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First Contact Pheromone Identified for a Longhorned Beetle (Coleoptera: Cerambycidae) in the Subfamily Prioninae

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Abstract Little is known of the reproductive behavior of longhorned beetles (Coleoptera: Cerambycidae) in the subfamily Prioninae. *Mallodon dasystomus* (Say), the hardwood stump borer, is a widely distributed prionine that is native to the southern U.S. Here, we explored the chemically-mediated mating behavior of *M. dasystomus*, and tested the hypothesis that males recognize females by a contact pheromone. In mating bioassays, all males tested attempted to mate with females only after contacting females with their antennae. Moreover, all males attempted to mate with solvent-washed dead females treated with as little as 0.15±0.03 female equivalents of conspecific cuticular extracts, confirming that compounds on the cuticle of females are essential for mate recognition. Cuticular hydrocarbon profiles of females contained 13 compounds that were not present in profiles of males. Among the female-specific compounds, two co-dominant methyl-branched alkanes, 2-methylhexacosane (2Me-C_{26}) and 2-methyloctacosane (2Me-C_{28}), accounted for 17% of the total hydrocarbons. Our strategy for identifying the contact pheromone was to synthesize and test the bioactivity of female specific compounds, starting with the most abundant. In bioassays, males displayed mating behavior in response to synthetic 2Me-C_{26} and 2Me-C_{28} when tested individually. Furthermore, when these compounds were tested in combination, they elicited the full progression of mating behaviors, suggesting that 2Me-C_{26} and 2Me-C_{28} make up the contact pheromone. These findings are further evidence of the critical role of contact pheromones in mating systems of longhorned beetles.

Key Words *Mallodon dasystomus* · Mating behavior · Contact pheromone · Chemoreception · Sex pheromone · Solid phase microextraction

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Introduction

The wax layer of insects contains a complex mixture of compounds such as aldehydes, ketones, esters, and hydrocarbons that not only protect the insect from desiccation, but also may serve as contact pheromones that mediate mate recognition (Gibbs, 1998). There is a growing body of evidence that hydrocarbons within the epicuticular wax layer of females serve as contact pheromones, and play important roles in the mating systems of longhorned beetles (Coleoptera: Cerambycidae; for review, see Ginzel and Hanks, 2003; Ginzel et al., 2006; Ginzel, 2010). Males from diverse subfamilies of the Cerambycidae orient to females only after contacting them with their antennae, suggesting that males recognize potential mates by contact chemoreception (Ginzel, 2010). Nevertheless, these signals have been identified only for species in the phylogenetically advanced subfamilies Cerambycinae and Lamiinae (see Ginzel, 2010). In many of these species, there is marked sexual dimorphism in antennal length; the antennae of males often are more than double the length of those of females (e.g., see volumes indexed by Linsley and Chemsak, 1997). These elongate antennae enable males to

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sweep out comparatively large areas as they patrol tree trunks and other substrates for females, so that they can locate mates efficiently (Hanks et al., 1996; Hanks, 1999). To our knowledge, contact pheromones have not yet been identified for beetles in the more ancestral subfamilies that lack elongate antennae, including the Prioninae, Parandrinae, and Aseminae (*sensu* Monné and Hovore, 2005). Although males of the prionine, *Prionus californicus* Motschulsky, recognize females via contact chemoreception (Cervantes et al., 2006; Barbour et al., 2007), the compounds that mediate mate recognition have not yet been identified. A greater understanding of the use of contact pheromones in ancestral subfamilies will lend insight into the evolution of mating systems in the Cerambycidae.

Mallodon dasystomus, the hardwood stump borer, is a widely distributed prionine that is native to the southern U.S. The elongate robust adults range in length from 23–47 mm and vary in color from reddish brown to black (Solomon, 1995). Females oviposit near the base of their hosts at sites where the wood is exposed, such as at wounds or areas of previous infestation. Larvae feed in the boles of trees, typically complete their development in 3–4 yr, and emerge as adults from May through July leaving large ovoid emergence holes in the bark (Linsley, 1962; Solomon, 1995). Preferred hosts *of M. dasystomus* include sugarberry, hackberry, oak, sycamore, hickory, willow, boxelder, sweetgum, and in some instances, conifers (Solomon, 1995; Yanega, 1996).

In this study, we explored the chemically-mediated mating behavior of *M. dasystomus*, and tested the hypothesis that males recognize females by contact chemoreception. We characterized the mating behavior of males, and report the identification and synthesis of the bioactive components. Additionally, we sampled cuticular hydrocarbons of males and females using both whole-body solvent extraction and solid phase microextraction (SPME). SPME is a solventless sampling technique that was used in conjunction with solvent extraction to identify the contact pheromones of the cerambycine species *Megacyllene robiniae* (Förster) (Ginzel et al., 2003b), *Megacyllene caryae* (Gahan) (Ginzel et al., 2006), and *Neoclytus acuminatus acuminatus* (F.) (Lacey et al., 2008). Wipe-sampling yields hydrocarbon profiles that are quantitatively different from whole-body solvent extraction (Ginzel et al., 2006). For example, the contact pheromone of *M. caryae,* (*Z*)-9-nonacosene, comprised ~16% of the total hydrocarbons in the hexane extracts of females, but represented ~34–36% of the hydrocarbons in SPME wipe samples of the elytra (Ginzel et al., 2006). These findings suggest that SPME samples more accurately represent those compounds that are accessible to the antennae of males and can be used to predict hydrocarbons that may act as contact pheromones. In this study we also test the hypothesis that contact pheromones are more abundant on the surface of the wax layer and are present in greater abundance in SPME samples when compared to whole-body extracts.

Methods and Materials

Source of Beetles Beetles used in experiments were reared from infested willow and sweetgum at the USDA Forest Service Center for Bottomland Hardwoods Research, Stoneville, MS, from late May to early August 2007 through 2009. As adult beetles emerged, they were shipped to Purdue University, West Lafayette, IN (USDA—APHIS permit No. P526P-09-01631). Upon arrival, beetles were housed individually in cylindrical cages of aluminum window screen (300 cm^3) with 9-cm glass Petri dishes covering top and bottom, and provided feeder vials of 10% sucrose solution (glass vial with a cotton dental roll; Patterson Dental Supply, South Edina, MN, USA). Beetles were kept in an environmental chamber (Mod. No. 1-30BLL, Percival Scientific, Boone IN, USA) on a 16 L –29°C:8D – 25°C cycle that was 12 hr out of phase with natural daylight, and maintained at 80% humidity. Reared beetles may have mated before they were individually caged. Beetles were isolated in these cages for at least 24 hr before being used in bioassays. Beetles used in bioassays were vigorous and active, and were used no more than once per day.

Characterization of Mating Behavior To characterize the mate recognition behavior of *M. dasystomus* in the laboratory, we observed individual males paired with individual females in Petri dish arenas (9 cm diam×2 cm tall) lined with filter paper (No. 1, Whatman, Maidstone, England). Observations were made in the dark under a red light and ambient laboratory conditions for a minimum of 2 min or until beetles mated. The behaviors of 17 pairs of female and male beetles in these arenas were recorded digitally using a Sony Handy-Cam, model DCR-SR42. All Petri dishes were cleaned, washed with acetone, and air dried between trials. We analyzed videotapes to determine whether one sex displayed directed movement toward the other, a behavior consistent with the use of either vision or volatile pheromones as mate location signals. We also observed whether males responded to females only after contacting them with their antennae, behavior that would suggest the use of contact pheromones.

Role of Contact Chemoreception To illustrate that male *M. dasystomus* recognize females via a contact pheromone, we used the following bioassay adapted from Ginzel *et al.* (2003b).

1. *Freeze-killed female.* An individual female was freeze-killed (−4°C for 20 min), allowed to warm to room temperature (~15 min), and presented to a vigorous male in a Petri dish arena to demonstrate that mate recognition signals were intact. The male was allowed to attempt to mate the female, but separated before copulation occurred.
2. *Solvent-extracted female carcass.* Non-polar compounds were removed from the dead female by immersing her in two successive 10-ml aliquots of analytical grade hexane for 5 min each. The aliquots then were combined and concentrated to 3 ml under nitrogen. The solvent-washed female was presented to the male to test whether he displayed mating behavior. Lack of a response by the male was taken as evidence that chemical recognition signals had been removed.
3. *Reconstituted female.* To test the bioactivity of the extract, we gradually pipetted 0.1 female equivalent (FE) of extract back onto the female carcass, coating the body with the extract. The solvent then was allowed to evaporate, and the reconstituted female was presented again to the same male to confirm that the recognition signal had been restored. If the male did not respond, we incrementally added 0.1 FE to the female, up to a maximum of 1 FE, and retested the female against the same male.

We conducted this videotaped bioassay with six dead females each paired with three different males (N= 18). Once a male touched a female with his antennae, a clear progression of behavioral steps lead to copulation (arrestment of male, body alignment with female, mounting, and copulation; see Results for details). A trial was scored as a "response" if the male, after making antennal contact with the female, displayed any of these behavioral steps. Bioassays were conducted in glass Petri dish arenas (see above), and numbers of males responding to reconstituted females were compared with those responding to freeze-killed females with a Fisher's exact test (Sokal and Rohlf, 1995).

Identification of Cuticular Hydrocarbons We sampled cuticular components of five female beetles by wiping the length of a SPME fiber (100 μm, polydimethylsiloxane; Supelco Inc., Cat. No. 57300-U, Bellefonte, PA, USA) across the elytra ten times, rotating the fiber between wipes. Samples were analyzed at Purdue University by coupled gas chromatography-mass spectrometry (GC-MS) with electron impact ionization (EI, 70 eV) using a Hewlett-Packard (HP) 6890N gas chromatograph (Hewlett-Packard, Sunnyvale, CA, USA) equipped with a DB-5 capillary column (30 m×0.25 mm×0.25 μm film, J&W Scientific, Folsom, CA, USA) in splitless mode, and interfaced to an HP 5973 mass selective detector (MSD), with helium as the carrier gas. The SPME fiber was thermally desorbed in the heated GC injection port for 1 min and the oven temperature was held at 50°C for 1 min and then ramped at 10°Cmin^{-1} to 280°C, with a hold at 280°C for 20 min. Injector and transfer line temperatures were 250°C.

Five female beetles and five males also were sampled by whole-body hexane extraction, allowing us to compare the hydrocarbon profiles of males and females, and the profiles of females produced by the two sampling methods. The cuticular chemicals were extracted from individual freeze-killed beetles by immersing each in two 10-ml aliquots of hexane for 5 min. The aliquots were combined and concentrated to 3 ml under nitrogen. Extracts were analyzed initially at Purdue University by coupled GC-MS as described above. The percentage that each peak contributed to the total hydrocarbons was calculated by integrating the areas under peaks of all hydrocarbons that were consistently present in the total ion chromatograms (Chemstation, Version D.05.01; Hewlett Packard Corp.). Quantitative data presented in Table 1 and Figs. 1 and 2 were produced by these analyses. Differences in mean relative abundance of compounds that comprise the contact pheromone (area under peak/total area of all peaks) between whole-body hexane extraction and SPME of females were compared by analysis of variance (StatSoft, Inc., 2005).

Compounds in whole-body extracts were identified at the University of California, Riverside by GC-MS using the same model of GC-MS instrument and the same type of column as described above. The column was programmed from 100°C for 1 min, 10°Cmin^{-1} to 280°C, with a final hold for 20 min at this temperature. Injector and transfer line temperatures were 280°C. Linear and branched chain hydrocarbons were identified by comparing their retention times and mass spectra with those of standards, or by interpretation of the mass spectra in combination with Kovats retention indices. Retention times of methylalkanes relative to straight-chain compounds, and characteristic mass spectral fragments (M^+ ions in combination with diagnostic fragments from cleavage on either side of branch points) can be used to completely and unequivocally identify saturated hydrocarbons found in insect cuticular extracts, as described in detail by Nelson (1993), Nelson and Blomquist (1995), and Carlson et al. (1998).

Synthesis of 2-Methylhexacosane and 2-Methyloctacosane Solvents were Optima grade (Fisher Scientific, Pittsburgh, PA, USA). Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under Ar. Reactions with air- or water-sensitive reagents were carried out in oven-dried glassware under Ar. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian INOVA-400 (400 and 100.5 MHz,

Table 1 Cuticular hydrocarbons of male and female *Mallodon dasystomus*

		% of total hydrocarbons± SE[a]			
		Hexane Extracts		SPME	
Peak No.[b]	Hydrocarbon	Male	Female	Female	Diagnostic Ions[c]
1	C_{23} monoene	0.14±0.03	ND	ND	**322** (M^+)
2	C_{23}	2.6±0.28	4.52±0.39	1.69±0.51	**324** (M^+)
3	11Me-C_{23}	ND	0.5±0.17	0.15±0.05	338 (M^+), 168/196
4	C_{24} monoene	0.64±0.21	ND	ND	**336** (M^+)
5	C_{24}	2.44±0.14	0.45±0.06	1.65±1.13	**338** (M^+)
6	2Me-C_{24}	0.33±0.07	ND	ND	352 (M^+), 337, 309
7	C_{25} monoene	18.6±5.59	1.09±0.32	0.42±0.12	**350** (M^+)
8	C_{25} monoene	ND	0.34±0.18	0.31±0.09	**350** (M^+)
9	C_{25}	41.7±3.79	8.64±1.08	10.2±2.66	**352** (M^+)
10	Diunsaturated C_{25}	1.59±0.22	0.18±0.04	0.56±0.33	**348** (M^+)
11	11Me-C_{25}	3.83±0.52	1.63±0.34	1.8±0.32	**366** (M^+), 168/224
	13Me-C_{25}				**366** (M^+), 196
12	9,13-DiMe-C_{25}	1.27±0.23	0.4±0.11	ND	380 (M^+), 140/267, 196/211, 365
13	Diunsaturated C_{25}	ND	0.13±0.04	0.2±0.04	**348** (M^+)
14	C_{26}	0.72±0.06	0.98±0.12	1.61±0.63	**366** (M^+)
15	2Me-C_{26}	0.47±0.09	4.58±0.85	7.58±0.39	**380** (M^+), 365, 337
16	C_{27} monoene	0.92±0.21	7.13±1.07	3.95±1.03	**378** (M^+)
17	C_{27} monoene	ND	0.57±0.12	0.35±0.11	**378** (M^+)
18	C_{27} monoene (front shoulder)	7.01±0.72	18.3±1.78	17.6±4.94	**378** (M^+)
	C_{27}				**380** (M^+)
19	Diunsaturated C_{27}	ND	0.44±0.06	ND	**376** (M^+)
20	11Me-C_{27}	0.36±0.05	4.14±0.59	3.87±0.65	394 (M^+), 168/252
	13Me-C_{27}				394 (M^+), 196/224
21	Diunsaturated C_{27}	ND	0.24±0.03	0.17±0.05	**376** (M^+)
22	11,15-DiMe-C_{27}	ND	3.22±0.25	3.8±0.61	**408** (M^+), 168/267, 196/239
23	3Me-C_{27}	ND	0.82±0.16	1.37±0.19	394 (M^+), 365
24	C_{28}	1.37±0.92	1.02±0.12	1.65±0.79	**394** (M^+)
25	12Me-C_{28}	ND	1.1±0.21	1.15±0.25	**408** (M^+), 182/252
	13Me-C_{28}				**408** (M^+), 196/238
	14Me-C_{28}				**408** (M^+), 210/224
26	2Me-C_{28}	ND	13±2.77	12.5±2.68	**408** (M^+), 365, 393
27	C_{29} monoene	ND	7.22±1.51	3.5±1.03	**406** (M^+)
28	C_{29}	5.57±0.43	6.05±0.87	7.87±1.61	**408** (M^+)
29	11Me-C_{29}	ND	4.03±0.73	4.51±1.04	422 (M^+), 168/280
	13Me-C_{29}				422 (M^+), 196/252
	15Me-C_{29}				422 (M^+), 224
30	13,17-DiMe-C_{29}	ND	2.92±0.22	4.15±0.73	436 (M^+), 196/267
31	2Me-C_{30}	5.49±1.52	0.97±0.06	2.05±1.03	436 (M^+), 393, 421
32	Diunsaturated C_{31}	ND	3.58±1.67	1.89±0.46	**432** (M^+)
	C_{31} monoene	0.58±0.09		ND	**434** (M^+)
33	C_{31}	1.63±0.26	ND	ND	**436** (M^+)
34	11Me-C_{31}	0.85±0.15	1.33±0.23	1.91±0.31	450 (M^+), 168/308
	13Me-C_{31}				450 (M^+), 196/280
35	2Me-C_{32}	0.49±0.15	ND	ND	464 (M^+), 421, 449
36	13Me-C_{33}	1.46±0.34	0.69±0.11	1.51±0.53	478 (M^+), 196/308

[a] Percent of total hydrocarbons represents means for five individuals. ND, not detected, SPME, solid-phase microextraction

[b] Peaks are numbered in order of elution from a DB-5 capillary column (see Methods and Materials) and correspond to those in Fig. 1

[c] Molecular ions in bold were observed. Molecular ions in normal font were not observed but could be inferred from the diagnostic ions

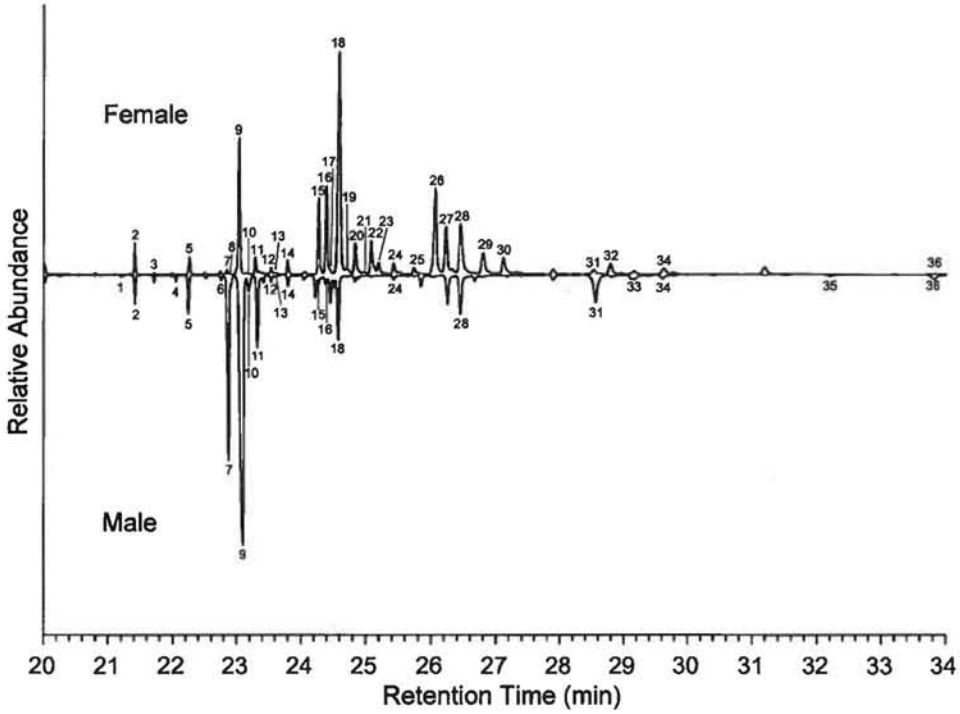

Fig. 1 Representative chromatograms of solvent extracts of female (*top*) and male (*bottom, inverted*) *Mallodon dasystomus*

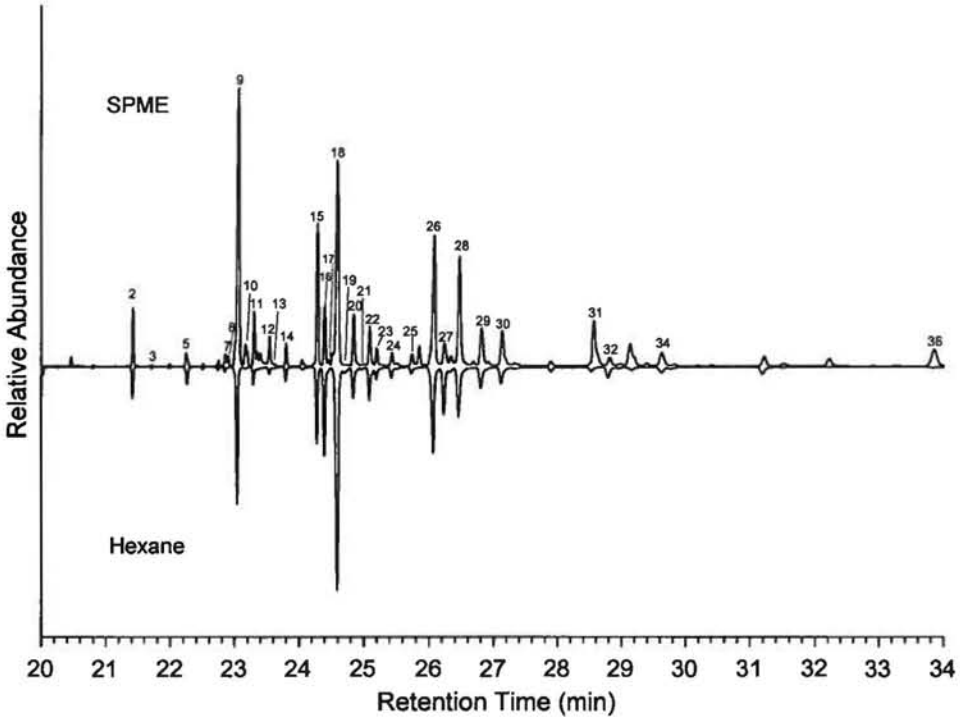

Fig. 2 Representative chromatograms of SPME (*top*) and solvent extracts (*bottom, inverted*) of female *Mallodon dasystomus* cuticular hydrocarbons

respectively) spectrometer (Palo Alto, CA, USA), as $CDCl_3$ solutions. ^1H NMR chemical shifts are expressed in ppm relative to residual $CHCl_3$ (7.27 ppm) and ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ (77.16 ppm). Unless otherwise stated, solvent extracts of reaction mixtures were dried by treatment with anhydrous Na_2SO_4 and concentrated by rotary evaporation under reduced pressure. Crude products were purified by vacuum flash chromatography (VFC) on silica gel (230–400 mesh; Fisher Scientific). Integer resolution mass spectra were obtained with a Hewlett-Packard (HP) 6890 GC (Avondale, PA, USA) interfaced to an HP 5973 mass selective detector, in EI mode (70 eV) with helium carrier gas. The GC was equipped with a DB5-MS column (25 m×0.20 mm ID×0.25 μm film, J&W Scientific, Folsom, CA, USA). Electrospray ionization exact mass data were obtained with an Agilent 6210 LCTOF instrument, introducing the sample via flow injection into a stream of 95% MeOH and 5% water (0.1% formic acid). The vaporizer and gas temperatures in the ESI interface were 200°C. Fragmenter voltage was 125 V. The instrument was operated in the multisource mode. GC-MS exact mass data were obtained on a Waters GCT Premier instrument operated in EI mode (70 eV) with ion source temperature 200°C and injector temperature 240°C. The GC was programmed from 50°C for 1 min, then 10°C min^{-1} to 300°C, using a DB-5 column as described above.

Synthesis of 2-methylhexacosane

12-(Tetrahydropyran-2-yloxy)-dodecan-1-ol **(2)** This compound was made by a modification of the method of Nishiguchi et al. (2000). Thus, 1,12-dodecanediol **1** (50.0 g, 247 mmol) was dissolved in a mixture of hexane (580 ml), dihydropyran (62.40 g, 741 mmol) and dimethylsulfoxide (21 ml). The mixture was stirred vigorously at 50–55°C while a solution of HCl (0.2 M, 49 ml) was slowly added, and the resulting mixture was stirred 14 h. After cooling, the aqueous phase was separated and extracted with hexane (3×50 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (3×50 ml) and brine (2×100 ml), dried, and concentrated. The crude product was purified by vacuum flash chromatography (VFC) on silica gel affording 57.10 g (81%) of the pure mono-protected product **2** (plus 15.4 g ~55% pure by GC). ^1H NMR: δ 1.20–1.40 (m, 16H), 1.46–1.64 (m, 8H), 1.66–1.75 (m, 1H), 1.76–1.88 (m, 1H), 3.37 (dt, 1H, J=6.8 and 9.6 Hz), 3.45–3.54 (m, 1H), 3.62 (t, 2H, J=6.8 Hz), 3.72 (dt, 1H, J=6.8 and 9.6 Hz), 3.82–3.90 (m, 1H), 4.56 (dd, 1H, J= 2.5 and 4.3 Hz). ^{13}C NMR: δ 19.92, 25.72, 25.96, 26.45, 29.65, 29.70, 29.78, 29.79 (3C), 29.81, 29.97, 31.00, 62.58, 63.28, 67.93, 99.09. MS: *m/z* 285 (M-1, 1), 201 (1), 125 (1), 111 (3), 101 (27, 85 (100), 69 (17), 55 (35), 41 (33).

12-(Tetrahydropyran-2-yloxy)-dodecyl-1-p-toluenesulfonate **(3)** Tosyl chloride (40.92 g, 215 mmol) was added in small portions over 2 hr to a solution of 12-(tetrahydropyran-2-yloxy)-dodecan-1-ol **2** (55.90 g, 195 mmol) in dry pyridine (30.86 g, 390 mmol) and chloroform (390 ml), while stirring at 0°C. The reaction was allowed to warm to room temperature, stirred for 1 d, and then *N,N*-dimethylaminopyridine (0.10 g) was added. After stirring 2 d, the mixture was concentrated under vacuum. The residue was taken up in ethyl ether (300 ml), and the solution was thoroughly washed with aqueous HCl (10%) (50 ml), saturated aqueous $NaHCO_3$ (2×50 ml), and brine (2×100 ml), then dried and concentrated. The resulting oil was purified by VFC (hexane: ethyl acetate, 9:1) giving the tosylate **3** as a white solid (64.31 g, 74.8% yield). ^1H NMR: δ 1.18–1.40 (m, 16H), 1.46–1.65 (m, 8H), 1.66–1.75 (m, 1H), 1.77–1.88 (m, 1H), 2.44 (s, 3H), 3.37 (dt, 1H, J=6.6 and 9.6 Hz), 3.46–3.53 (m, 1H), 3.62 (t, 2H, J=6.8 Hz), 3.72 (dt, 1H, J=6.8 and 9.6 Hz), 3.83–3.90 (m, 1H), 4.56 (dd, 1H, J=2.5 and 4.3 Hz), 7.31–7.35 (m, 2H), 7.76–7.80 (m, 2H). ^{13}C NMR: δ 19.95, 21.87, 25.54, 25.73, 26.46, 29.03, 29.15, 29.60, 29.70 (2C), 29.76, 29.78, 29.98, 31.02, 62.61, 67.92, 70.94, 99.12, 128.12 (2C), 130.02 (2C), 133.45, 144.83. HRMS (ESI): calc. *m/z*=463.2489 $(M+Na)^+$; found: *m/z*=463.2486.

15-Methyl-1-(tetrahydropyran-2-yloxy)-hexadecane **(4)** A solution of 1-bromo-3-methylbutane (5.14 g, 34.0 mmol) in dry THF (15 ml) was added dropwise (ca 3 hr) to a suspension of Mg turnings (1.24 g, 51 mmol) in dry THF (19 ml) at room temperature under Ar. After 5 hr, the resulting Grignard reagent solution was diluted with dry THF (30 ml) and added dropwise at –60°C to a previously prepared solution of tosylate **4** (5.00 g, 11.3 mmol) in dry THF (85 ml) under Ar, followed by addition of a solution of Li_2CuCl_4 in THF (0.1 M, 5.7 ml, 0.57 mmol; Aldrich Chemical Co., Milwaukee, WI, USA). The mixture was warmed to room temperature over 2 hr and stirred overnight. The reaction was quenched with saturated aqueous NH_4Cl (50 ml), then extracted with ethyl acetate (4×30 ml). The organic layer was washed with saturated aqueous $NaHCO_3$ (50 ml) and brine (50 ml), dried, and concentrated. The residue was purified by VFC (hexane: ethyl acetate, 95:5) affording compound **4** (3.65 g) in 94.5% yield. ^1H NMR: δ 0.85 (d, 6H, J=6.6 Hz), 1.10–1.20 (m, 2H), 1.20–1.40 (m, 21H), 1.44–1.62 (m, 8H), 1.66–1.75 (m, 1H), 1.78–1.88 (m, 1H), 3.37 (dt, 1H, J=6.8 and 9.6 Hz), 3.45–3.53 (m, 1H), 3.72 (dt, 1H, J=6.8 and 9.6 Hz), 3.83–3.91 (m, 1H), 4.57 (dd, 1H, J=2.7 and 4.3 Hz). ^{13}C NMR: δ 19.92, 22.88 (2C), 25.73, 26.47, 27.65, 28.19, 29.72, 29.83, 29.84, 29.89, 29.91, 29.92 (2C), 29.95, 29.99, 30.17, 31.01, 39.28, 62.55, 67.92, 99.06. MS: *m/z* 339 (M – H, 1), 267 (1), 115 (2), 101 (7), 85 (100), 71 (10), 57 (25), 43 (31) 41 (25).

15-Methylhexadecan-1-ol **(5)** *p*-Toluenesulphonic acid (100 mg) was added to a solution of 15-methyl-1-(tetrahydropyran-2-yloxy)-hexadecane **4** (3.60 g, 10.6 mmol) in methanol (20 ml) and the mixture was stirred at room temperature overnight. Most of the methanol was removed under reduced pressure, water (20 ml) was added to the residue, and the mixture was extracted with hexane (3× 20 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. The residue was purified by VFC (hexane: ethyl acetate, 95:5) affording alcohol **5** (2.53 g) in 93.2% yield. ^{1}H NMR: δ 0.85 (d, 6H, J=6.6 Hz), 1.10–1.20 (m, 2H), 1.20–1.40 (m, 22H), 1.51 (non, 1H, J=6.6 Hz) 1.53–1.60 (m, 2H), 3.63 (t, 2H, J=6.7 Hz). ^{13}C NMR: δ 22.89 (2C), 25.96, 27.65, 28.20, 29.66, 29.83, 29.85, 28.89, 29.91, 29.92 (2C), 29.96, 30.18, 33.04, 39.29, 63.33. MS: *m/z* 238 (M – 18, 1), 223 (1), 210 (3), 182 (3), 168 (1), 153 (2), 140 (2), 125 (8), 111 (21), 97 (36), 83 (55), 71 (26), 57 (96), 43 (100) 41 (87).

1-Iodo-15-methylhexadecane **(6)** Iodine (1.34 g, 5.3 mmol) was added in small portions to a mixture of 15-methylhexadecan-1-ol **5** (0.90 g, 3.5 mmol), triphenylphosphine (1.38 g, 5.3 mmol), and imidazole (0.36 g, 5.3 mmol) in ethyl ether-acetonitrile (3:1–12 ml), and the resulting slurry was stirred at room temperature for 1 hr. The mixture then was diluted with ethyl ether (50 ml), washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. The residue was purified by VFC (hexane) affording 1-iodo-15-methylhexadecane **6** (1.21 g) in 94.1% yield. ^{1}H NMR: δ 0.86 (d, 6H, J=6.6 Hz), 1.10–1.20 (m, 2H), 1.20–1.34 (m, 20H), 1.34–1.44 (m, 2H), 1.51 (non, 1H, J=6.6 Hz), 1.77–1.86 (m, 2H), 3.18 (t, 2H, J=7.0 Hz). ^{13}C NMR: δ 7.56, 22.90 (2C), 27.66, 28.20, 28.79, 29.66, 29.79, 29.85, 29.89, 29.92, 29.93, 29.96, 30.18, 30.75, 33.82, 39.30. MS: *m/z* 239 (M – I, 5), 183 (2), 169 (3), 155 (6), 141 (4), 127 (6), 113 (8), 99 (14), 85 (43, 71 (63, 57 (100), 43 (80).

25-Methylhexacos-9-yne **(8)** *n*-BuLi (1.6 M in hexanes, 4.30 ml, 6.9 mmol) was added dropwise to a solution of 1-decyne **7** (0.91 g, 6.6 mmol) in dry THF (7.0 ml) under Ar at –10°C, and the mixture was stirred for 2 hr, forming a suspension. Dry THF (6 ml) and DMPU (3.36 g, 26 mmol) were added, the mixture was stirred for 1 hr at 0°C, then recooled to –10°C, and a solution of 1-iodo-15-methylhexadecane **6** (1.20 g, 3.3 mmol) in dry THF (2.0 ml) was added dropwise. The reaction was quenched with saturated aqueous NH_4Cl (10 ml) and extracted with hexanes (4× 10 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. The residue was purified by VFC (hexane), affording alkyne **8** contaminated with excess 1-decyne **7**. The impure product was used directly in the next step. ^{1}H NMR: δ 0.86 (d, 6H, J=6.6 Hz), 0.88 (t, 3H, J=7.0 Hz), 1.15–1.19 (m, 2H), 1.22–1.31 (m, 27H), 1.31–1.42 (m, 4H), 1.42–1.56 (m, 4H), 2.10–2.17 (m, 4H). ^{13}C NMR: δ 14.33, 18.99, 22.89, 27.65, 28.19, 29.10, 29.37, 29.40, 29.46, 29.80, 29.87, 29.91, 29.93, 29.96, 30.18, 32.08, 39.29, 77.55, 80.47. MS: *m/z* 376 (M^+, 12), 281 (3), 193 (8), 152 (7), 137 (18), 124 (24), 109 (36), 95 (70), 81 (100), 67 (92), 43 (98), 41 (68). HRMS (ESI): calc. *m/z*=375.3985 $(M\text{-}H)^+$; found: *m/z*=375.3991.

2-Methylhexacosane **(9)** A mixture of 25-methylhexacos-9-yne **8** (+ 1-decyne, 0.90 g) and 5% Pd/C (0.090 g,) in hexane (15 ml) was stirred at room temperature under hydrogen for 16 hr. The mixture was filtered through Celite, and the filtrate was concentrated. The resulting mixture of hydrocarbons was subjected to Kugelrohr distillation (oven temp to 140°C, 0.05 mm Hg) to remove decane, affording 0.75 g (60% over 2 steps) of pure 2-methylhexacosane **9**. ^{1}H NMR: δ 0.86 (d, 6H, J=6.8 Hz), 0.88 (t, 3H, J=6.8 Hz), 1.11–1.18 (m, 2H), 1.20–1.34 (m, 44H), 1.45–1.57 (m, 1H). ^{13}C NMR: δ 14.35, 22.89, 27.66, 28.20, 29.59, 29.89, 29.93, 29.96, 30.18, 32.16, 39.29. MS: *m/z* 380 (M^+, 1), 365 (3), 337 (16), 239 (3), 225 (2), 211 (3), 197 (4), 183 (4), 169 (5), 155 (6), 141 (8), 127 (9), 113 (12), 99 (19), 85 (42), 71 (63), 57 (100), 43 (93).

Synthesis of 2-methyloctacosane

2-Methyltetradec-5-yne **(11)** *n*-BuLi (2.88 M, 35 ml, 100 mmol) was added dropwise under Ar to a solution of 1-decyne **7** (13.8 g, 100 mmol) in 200 ml THF cooled in a dry ice-acetone bath. The resulting slurry was warmed to room temperature, and NaI (1.5 g, 10 mmol) and 1-bromo-3-methylbutane **10** (14.3 g, 95 mmol) were added. The resulting mixture was refluxed under Ar for 40 hr, then cooled and quenched with saturated aqueous NH_4Cl. The mixture was extracted with hexane, and the organic layer was washed with brine, dried, and concentrated. The residue was Kugelrohr distilled, collecting a forerun (oven temp <50°C, 0.1 mm Hg) consisting mostly of unreacted 1-decyne **7**. The desired product **11** then was collected in a clean bulb (15.33 g, oven temp 70–80°C, 0.1 mm Hg) in 73% overall yield (86% based on recovered 1-decyne). ^{1}H NMR: δ 0.89 (t, 3H, J= 6.8 Hz), 0.89 (d, 6H, J=6.8 Hz), 1.25–1.35 (m, 10H), 1.35–1.52 (m, 4H), 1.63–1.72 (m, 1H), 2.12–2.22 (m, 4H). ^{13}C NMR: δ 14.30, 16.95, 18.95, 22.39, 22.86, 27.37, 29.05, 29.32, 29.36, 29.41, 32.04, 38.37, 80.30, 80.40. MS: *m/z* 208 (M^+, 1), 193 (18), 166 (1), 152 (2), 137 (3), 123 (7), 109 (26), 95 (100), 81 (76), 69 (26), 67 (46), 55 (36), 43 (23). HRMS (ESI): calc. *m/z*=208.2186 (M^+); found: *m/z*=208.2193.

13-Methyltetradec-1-yne **(12)** A dry 1 liter 3-neck flask was flushed with Ar and charged with 225 ml dry 1,3-diaminopropane, followed by Li wire (3.2 g, 460 mmol). The mixture was stirred 1 hr at room temperature during which time the Li partially dissolved to give a blue solution. The mixture was heated to 70°C, and stirred for 2 hr, by which time all the Li had dissolved and the blue solution changed to a white suspension. The mixture was cooled to room temperature, and potassium *t*-butoxide (33 g, 295 mmol) was added in one portion with stirring, giving a yellow suspension. After stirring 30 min, 2-methyltetradec-5-yne **11** (15.2 g, 73 mmol) was added over 10 min, and the mixture became warm and turned red-brown. The progress of the isomerization was followed by GC, and after ~1 hr, there was no further change. The mixture was poured into 1 L of a slurry of crushed ice and water, and the mixture was extracted with hexane (3× 250 ml). The combined hexane extracts were washed with water and brine, dried, concentrated, and purified by Kugelrohr distillation (forerun, oven temp <60°C at 0.5 mm Hg; product, oven temp≤100°C, 0.2 mm Hg), yielding 12.76 g of alkyne **12** (83%). ^{1}H NMR: δ 0.87 (d, 6H, J=6.8 Hz), 1.11–1.18 (m, 2H), 1.22–1.34 (m, 12H), 1.35–1.44 (m, 2H), 1.47–1.57 (m, 3H), 1.94 (t, 1H, J= 2.5 Hz), 2.19 (td, 2H, J=7.0, 2.5 Hz). ^{13}C NMR: δ 18.6, 22.9 (2C), 27.6, 28.2, 28.7, 29.0, 29.3, 29.7, 29.8, 29.9, 30.1, 39.2, 68.2, 85.0. MS: *m/z* 193 (M^+-15, trace), 179 (trace), 137 (1), 123 (4), 109 (18), 95 (48), 81 (100), 69 (36), 67 (64), 57 (33), 55 (48), 43 (59). HRMS (ESI): calc. *m/z*=207.2107 $(M-H)^+$; found: *m/z*=207.2103.

2-Methyloctacos-13-yne **(14)** A solution of tetradecanol (2.14 g, 10 mmol) and pyridine (0.85 g, 10.5 mmol) in CH_2Cl_2 was cooled to <-10°C in an ice-salt bath, and triflic anhydride (2.2 ml, 13 mmol) was added dropwise. The resulting solution was slowly warmed to room temperature, by which time all the starting alcohol had been consumed. The solution was diluted with 100 ml hexane, and filtered through a 2 cm pad of Celite. The colorless filtrate was concentrated and pumped under vacuum for a few minutes to remove traces of solvent, then the crude triflate **13** was taken up in 10 ml THF and used immediately.

In a separate flask, a solution of 13-methyltetradec-1-yne **12** (2.6 g, 12.5 mmol) in 25 ml THF under Ar was cooled in an ice bath and n-BuLi (2.8 M, 4.8 ml, 13.4 mmol) was added dropwise. After stirring for 15 min, the mixture was cooled to –20°C, the solution of freshly prepared triflate was added dropwise, and the resulting slurry was slowly warmed to room temperature and then stirred for 4 hr. The mixture was quenched with ice water and extracted with hexane. After washing with brine, the hexane solution was dried and concentrated, and the residue was purified by VFC (hexane), followed by Kugelrohr distillation (oven temp up to 100°C, 0.2 mm Hg) to remove low-boiling impurities. The pot residue consisting of essentially pure alkyne **14** was used directly in the next step. ^{1}H NMR: δ 0.84–0.91 (m, 9H), 1.12–1.41 (m, 43H), 2.15 (m, 4H). ^{13}C NMR: δ 14.31, 18.56, 22.86, 27.62, 28.17, 29.07, 29.38, 29.56, 29.78, 29.89, 30.14, 31.79, 32.13, 39.26, 80.44. MS: *m/z* 404 (M^+,6), 292 (1), 291 (1), 278 (5), 277 (5), 264 (5), 263 (5), 236 (3), 221 (8), 207 (11), 194 (7), 180 (4), 166 (4), 151 (8), 137 (19), 123 (33), 109 (54), 97 (55), 96 (89), 95 (97), 83 (60), 82 (91), 81 (100), 69 (46), 67 (65), 57 (38), 55 (43), 43 (47). HRMS (ESI): calc. *m/z*=403.4298 $(M-H)^+$; found: *m/z*= 403.4291.

2-Methyloctacosane **(15)** Alkyne **14** was taken up in 50 ml hexane, and 5% Pd on activated charcoal (0.5 g) was added. The flask was fitted with a balloon filled with H_2, and the headspace was flushed with H_2 before sealing and stirring under H_2 until hydrogenation was complete (3 hr). The resulting mixture was filtered through a pad of Celite, rinsing with 20 ml hexane, and the combined filtrate (~70 ml) was cooled overnight at –20°C. The resulting white crystals were filtered with suction in a cold room, yielding 1.38 g of 2-methyloctacosane **15** (>99.5% pure), and 2 g of impure product from the remaining liquor after concentration. ^{1}H NMR: δ 0.86 (d, 6H, J=6.8 Hz), 0.88 (t, 3H, J=6.8 Hz), 1.11–1.18 (m, 2H), 1.20–1.32 (m, 48H), 1.45–1.57 (m, 1H). ^{13}C NMR: δ 14.33, 22.86, 27.64, 28.17, 29.58, 29.90, 30.17, 32.13, 39.29. MS: *m/z* 408 (M^+,1), 393 (9), 365 (30), 351 (2), 337 (2), 323 (3), 309 (3), 295 (4), 281 (4), 267 (4), 253 (5), 239 (5), 225 (6), 211 (7), 197 (8), 183 (8), 169 (10), 155 (12), 141 (15), 127 (19), 113 (25), 99 (36), 85 (77), 71 (88), 57 (100), 43 (55).

Bioassays of Hydrocarbon Standards We tested the bioactivity of female-specific compounds in bioassays similar to those described above. The bioactivity of standards of 2Me-C_{26} and 2Me-C_{28} were tested individually and in combination at 1 FE doses in ratios similar to those of SPME samples (28 and 43 ug, respectively). Compounds were quantified by comparing their peak areas in total ion chromatograms with those of an external standard (*n*-docosane). Numbers of males responding to treatments were compared with those responding to freeze-killed females using a Fisher's exact test (Sokal and Rohlf, 1995). Bioassays were conducted in the dark under a red light between 12.00 and 17.00 hr during late June through July, 2009. The remaining female specific compounds were not synthesized and tested because these two synthetic compounds elicited a response in males equal to that of freeze-killed females (see Results).

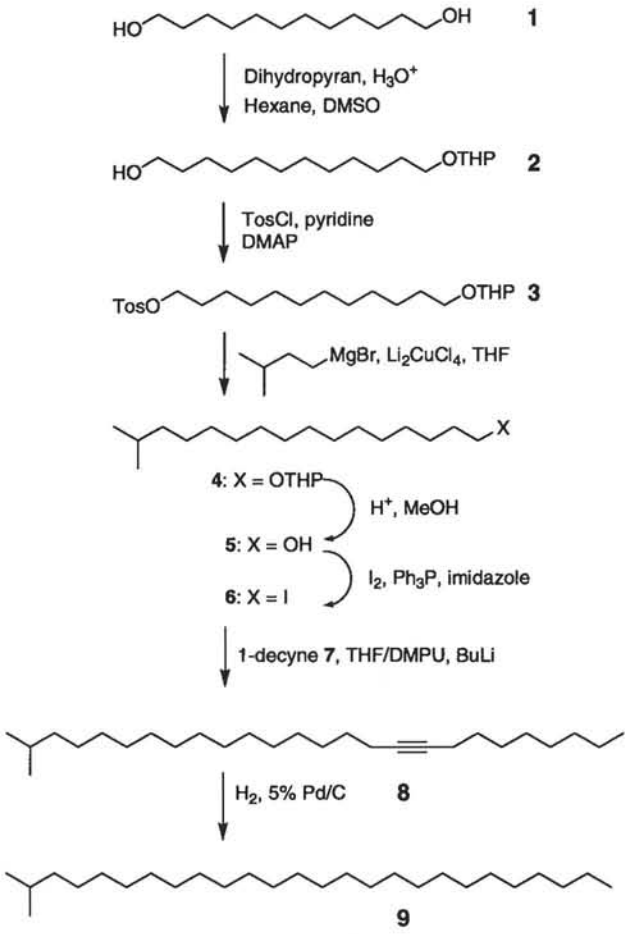

Fig. 3 Synthesis of 2-methylhexacosane

Results

Characterization of Mating Behavior Once a male touched a female with his antennae (N=17), a clear progression of cumulative behavioral steps led to copulation: Step 1) male stopped walking and antennated the female; Step 2) the male aligned his body with the female; Step 3) the male mounted the female; Step 4) the male bent his abdomen to connect his genitalia to those of the female. Out of 17 males tested, 15 responded to live females with the full range of behavioral steps after making antennal contact. The remaining two males either ignored the female or responded aggressively (e.g., head raised, biting female's elytra and/or legs) upon encountering the female. In preliminary assays, males were also paired with other males (N=10) and, after brief antennal contact, responded aggressively to each other (e.g., biting and kicking) and made no attempt to mate.

Role of Contact Chemoreception All males that were tested displayed a Step 4 behavioral response to freeze-killed females, confirming that signals mediating mate recognition were intact and behavioral signals were not important in mate recognition. None of the males responded to the solvent-washed females, demonstrating that the compounds that mediate mate recognition had been removed by solvent extractions. Of the 18 males tested, 16 displayed Step 4 behavior toward female carcasses to which solvent extracts had been reapplied (response not different from response to freeze-killed females; Fisher's exact test, F=14.06, P> 0.05). Males displayed mating behavior to female carcasses after application of an average (±SE) of 0.15±0.03 FE of crude extract. Two males did not display any mating behaviors to females reconstituted with as much as 0.9 FE of crude extract.

Identification of Cuticular Hydrocarbons Thirty-one compounds were identified in hexane extracts of females, including a series of straight chain compounds from C_{23} to C_{29}. Thirteen compounds were essentially specific to females (Table 1, Fig. 1). Although there were many qualitative differences between hydrocarbon profiles of extracts of females and males, those of females contained two dominant compounds (2Me-C_{26} and 2Me-C_{28}) that were virtually absent in extracts of males. Of these two compounds, the relative abundance of 2Me-C_{26} in SPME wipe samples increased by 65% over solvent extracts (peak 15; Table 1, Fig. 2, means for two sampling methods significantly different, ANOVA: $F_{(1,8)}$=10.28, P=0.01),

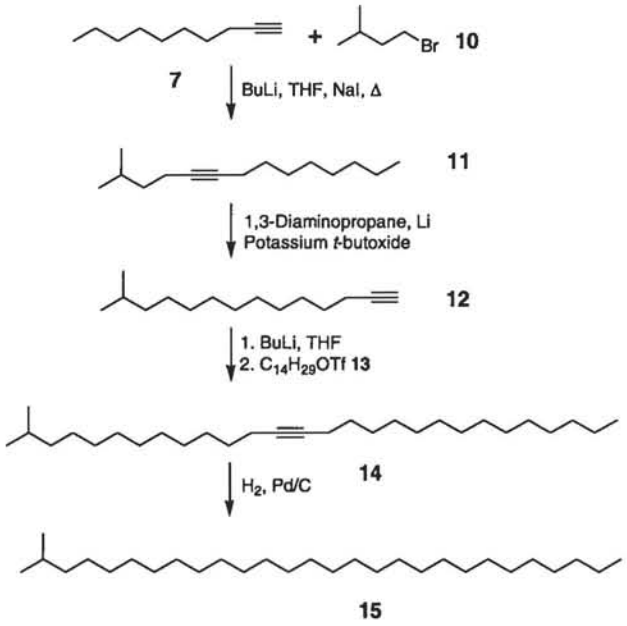

Fig. 4 Synthesis of 2-methyloctacosane

Table 2 Responses of male *Mallodon dasystomus* to freeze-killed females (controls) and to solvent-washed females that had been treated with one female equivalent of hydrocarbon standards

Compound(s)	No. of males tested	Percent of males responding per step in behavioral sequence[a]			
		Step 1	Step 2	Step 3	Step 4[b]
2Me-C_{26}	21	90 (100)	71 (100)	52 (100)	38 (86)*
2Me-C_{28}	24	96 (100)	79 (100)	67 (100)	58 (100)**
2Me-C_{26}+2Me-C_{28}	27	100 (100)	100 (100)	100 (100)	81 (100)

[a] Response of males to treatments *versus* controls (values in parentheses) were tested with Fisher's exact test (*$P<0.05$, **$P<0.001$)

[b] Numbers of males reaching behavioral step 4 were compared to the response to controls (H_o=no difference in bioactivity between treatment and freeze-killed female)

suggesting that it may be an important component of the contact pheromone. Other less abundant, female-specific compounds included: a C_{25} diene, 11-methyl- and 13-methyl-C_{27}, 11,15-dimethyl-C_{27}, 12-methyl-, 13-methyl-, and 14-methyl-C_{28}, 11-methyl-, 13-methyl-, and 15-methyl-C_{29}, and 13,17-dimethyl-C_{29} (Table 1, Fig. 1). Most of the compounds present in the solvent extracts also were present in the SPME samples of females.

Synthesis of 2Me-C_{26} and 2Me-C_{28} Two different routes for making 2-methylalkanes were explored. In the first (Fig. 3), 1,12-dodecanediol **1** was mono-protected with 3,4-dihydropyran (DHP) to give **2** in 81% isolated yield (Nishiguchi et al., 2000), and the remaining hydroxyl group then was converted to the tosylate **3** (75%). Coupling of tosylate **3** with 3-methylbutyl magnesium bromide with Li_2CuCl_4 catalysis installed the methyl branch, giving 15-methyl-1-(tetrahydropyran-2-yloxy)-hexadecane **4** (95%), which was deprotected in acidic MeOH to give 15-methyl-hexadecan-1-ol **5**. The alcohol was converted to iodide **6** in 94% yield (Corey et al., 1983), giving a synthon that could be converted to any desired long-chain 2-methylalkane. Thus, treatment of 1-decyne **7** in DMPU/THF with *n*-butyllithium followed by addition of iodide **6** gave crude 25-methylhexadec-9-yne **8** contaminated with unreacted 1-decyne. This mixture was reduced with H_2 and 5% Pd/C catalyst, giving 2-methylhexacosane **9** in 60% isolated yield over 2 steps after purification.

2-Methyloctacosane was made by a shorter route (Fig. 4). Thus, reaction of 1-bromo-3-methylbutane **10** with 1-decynyllithium in refluxing THF with NaI catalysis (Buck and Chong, 2001) gave 2-methyltetradec-5-yne **11** in 73% yield (86% based on recovered starting material). Isomerization of the alkyne to the terminal position with the acetylene zipper reaction (Abrams and Shaw, 1988) then gave 13-methyltetradec-1-yne **12**, an alternate synthon for synthesis of a variety of long-chain 2-methylalkanes. Deprotonation of 13-methyltetradec-1-yne **12** with butyllithium followed by reaction with the triflate **13** derived from tetradecan-1-ol in THF gave 2-methyloctacos-13-yne **14** in good yield (Wang and Zhang, 2007), which was then hydrogenated with H_2 and 5% Pd/C catalyst to give the desired 2-methyloctacosane **15**. This route was shorter and more efficient overall than the route used to make 2-methylhexacosane.

Bioassays of Hydrocarbon Standards In bioassays, 2Me-C_{26} and 2Me-C_{28} as individual components elicited some mating responses in males (Step 4 behavior). However, males responded at a rate similar to that elicited by freeze-killed females only when the two compounds were presented as a blend (Table 2).

Discussion

Our results support the hypothesis that male *M. dasystomus* recognize females by a cuticular contact pheromone blend. Males attempted to mate with females only after contacting them with their antennae. In bioassays with freeze-killed females, all males displayed a full progression of mating behaviors and attempted to connect the genitalia, excluding behavioral signals as a component of mate recognition. Additionally, no males attempted to mate with a solvent washed female, suggesting that the mate recognition signals had been removed by the solvent, and that visual signals were not involved. When paired with extracted female carcasses that had been treated with the cuticular extract, 15 of 17 males attempted to mate only after making antennal contact, providing further evidence for an extractable contact pheromone.

Because of the marked differences in the profiles of cuticular lipids between the sexes, our strategy for identifying the contact pheromone components was to synthesize and test the bioactivity of female specific compounds, starting with the most abundant. This approach was effective in the identification of the contact pheromones of *Xylotrechus colonus* F. (Ginzel et al., 2003a) and,

more recently, *N. a. acuminatus* (Lacey et al., 2008). The first two compounds that were synthesized (2Me-C_{26} and 2Me-C_{28}) comprised over 17% of the total hydrocarbons in the cuticular extracts of females. Moreover, there was a significant increase in the proportion of 2Me-C_{26} in the SPME samples when compared to the whole-body hexane extracts, suggesting that it may be an important component of the contact pheromone.

Male *M. dasystomus* responded to both 2Me-C_{26} and 2Me-C_{28} alone in bioassays. However, a blend of the two compounds elicited the greatest response in males, and the response was similar to that of the crude extract, suggesting that these compounds together are the most important components of the contact pheromone. Other cerambycids also have contact pheromones that are multicomponent blends. For example, the contact pheromone of *X. colonus* is comprised of at least three compounds: *n*-C_{25}, 9Me-C_{25}, and 3Me-C_{25} (Ginzel et al., 2003a). Moreover, male *N. a. acuminatus* recognize mates by the presence of 7Me-C_{27}, with two additional branched compounds, 9Me-C_{27} and 7Me-C_{25}, acting as synergists (Lacey et al., 2008). These multi-component blends often are composed of compounds that are homologues, as is the case here with 2Me-C_{26} and 2Me-C_{28}. However, to our knowledge, these compounds are the first 2-methylalkanes and even-chain length hydrocarbons to be identified as contact pheromones in the Cerambycidae. Nevertheless, both 2Me-C_{26} and 2Me-C_{28} are part of the wax layer of other cerambycid species, suggesting that some hydrocarbons may be common to the family. For example, 2Me-C_{26} was found in the cuticular hydrocarbons of male and female *N. a. acuminatus* (Lacey et al., 2008), both sexes of *M. robiniae* (Ginzel et al., 2003b), and in the wax layer of female *M. caryae* (Ginzel et al., 2006). Interestingly, 2Me-C_{28} is more abundant in the wax layer of male than female *M. robiniae* (Ginzel et al., 2003b), whereas it is more abundant in the cuticular hydrocarbons of female than male *M. caryae* (Ginzel et al., 2006).

The solvent extracts of female *M. dasystomus* contained 13 compounds that were essentially absent or greatly reduced in extracts of males. Similar sexual differences were noted in the cuticular lipid profiles of *M. robiniae* and *X. colonus*, in which the compounds constituting the contact pheromones were not completely absent from extracts of males, but were greatly reduced in abundance when compared to extracts of females (Ginzel et al., 2003a, 2006). Here, the differences in the relative amounts of the *M. dasystomus* contact pheromone components, 2Me-C_{26} and 2Me-C_{28}, were obvious, with these two compounds being co-dominant in the hydrocarbon profiles of females. Furthermore, 2Me-C_{26} was significantly more abundant in SPME wipe samples than in hexane extracts of female cuticular hydrocarbons, supporting our previous observations that contact pheromones are present in greater abundance in SPME samples when compared to whole-body extracts. SPME may more accurately represent the compounds that are accessible to the male antennae on the surface of the wax layer, and provide a clearer representation of those hydrocarbons that act as contact pheromones.

During this study, we also observed females displaying behaviors associated with long-range pheromone production. These "calling" behaviors included lowering the head, raising the abdomen, and extending the ovipositor, often in a rhythmic pumping motion. While "calling", one female everted a clear cylindrical, fluid-filled sac from her ovipositor. Female *Prionus californicus* release a powerful sex pheromone from a similar structure (Cervantes et al., 2006; Rodstein et al., 2009). In fact, the calling behaviors of female *M. dasystomus* are almost identical to those of *P. californicus* (Barbour et al., 2006), and similar to other species of insects in which females produce volatile pheromones (e.g., Burkholder et al., 1974; Hammack and Burkholder, 1981). In the subfamily Cerambycinae, the scanty available evidence suggests that mate location might involve three sequential behavioral steps: 1) both sexes are independently attracted to volatiles emanating from the larval host plant; 2) once on the host, males attract both sexes from some distance with aggregation pheromones; and 3) having recruited conspecifics, males recognize females by contact pheromones (Ginzel and Hanks, 2005). In contrast, beetles in the subfamily Prioninae may rely exclusively on sex pheromones to bring the sexes together (Barbour et al., 2006; Cervantes et al., 2006; Rodstein et al., 2009), and then, once in close proximity, the final steps of mate recognition may be mediated by contact pheromones. Although this is the first contact pheromone identified from a cerambycid beetle in the subfamily Prioninae, we anticipate that the use of contact pheromones will be widespread in this subfamily (e.g., Barbour et al., 2007), as it is in many other insect groups.

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