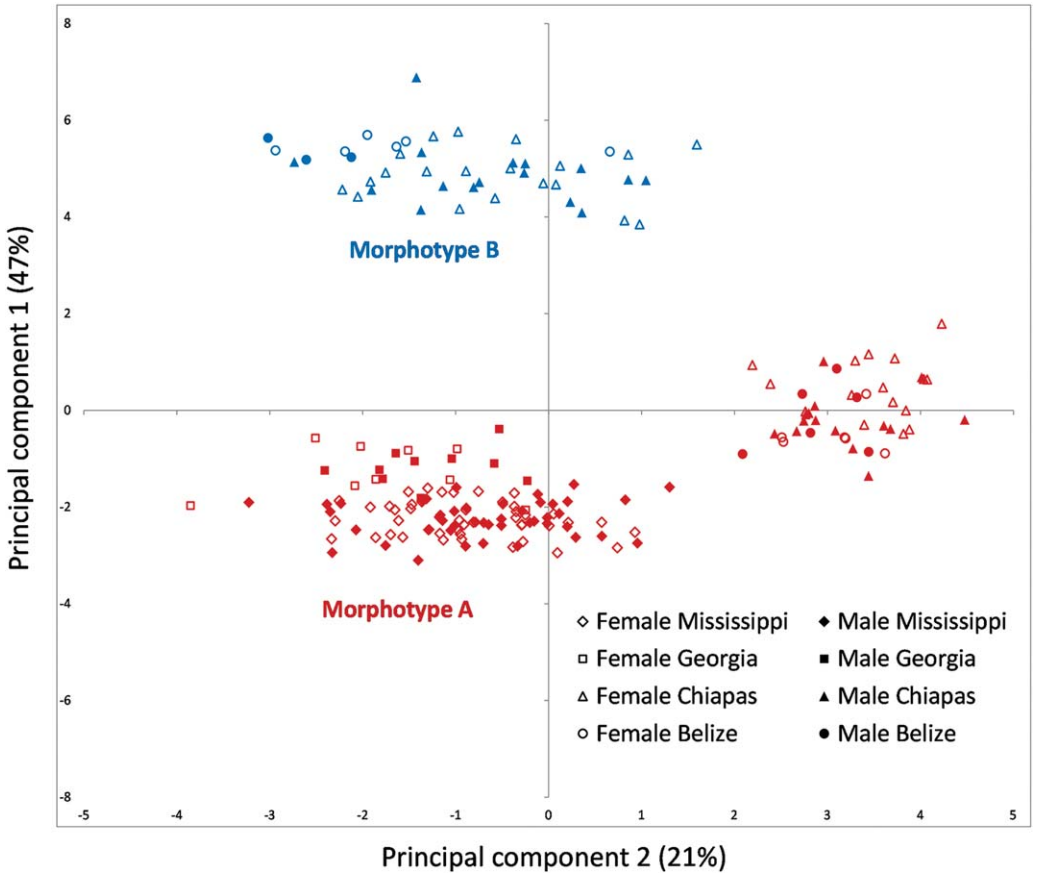

Fig. 4. Principal component analysis plot computed from the relative quantities of 18 different methyl-branched hydrocarbons extracted from the cuticles of individual adult *D. frontalis* collected in the southeastern United States, Belize, and Chiapas, Mexico. Marker colors indicate individuals possessing external diagnostic character states of morphotypes A and B (red and blue, respectively).

distinct, complex phenotypes in the same locations, on the same species of host, and commonly the same tree (e.g., Table 1) suggests that the morphotypes are reproductively isolated and thus represent distinct species. However, direct studies of genotypes and reproductive compatibility of the two morphotypes are ultimately necessary to definitively reject the alternative hypothesis that the morphotypes represent an extraordinary, complex polymorphism within *D. frontalis.*

The hypothesis of sibling species is complemented by the possibility that the detected biochemical differences could themselves mediate reproductive isolation between the morphotypes. The volatiles produced by the two morphotypes, including pheromone components for *D. frontalis*, differed quantitatively and qualitatively, suggesting that these blends could function as cues for assortative aggregation and mating. Volatiles profiles of morphotype A beetles in Chiapas largely resembled those reported for *D. frontalis* in the southeastern United States (Smith et al. 1993, Sullivan 2005, Pureswaran et al. 2007) and in Arizona (Pureswaran et al. 2008): notably, females produced frontalin both when newly emerged or mining but little or no *endo*-brevicomin, which was produced by the males alone. By contrast, morphotype B females produced both compounds. Frontalin is the major component of the aggregation attractant for *D. frontalis*, which is synergized by female-produced *trans*-verbenol or by odors of host resin (Renwick and Vité 1969, Payne et al. 1978). Male-produced *endo*-brevicomin can function either as a potent aggregation synergist or inhibitor depending on its rate of release and proximity to active infestations or other sources of semiochemicals (Sullivan and Mori 2009, Sullivan et al. 2011). Responses by morphotype A to trap baits in Chiapas largely were similar to those of *D. frontalis* in studies in Mississippi conducted with similar procedures (i.e., away from active infestations and with wide trap spacing), namely, frontalin and turpentine were attractive to males and synergized by *endo*-brevicomin (Sullivan et al. 2007). In Chiapas, *D. frontalis* morphotype B males also were attracted to a turpentine/frontalin and *endo*-brevicomin combination, but unlike morphotype A this attraction was statistically significant only when the *endo*-brevicomin bait was

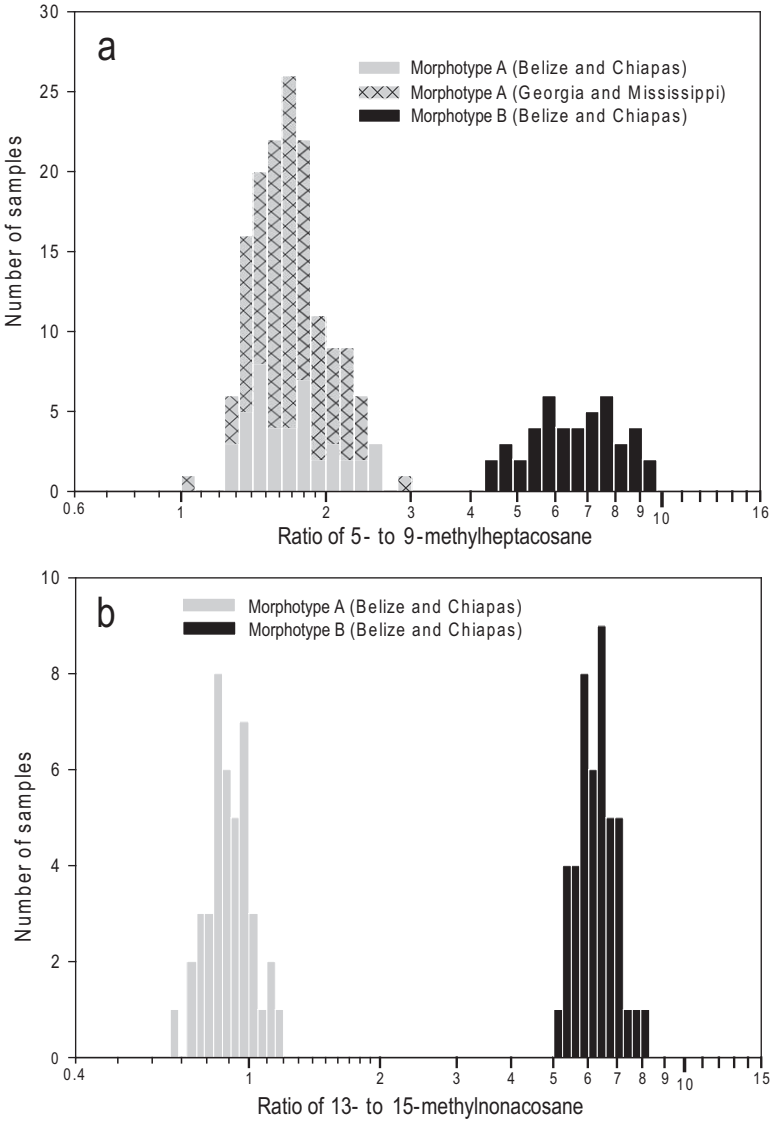

Fig. 5. Frequency distributions of the abundance ratios of branched hydrocarbons in the cuticles of two morphotypes of *D. frontalis*: (a) ratio of 5- to 9-methylheptocosane, the ratio which produced the greatest discrimination (based on estimated distributional nonoverlap) between the two morphotypes at all sampled sites, and (b) ratio of 13- to 15-methylnonacosane, the ratio which produced the greatest discrimination between the morphotypes at sites of sympatry (i.e., Chiapas and Belize). Histogram x-axes and bin sizes are log-scaled.

displaced 4 m from the trap. Experiments with *D. frontalis* in Mississippi have shown that the synergistic effect of an *endo*-brevicomin bait can sometimes be enhanced by displacing it a short distance (4–16 m) from a frontalin and turpentine-baited trap (Sullivan and Mori 2009). Because frontalin and *endo*-brevicomin are found in opposite sexes in Mississippi *D. frontalis*, it was hypothesized that colocated sources might be less attractive (particularly to males) because they would signal that females on a host resource were paired and, concurrently, that the resource was approaching its colonization capacity. In Chiapas, our volatiles analyses indicated that attacks with solitary B females and either A or B pairs should produce both frontalin and *endo*-brevicomin, whereas attacks by A females should produce frontalin alone. If this difference were mediating assortative attraction by the morphotypes, we would predict that morphotype B males should prefer colocated sources of frontalin and *endo*-brevicomin (associated with solitary B females), whereas morphotype A males should prefer separated sources (associated with the presence of solitary A females). However, the opposite occurred: in Chiapas we observed morphotype B males exhibiting a preference for separated sources of *endo*-brevicomin and frontalin but not morphotype A males.

Hence, even though morphotypes differed significantly in their attractive responses to *D. frontalis* pheromone components, this difference would not appear to promote reproductive isolation. The relevance of the trapping tests to natural behaviors is subject to question, however, because the release rates and enantiomeric composition of the artificial baits almost certainly differed from those of natural beetle attacks. Furthermore, the volatiles analyses indicated that the morphotypes differed in their production of many compounds in addition to frontalin and *endo*-brevicomin, and these other compounds could also modify cross-morphotype responses. Determination of a role for aggregation pheromones in reproductive isolation between the morphotypes will require cross-attraction and inhibition studies using artificially-infested host tissue as bait. Even if distinct species, the morphotypes may resemble certain sympatric *Dendroctonus* species for which differing aggregation pheromone compositions apparently confer little or no reproductive isolation, as these pheromones are cross-attractive to flying individuals (Lanier and Burkholder 1974, Smith et al. 1990, Hofstetter et al. 2008). Our evidence that both morphotypes can be trapped by baits of mutually-produced compounds frontalin and *endo*-brevicomin (when combined with host odors in the form of turpentine) indicates that some degree of cross-attraction between morphotypes probably exists. However, the very low catches of morphotype B, despite the abundant attacks by this morphotype in the surrounding forest, suggest that the three component bait is an inefficient and likely incomplete or otherwise inadequately formulated lure for these insects.

Nonetheless, production of different volatile blends by females of the two morphotypes may allow discrimination of gallery entrances and thus assortative mating by males that have already landed on the host tree. In studies of *D. frontalis* from the southeastern United States, presence of *endo*-brevicomin caused walking males to reject artificial gallery entrances releasing female pheromone components *trans*-verbenol and frontalin, presumably because *endo*-brevicomin signals that the female has already paired (Rudinsky et al. 1974). If morphotype A males in zones of sympatry with morphotype B share this response, then the *endo*-brevicomin produced by mining female morphotype B females might likewise inhibit entry of morphotype A males and thereby prevent such pairings. Short-range male discrimination of species-specific blends produced by solitary female entrances could confer reproductive isolation between different species despite long-range cross-attraction to the same trees caused by shared aggregation pheromone components. The importance of the volatile pheromones of *Dendroctonus* in reproductive isolation is implied by phylogenetic analyses indicating that sibling species are characterized by saltational shifts in pheromone composition (Symonds and Elgar 2004).

The Mississippi and Georgia *D. frontalis* examined in this study were entirely morphotype A, and we have not collected specimens of morphotype B outside of southern Mexico and Central America. Morphotype A beetles from Belize and Chiapas clustered separately from those of Mississippi and Georgia in the principal component plot of cuticular hydrocarbon compositions (Fig. 4), and this separation can be attributed to the large geographic distance and broad host-free zones (e.g., the Texas plains) that likely impede gene flow between these populations. The principal components plot furthermore suggests an approximately similar degree of divergence in cuticular hydrocarbon chemistry between morphotypes A and B as between the southeastern United States and Central American populations. One previous study that contrasted the cuticular hydrocarbon compositions of pairs of closely related *Dendroctonus* species (*Dendroctonus ponderosae* Hopkins and *D. jeffreyi* Hopkins; *D. frontalis* and *D. brevicomis* LeConte) identified multiple qualitative as well as quantitative differences in hydrocarbon profiles (Page et al. 1990b). Thus, the apparent absence of qualitative compositional differences in cuticular hydrocarbons between the two *D. frontalis* morphotypes suggests a smaller phenotypic divergence between the *D. frontalis* morphotypes than between the aforementioned species pairs. Cuticular hydrocarbons function as contact cues for mate recognition in many insects (Howard 1993), and the species-specific composition of these cues can mediate reproductive isolation (Coyne et al. 1994). However, there is no evidence that bark beetles use cuticular hydrocarbon contact pheromones in mate selection and species recognition, and thus no reason to expect that selective pressures exist for divergence of cuticular hydrocarbon compositions between sympatric sibling species of bark beetles.

The morphotypes apparently represent an instance of closely-related *Dendroctonus* species colonizing the same hosts within infestations. *Dendroctonus frontalis* has been reported to cohabit trees with other close relatives including *D. mexicanus* (Zúñiga et al. 1995, Moser et al. 2005); *D. vitei* (Lanier et al. 1988); and *D. brevicomis* LeConte (Davis and Hofstetter 2009). Complexes of bark beetle species with cross-attraction to aggregation pheromones may mutually increase host availability by collectively participating in mass-attacks, and these benefits may outweigh the negative effects of increased interspecific competition. One major implication of our findings is that the devastating bark beetle outbreaks in the Central American region that are currently attributed to *D. frontalis* alone may be the result of two species possibly acting in concert. A forthcoming publication currently being prepared by the authors will address evidence of genetic differences between the two morphotypes and report other critical information necessary for an anticipated taxonomic revision of *D. frontalis.*

Acknowledgments

We thank Joanne Barrett, Zachary Oliver, Roberto Castellanos, and field staff of the Lagunas de Montebello National Park, Mexico for providing essential technical assistance with this work. Mark Dalusky graciously provided

beetles from Georgia. Jeremy Allison, and Tom Atkinson provided helpful reviews of earlier versions of this manuscript. Figs. 1 and 2 photos are by Erich G. Vallery and Joanne P. Barrett, respectively. This work was funded in part by Cooperative Agreements between the U.S. Forest Service Southern Research Station and El Colegio de la Frontera Sur, 04-IC-11330129-131 and 07-IC-11330129-056.

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Received 13 March 2012; accepted 5 September 2012.