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2,3-Hexanediols as Sex Attractants and a Female-produced Sex Pheromone for Cerambycid Beetles in the Prionine Genus *Tragosoma*

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Abstract Recent work suggests that closely related cerambycid species often share pheromone components, or even produce pheromone blends of identical composition. However, little is known of the pheromones of species in the subfamily Prioninae. During field bioassays in California, males of three species in the prionine genus *Tragosoma* were attracted to 2,3-hexanediols, common components of male-produced aggregation pheromones of beetles in the subfamily Cerambycinae. We report here that the female-produced sex pheromone of *Tragosoma depsarium* "sp. nov. Laplante" is (2*R*,3*R*)-2,3-hexanediol, and provide evidence from field bioassays and electroantennography that the female-produced pheromone of both *Tragosoma pilosicorne* Casey and *T. depsarium* "*harrisi*" LeConte may be (2*S*,3*R*)-2,3-hexanediol. Sexual dimorphism in the sculpting of the prothorax suggests that the pheromone glands are located in the prothorax of females. This is the second sex attractant pheromone structure identified from the subfamily Prioninae, and our results provide further evidence

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of pheromonal parsimony within the Cerambycidae, in this case extending across both subfamily and gender lines.

Keywords 2,3-hexanediol · Mating behavior · Prioninae · Cerambycidae · Longhorned beetle · Pheromone

Introduction

A rapidly growing body of literature indicates that species in the beetle family Cerambycidae exhibit considerable parsimony in pheromone structures, with closely related species often sharing pheromone components, or even apparently using pheromones of identical composition. For example, males of many species in the subfamily Cerambycinae produce pheromones composed of isomers of 3-hydroxy-2-hexanone and/or 2,3-hexanediol, to which both sexes are attracted (e.g., Lacey et al., 2004, 2007, 2008, 2009; Hanks et al., 2007; Ray et al., 2009). Similarly, the terpenoid alcohol (*E*)-6,10-dimethyl-5,9-undecadien-2-ol, termed "fuscumol", is a male-produced pheromone of some species in the subfamily Spondylidinae (Silk et al., 2007), and a species in the subfamily Lamiinae (Fonseca et al., 2010). Fuscumol and its acetate also attract both sexes of many other lamiine species (e.g., Mitchell et al., 2011), suggesting that these compounds may be pheromone components for these species as well. A single pheromone structure also is shared among species of the lamiine genus *Monochamus*, with males of at least five species producing 2-undecyloxy-1-ethanol (Pajares et al., 2010; Teale et al., 2011, Fierke et al. 2012; Allison et al. unpublished).

We recently identified the first sex attractant pheromone, (3*R*,5*S*)-3,5-dimethyldodecanoic acid, secreted in nanogram quantities by a gland on the ovipositor, from a cerambycid species in the subfamily Prioninae (Tribe Prionini), *Prionus californicus* Motschulsky (Rodstein et al., 2009, 2011). Males of at least seven other North American and European *Prionus* species are attracted by a blend of the four stereoisomers of 3,5-dimethyldodecanoic acid, suggesting that (3*R*,5*S*)-3,5-dimethyldodecanoic acid, or one of its isomers, are likely pheromone components for those species as well (Barbour et al., 2011). These findings further suggest that 3,5-dimethyldodecanoic acid represents another pheromone motif that is shared by multiple species in the family Cerambycidae.

In field bioassays, screening a series of known cerambycid pheromones, males of three prionine species in the genus *Tragosoma* (Tribe Meroscelisini) were attracted to 2,3-hexanediols, the same compounds that are male-produced aggregation pheromones of many cerambycine species (Fettköther et al., 1995; Lacey et al., 2004, 2008, 2009; Ray et al., 2009). At the time of writing, the genus is under revision by S. Laplante (Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada). Here, we describe the identification of a female-produced sex attractant pheromone for *Tragosoma depsarium* "sp. nov. Laplante", and provide evidence from field bioassays and electroantennography that suggests that the congeners *T. pilosicorne* Casey and *T. depsarium* "*harrisi*" LeConte may also use a 2,3-hexanediol isomer as a sex pheromone.

Methods and Materials

Pheromone Chemicals 2,3-Hexanediol, as a mixture of all 4 stereoisomers, was prepared by $LiAlH_4$ reduction of 2,3-hexanedione (Aldrich Chemical, Milwaukee, WI, USA), as described in Hanks et al. (2007). Diastereomerically pure, but racemic, (2*R**,3*R**)- and (2*R**,3*S**)-2,3-hexanediols were made by OsO_4-catalyzed dihydroxylation of (*Z*)- and (*E*)-2-hexenes (GFS Chemicals, Powell, OH, USA), respectively, as described by Lacey et al. (2004), whereas racemic 3-hydroxy-2-hexanone was made as described in Imrei et al. (2012). The four individual 2,3-hexanediol stereoisomers were synthesized by $NaBH_4$ reduction of (*R*)- or (*S*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-3-hexanones, followed by acid-catalyzed hydrolysis of the THP-protecting groups and chromatographic separation of the resulting pairs of diol diastereomers (Lacey et al., 2008). The resulting diols had stereoisomeric purities as follows: (2*R*,3*R*), >98 % de; (2*R*,3*S*), >97 % de; (2*S*,3*S*), >98 % de; (2*S*,3*R*), >96 % de.

Field Bioassays Field bioassays were conducted in California and Idaho during July and August of 2005, 2009, 2010, and 2011 (maximum daily temperatures 27–33 °C, at most a trace of precipitation). Black flight-intercept panel traps (see Hanks et al., 2007) were used, suspended from tree branches or from frames of plastic irrigation pipe. Traps were positioned 5–10 m apart in linear transects and checked every 2–9 d, at which time treatments were rotated down transects, to control for location effects, and lures replaced as needed. Trap lures were suspended in the center of traps, but the design of lures changed over seasons as more effective lures were developed (see below).

Tragosoma pilosicorne males were first caught during a field-screening bioassay, conducted 25 July to 10 August 2005 at the University of California's James San Jacinto Mountain Reserve (Riverside Co., CA, USA; Table 1), testing attraction to racemic six-, eight-, and ten-carbon 2,3-alkanediols, 2-hydroxy-3-alkanones, and 3-hydroxy-2-alkanones. Lures consisted of cotton dental rolls loaded with 80 mg of test compounds in 1 ml of hexane in uncapped 3.7 ml glass vials, with controls consisting of hexane only.

Attraction of *T. pilosicorne* to (2*R**,3*S**)-2,3-hexanediol was confirmed with a follow-up study conducted at the same site from 10–15 August 2005. Traps were modified so as to capture beetles alive for pheromone analysis

Table 1 Study sites where field bioassays were conducted. All field sites were mixed conifer-oak woodlands

Site name (**abbreviation**)	Location	Position of first trap in transect
Univ. California James San Jacinto Mountain Reserve (**JMR**)	San Jacinto Mountains, Riverside Co., CA	N 33° 48′ 30.00″, W 116° 46′ 40.02″; 1,638 m elevation
Barton Flats (**BF**)	West Jenks Lake Road, San Bernardino National Forest, Mountaintop Ranger District, San Bernardino Co., CA	N 34° 9′ 47″ W116° 53′ 57″; 2,020 m elevation
Blue Ridge (**BR**)	Blue Ridge Truck Trail, Angeles National Forest, Mojave River Ranger District, Los Angeles Co., CA	N 34°22′12″, W 117°42′16.82″, 2,200 m elevation
Rancho los Mochos Boy Scout Camp (**RLM**)	Mines Rd., Foothills of Diablo Mountain Range, Alameda Co., CA	Replicate 1: N37° 32′35″ W121° 34′19″ 763 m elevation; replicate 2: N37° 32′38″ W121° 34′22″ 742 m elevation
University of California Lick Observatory (**UCLO1**)	Old Spring Rd. Mt. Hamilton, Diablo Mountain Range, Santa Clara Co., CA	N37° 20′23″ W121° 38′08″ 1,166 m elevation
University of California Lick Observatory (**UCLO2**)	Mule Trail Rd., Mt. Hamilton, Diablo Mountain Range, Santa Clara Co., CA	N37° 20′58″ W121° 37′12″ 1,155 m elevation
Lone Pine Canyon (**LP**)	Lone Pine Canyon Rd., San Gabriel Mountains, San Bernardino Co., CA	N34° 20′19. 7″ W117° 36′06.4″ 1,825 m elevation
Shafer Butte (**SB**)	National Forest Service Rd. 374, Boise Co., ID	N43° 47′0.28″ W116° 5′12.58″ 2,041 m elevation
Sage Hen Reservoir (**SR**)	National Forest Service Rd. 614, Gem Co., ID	N44° 20′12.17″ W116° 10′43.42″ 2,041 1,552 m elevation

(erroneously assuming that both sexes would be attracted; see Results and Hanks et al., 2007 for details about modification of traps). Lures were cotton rolls, loaded with 5 mg of (2*R**,3*S**)-2,3-hexanediol in 1 ml of absolute ethanol, held in uncapped 3.7 ml glass vials, with controls treated only with 1 ml ethanol. Treatment and control traps were alternated along a transect of 14 traps.

Further bioassays were conducted in 2009 at field sites in Alameda County, CA, USA, where a population of *T. pilosicorne* was detected by light trapping. Traps were set at two sites: Rancho los Mochos Boy Scout Camp (RLM) and two areas on the grounds of the University of California's Lick Observatory (UCLO1 and UCLO2; Table 1). Bioassays were conducted fom 29 July to 8 August 2009. Pheromone lures consisted of low-density polyethylene sachets as described by Ray et al. (2011), loaded with 20 mg of (2*R**,3*S**)- or (2*R**,3*R**)-2,3-hexanediols in 1 ml of ethanol, or ethanol controls. Treatments and controls were replicated three times at each study site. Traps were as described above.

A further bioassay to identify the biologically active enantiomer for *T. pilosicorne* was conducted at UCLO1 and UCLO2 from 8–31 August 2009, using the same methods and numbers of replicates as the previous bioassay, but with lures loaded with (2*R**,3*S**)-, (2*R*,3*S*)-, or (2*S*,3*R*)-2,3-hexanediol (racemate, 50 mg in 1 ml of ethanol; enantiomers, 25 mg in 1 ml ethanol), or ethanol controls.

Bioassays conducted during the 2009 and 2010 seasons tested whether 2,3-hexanediols would also attract other *Tragosoma* species, in particular *T. depsarium* (L.). Potential field sites were identified by examining collection records in the Entomology Research Museum at the University of California, Riverside, the Essig Museum of Entomology at the University of California, Berkeley, and private insect collections. These new field sites included Barton Flats (BF), Blue Ridge (BR), and Lone Pine Canyon (LP) areas in southern California, and sites near Shafer Butte (SB) and Sage Hen Reservoir (SR) in Idaho (Table 1). Trap and lure designs were the same as those used in the study at UCLO (above), with the exception that traps were treated with a fluoropolymer dispersion emulsion (Fluon®; Graham et al., 2010).

Bioassays at BF and BR were conducted from 5 July to 17 August 2009 with traps baited with either the complete blend of all four 2,3-hexanediol isomers, (2*R**,3*R**)- or (2*R**,3*S**)-2,3-hexanediols, or a solvent control. At both sites, traps were set in three separate transects, with each transect containing one trap of each treatment.

Bioassays at SB and SR in Idaho were conducted 12–27 August 2009, as part of a larger survey testing attraction of cerambycid species to traps baited with a variety of candidate pheromones (unpublished data), but including (2*R**,3*R**)- and (2*R**,3*S**)-2,3-hexanediols, and a solvent control. Trap and lure designs were as described above, with each site containing a single replicate.

Bioassays in 2010 were conducted at LP and BF from 7 July to 23 August. The treatments were (2*R**,3*R**)-, (2*R**,3*S**)-2,3-hexanediols, and a solvent control. Trap and lure designs were as previously described, with two replicates used at each site.

A final bioassay was conducted at the BF site from 20 July to 9 September 2011, with two replicates, and treatments of (2*R*,3*R*)-, (2*S*,3*S*)-, (2*R*,3*S*)-, or (2*S*,3*R*)-2,3-hexanediols. Trap and lure designs were as previously described.

Statistical Analysis Differences among treatments were tested separately for each site and year, and blocked by date,

using the nonparametric Friedman's Test (PROC FREQ, option CMH; SAS Institute, 2001). Differences between pairs of means were tested with the REGWQ means-separation test to control maximum experiment-wise error rates (PROC GLM; SAS Institute, 2001). We included in each analysis, only replicates that had a minimum number of specimens in order to assure sufficient replication for a robust analysis (at least 10 replicates; number of specimens ranged from 1–12, depending on total number captured).

Analysis of Headspace Volatiles Eight pupae of *T. depsarium* "sp. nov. Laplante" were collected on 3 June 2010 from downed *Pinus* sp. in the vicinity of the BF field site. Pupae were placed individually in artificial pupation chambers constructed of rolled newsprint (7 cm long×3 cm diam.) that were plugged with tissue paper, misted with water, and stored in a plastic bag at ambient temperature. Pupae were examined 2–3 times per week to monitor development. Adults eclosed from 26 June to 12 July 2010; teneral adults were returned to their paper tubes and temporarily stored at 4 °C until needed for experiments.

Headspace odors of individual adult *T. depsarium* "sp. nov. Laplante" (virgin-reared females, and both field-caught and virgin-reared males) were collected in an environmentally controlled room (26 °C, 65 % RH), using apparatus and methods previously described (Ray et al., 2011). Aerations were run for 4 d, after which the activated-charcoal collectors were extracted with dichloromethane (3 aliquots totaling 500 μl). Extracts were stored in a freezer (−4 °C) until analyzed.

To determine which 2,3-hexanediol stereoisomer(s) were produced by female *T. depsarium* "sp. nov. Laplante", an aliquot of headspace extract and samples of all four isomers were analyzed on a chiral stationary phase Cylodex B column (30 m×0.25 mm ID, 0.25 μ film; J&W Scientific, Folsom, CA, USA) with an oven-temperature program of 50 °C for 1 min, 3 °C.min^{-1} to 200 °C; injector temperature was 150 °C, and column head pressure 175 kPa. Under these conditions, the 2,3-hexanediol stereoisomers eluted in the following order: (2*S*,3*S*) 17.45 min, (2*R*,3*R*) 17.70 min, (2*R*,3*S*) 18.33 min, (2*S*,3*R*) 18.52 min.

Gas Chromatography/Electroantennography and Gas Chromatography/Mass Spectrometry Antennae from male beetles were used for coupled gas chromatography/electroantennogram detection assays (GC/EAD) of the 2,3-hexanediol stereoisomers, using specimens of *T. pilosicorne* captured in traps in 2009 (RLM field site), and *T. depsarium*

Table 2 Number of specimens of three species of *Tragosoma* captured in field bioassays of chiral and racemic 2,3-hexanediols

Species	Mean (± SE) number of males per trap									
Site (yr)	# individuals captured	2,3[a]	*R**,*R**	*R,R*	*S,S*	*R**,*S**	*R,S*	*S,R*	Blank	Fried.*Q*[b]
T. pilosicorne										
JMR (05)	12					**2.4±0.87a**			0b	8.0**
RLM (09)	56		0b			**8.0±2.4a**			0b	19.0***
UCLO (09)	137					**3.9±0.98a**	0b	**5.3±1.8a**	0b	40.8***
LPC (10)	23		0b			**3.29±1.3a**			0b	10.4**
T. depsarium "harrisi"										
BF (09)	13	0.14±0.14b	0b			**1.7±0.29a**			0b	24.3***
BF (10)	6		0b			**2.0±0.58a**			0b	7.62*
BF (11)	10			0b	0b		0.17±0.17b	**1.5±0.43a**		13.4**
SB, SR (09)	16		0b			**2.5±1.0a**			0.17b	10.3***
T. depsarium "sp. nov. Laplante"										
BF (09)	49	0b	**3.3±0.7a**			0b			0b	47.8***
BF (09)	74			**9.1±1.9a**	0.13±0.1b					12.3***
BR (09)	20	0.17±0.17b	**3.2±1.6a**			0b			0b	19.8***
SB, SR (09)	28		**6.75±2.9a**			0b			0b	12.0***
BF (10)	79		**19.8±5.9a**			0b			0b	10.5***
LPC (10)	33		**1.74±0.88a**			0b			0b	7.37*

Abbreviations for field sites as in Table 1. Means within species, sites, and dates with the same letters are not different (REGWQ means-separation test) at $P<0.05$; the greatest means are in bold

[a] Blend of (2*R**,3*R**)- and (2*R**,3*S**)-2,3-hexanediol

[b] Asterisks indicate a significant value of *Q*: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

"*harrisi*" and *T. depsarium* "sp. nov. Laplante" trapped in 2010 (LP and BF field sites). GC/EAD analyses were performed using DB-5 and DB-Wax columns (both 30 m× 0.25 mm i.d., 0.25 μm film; J&W Scientific, Folsom, CA, USA) with He as carrier gas. The GC was programmed from 40 °C for 1 min, 10 °C. min^{-1} to 275 °C for DB-5 and 250 °C for DB-Wax, and held for 45 min. Solvent extracts and solutions of standards (1 μl aliquots) were analyzed in splitless mode. The EAD apparatus and antennal preparations were as described in Ray et al. (2011).

Gas chromatography/mass spectrometry (GC/MS) analyses were carried out with an Agilent 6890N GC interfaced to a 5975C mass selective detector (Agilent, Santa Clara, CA, USA). The GC was fitted with an HP5-MS column (30 m× 0.25 mm i.d., 0.25 μm film), and the same temperature program and injection conditions as described above were used. Retention indices (RI) were calculated relative to blends of straight-chain hydrocarbons. For increased precision, KI values on the DB-Wax column were obtained using a GC oven program rate of 5 °C rather than 10 °C.min^{-1}.

Scanning Electron Microscopy Scanning electron microscopy was used to examine the prothoraces of *T. pilosicorne* adults for the presence of pores that are associated with the production of 2,3-hexanediol pheromones in cerambycine species (e.g., Ray et al., 2006). The male specimen had been collected during bioassays in 2005 (see above), while the female specimen was donated by the Entomology Research Museum University of California, Riverside (no collection data available; identifications confirmed using the characters of Linsley, 1962). Specimens were prepared following the methods of Ray et al. (2006), and were imaged with an environmental scanning electron microscope equipped with a field-emission electron gun (Philips XL30, FEI Company, Hillsboro, OR, USA) at 5.0 kV. Remnants of specimens have been retained by AMR at Xavier University and scanning electron micrographs have been submitted to Morphbank (www.morphbank.net, image numbers 549802–549806).

Voucher specimens of all three *Tragosoma* species have been submitted to the Entomology Research Museum (UC Riverside, Riverside, CA, USA) with the following identification codes: *T. pilosicorne* (from study site JMR) UCRC ENT 291760-81; *T. pilosicorne* (UCLO, RLM) UCRC ENT 301599-607; *T. depsarium "harrisi"* (SB) UCRC ENT 301608-611; *T. depsarium* "sp. nov. Laplante" (SB, BF) UCRC ENT 301612-620.

Results and Discussion

During a preliminary field-screening bioassay conducted in 2005, four male *T. pilosicorne* were caught in two traps baited with (2*R**,3*S**)-2,3-hexanediol, whereas no *T. pilosicorne* were caught in any of the other traps. In a follow-up bioassay at the JMR site, 12 male *T. pilosicorne* were captured, all in traps baited with (2*R**,3*S**)-2,3-hexanediol (treatment different than control, Table 2). Attraction to that racemic blend was confirmed by subsequent trials in 2009 and 2010 at three other field sites, with the active enantiomer determined as (2*S*,3*R*)-

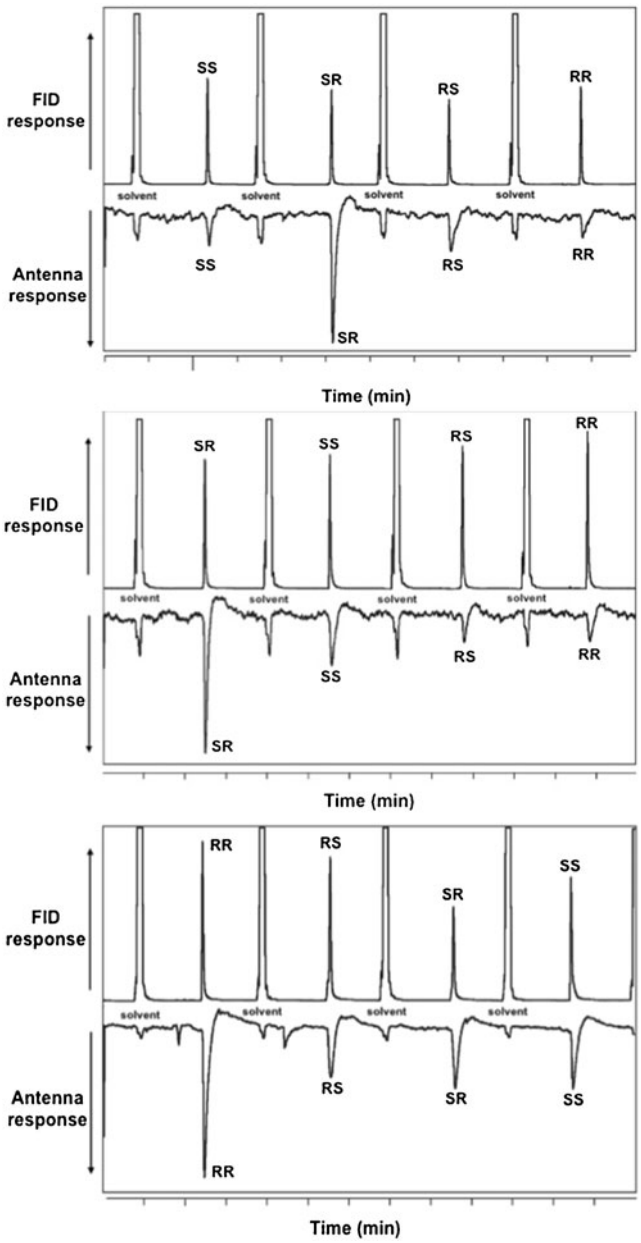

Fig. 1 Representative gas chromatography/electroantennogram detection traces showing responses to stereoisomers of 2,3-hexanediols by antennae of male *Tragosoma pilosicorne* (*top*), *T. depsarium* "*harrisi*" (*middle*), and *T. depsarium* "sp. nov. Laplante" (*bottom*). In each frame, the top trace shows the GC peaks (flame ionization detection; FID) from sequential injection of the stereoisomers in random order on a DB-5 column (30 m×0.25 mm×0.25 micron film, 100 °C isothermal), and the inverted, bottom trace shows the antennal response

2,3-hexanediol (Table 2). The single enantiomer (2*S*,3*R*)-2,3-hexanediol and racemic (2*R**,3*S**)-2,3-hexanediol were equally attractive in the 2009 trial, indicating that the unnatural enantiomer, (2*R*,3*S*)-2,3-hexanediol, did not inhibit attraction of male beetles. Also, antennae of male *T. pilosicorne* responded most strongly to the (2*S*,3*R*)-2,3-enantiomer, consistent with it being a sex pheromone component. The other enantiomers elicited detectable, but lower, responses from antennae (Fig. 1). This suggests some interaction between the unnatural stereoisomers and the olfactory receptors, as has been shown to occur with chiral pheromone components of other cerambycid species (e.g., Mitchell et al., 2012). However, the lower responses also may have been due, in part, to contamination of the chiral standards with trace amounts of other stereoisomers.

Specimens of *T. depsarium* captured at Barton Flats in 2009 appeared to represent two distinct morphologies that were consistently associated with different trap treatments. We subsequently discovered that these two 'morpho-species' had been recognized by S. Laplante. In the present article, these species are designated as *T. depsarium* "*harrisi*" (antennae gracile and barely attaining elytral apex, integument of elytra medium brown, pronotum clothed in dense, golden setae) and *T. depsarium* "sp. nov. Laplante" (antennae robust and attaining or exceeding elytral apex, integument of elytra dark brown, setae on pronotum less dense [AMR, pers. obs.]).

As was the case with *T. pilosicorne*, male *T. depsarium* "*harrisi*" were attracted to (2*R**,3*S**)-2,3-hexanediol (Table 2), with subsequent bioassays confirming that males were specifically attracted to (2*S*,3*R*)-2,3-hexanediol. This stereoisomer also elicited stronger responses from antennae of males in GC/EAD analyses than did the other three stereoisomers (Fig. 1), further suggesting that it may be a sex pheromone component of this species.

Male *T. depsarium* "sp. nov. Laplante" differed from the other two species in being attracted consistently to racemic (2*R**,3*R**)-2,3-hexanediol, and tests with the two enantiomers showed that males were specifically attracted to the (2*R*,3*R*)-2,3-hexanediol enantiomer (Table 2). Although no direct comparison was made between (2*R**,3*R**)-2,3-hexanediol and (2*R*,3*R*)-2,3-hexanediol, the fact that the racemate attracted significant numbers of beetles in several different bioassays suggests that the (2*S*,3*S*)-2,3-hexanediol enantiomer was not inhibitory. In contrast, the mixture of all four stereoisomers of 2,3-hexanediol attracted very few males (Table 2), indicating that (2*R*,3*S*)- and/or (2*S*,3*R*)-2,3-hexanediol inhibited attraction. Antennae of male *T. depsarium* "sp. nov. Laplante" responded most strongly to (2*R*,3*R*)-2,3-hexanediol in GC/EAD assays (Fig. 1), consistent with it being the most attractive enantiomer.

Identification of the pheromones produced by females of *Tragosoma* species was hindered by the difficulty in obtaining females. Attempts to capture females by light trapping at the RLM, UCLO, and BF field sites during the known activity periods of the adults in 2009 were unsuccessful. However, eight pupae of *T. depsarium* "sp. nov. Laplante" were obtained from infested logs, and the adults that emerged were used for collection of headspace volatiles. The volatiles collected from female *T. depsarium* "sp. nov. Laplante" contained one major sex-specific peak, confirmed as (2*R**,3*R**)-2,3-hexanediol by retention time (DB-5) and mass spectral matches with those of a standard. On this column, we achieved baseline separation of the (2*R**,3*R**)- and (2*R**,3*S**)-diastereomers. Further analysis on a chiral stationary phase Cyclodex B GC column, comparing the retention time of the insect-produced compound to those of all four 2,3-hexanediol stereoisomers, further confirmed the identification, and revealed that the insect produced (2*R*,3*R*)-2,3-hexanediol exclusively. Analogous extracts of headspace volatiles of male *T. depsarium* "sp. nov. Laplante" did not show this compound, indicating that production of (2*R*,3*R*)-2,3-hexanediol was female-specific.

Scanning electron microscopy revealed that female *T. pilosicorne* had numerous pores on the ventral side of the lateral spine of the prothorax that were entirely absent in males (Fig. 2). Similar pores on the prothoraces of female *T. depsarium "harrisi"* and *T. depsarium* "sp. nov. Laplante" were located by light microscopy. This sexual dimorphism is analogous to that of cerambycine species that produce pheromones comprised of 2,3-alkanediols and related 3-

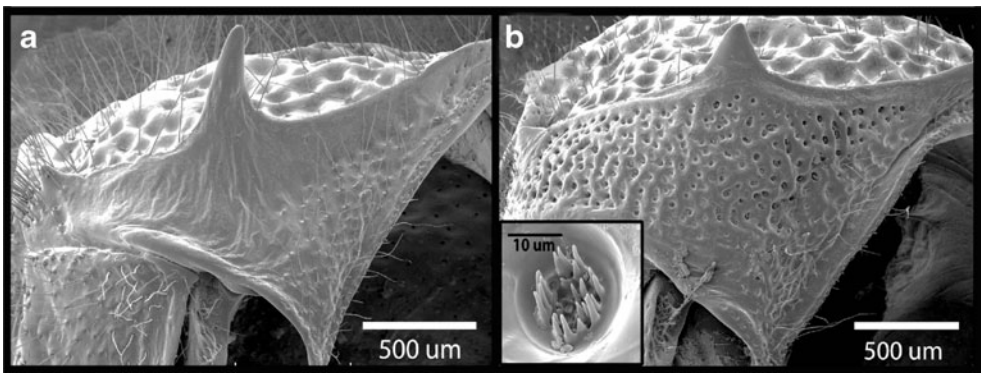

Fig. 2 Scanning electron micrographs of prothoraces of adult male (**a**) and female (**b**) *Tragosoma pilosicorne*. Prothoraces of females have pores lying within pits (see inset) that are absent in males

hydroxyalkan-2-ones; however, in the cerambycines it is the males that produce volatile pheromones and have the prothoracic pheromone gland pores (Hanks et al., 2007; Lacey et al., 2007; Ray et al., 2009). This suggests that there may have been a switch in the sex that produces pheromone between the *Tragosoma* spp. and the cerambycines, but the pheromone chemistry and the pheromone production sites appear to have remained unchanged.

In summary, this study indicates that the female-produced sex attractant pheromone for *Tragosoma depsarium* "sp. nov. Laplante" is (2*R*,3*R*)-2,3-hexanediol, and provides evidence from field bioassays and electroantennography that (2*S*,3*R*)-2,3-hexanediol may be a sex pheromone component of the congeners *T. pilosicorne* and *T. depsarium* "*harrisi*". These experiments suggest a second shared pheromone motif within the cerambycid subfamily Prioninae. Whereas the available evidence suggests that female beetles in the prionine genus *Prionus* produce similar, or perhaps identical, pheromones (Barbour et al., 2011), the results described above suggest that females of at least two species of *Tragosoma* may share a pheromone structure, whereas a third species may use a diastereomer of the same basic structure as its pheromone. Remarkably, the same compounds are being used as pheromones by species from two different cerambycid subfamilies, as well as by different sexes. These facts suggest a close phylogenetic relationship between the two subfamilies, which indeed has been suggested in a recent phylogenetic analysis (Sykorova, 2008). The results from the three *Tragosoma* species also suggest that cerambycids take advantage of available stereoisomers to create unique pheromone channels, thus minimizing the possibility of cross-attraction. However, host plant volatiles, minor components of the insect-produced blends, or contact pheromones may also play important roles in maintaining reproductive isolation, particularly among sympatric species.

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References

Barbour, J. D., Millar, J. G., Rodstein, J., Ray, A. M., Alston, D. G., Rejzek, M., Dutcher, J. D., and Hanks, L. M. 2011. Synthetic 3,5-dimethyldodecanoic acid serves as a general attractant for multiple species of *Prionus* (Coleoptera: Cerambycidae). *Ann. Entomol. Soc. Am.* 104:588–593.

Fettköther, R., Dettner, K., Schröder, F., Meyer, H., Francke, W., and Noldt, U. 1995. The male pheromone of the old house borer *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae): identification and female response. *Experientia* 51:270–277.

Fierke, M. K., Skabeikis, D. D., Millar, J. G., Teale, S. A., MCelfresh, J. S., and Hanks, L. M.. 2012. Identification of a male-produced pheromone for *Monochamus scutellatus scutellatus* and *Monochamus notatus* (Coleoptera: Cerambycidae). *J. Econ. Entomol.* (in press).

Fonseca, M., Vidal, D. M., and Zarbin, P. H. G. 2010. Male-produced sex pheromone of the cerambycid *Hedypathes betulinus*: chemical identification and biological activity. *J. Chem. Ecol.* 36:1132–1139.

Graham, E. E., Mitchell, R. F., Reagel, P. F., Barbour, J. D., Millar, J. G., and Hanks, L. M. 2010. Treating panel traps with a fluoropolymer enhances their efficiency in capturing cerambycid beetles. *J. Econ. Entomol.* 103:641–647.

Hanks, L. M., Millar, J. G., Moreira, J. A., Barbour, J. D., Lacey, E. S., McElfresh, J. S., Reuter, F. R., and Ray, A. M. 2007. Using generic pheromone lures to expedite identification of aggregation pheromones for the cerambycid beetles *Xylotrechus nauticus*, *Phymatodes lecontei*, and *Neoclytus modestus modestus*. *J. Chem. Ecol.* 33:889–907.

Imrei, Z., Millar, J. G., Janik, G., and Tóth, M.2012. Field screening of known pheromone components of longhorned beetles in the subfamily Cerambycinae (Coleoptera: Cerambycidae) in Hungary. *Z. Naturforsch*. (in press).

Lacey, E. S., Ginzel, M. D., Millar, J. G., and Hanks, L. M. 2004. Male-produced aggregation pheromone of the cerambycid beetle *Neoclytus acuminatus acuminatus*. *J. Chem. Ecol.* 30:1493–1507.

Lacey, E. S., Moreira, J. A., Millar, J. G., Ray, A. M., and Hanks, L. M. 2007. Male-produced aggregation pheromone of the cerambycid beetle *Neoclytus mucronatus mucronatus*. *Entomol. Exp. Appl.* 122:171–179.

Lacey, E. S., Moreira, J. A., Millar, J. G., and Hanks, L. M. 2008. A male-produced aggregation pheromone blend consisting of alkanediols, terpenoids, and an aromatic alcohol from the cerambycid beetle *Megacyllene caryae*. *J. Chem. Ecol.* 34:408–417.

Lacey, E. S., Millar, J. G., Moreira, J. A., and Hanks, L. M. 2009. Male-produced aggregation pheromones of the cerambycid beetles *Xylotrechus colonus* and *Sarosesthes fulminans*. *J. Chem. Ecol.* 35:733–740.

Linsley, E. G. 1962. The Cerambycidae of North America. Part II. Taxonomy and classification of the Parandrinae, Prioninae, Spondylinae and Aseminae. *Univ. Calif. Publ. Entomol.* 19:1–102.

Mitchell, R. F., Graham, E. E., Wong, J. C. H., Reagel, P. F., Striman, B. L., Hughes, G. P., Paschen, M. A., Ginzel, M. D., Millar, J. G., and Hanks, L. M. 2011. Fuscumol and fuscumol acetate are general attractants for many species of cerambycid beetles in the subfamily Lamiinae. *Entomol. Exp. Appl.* 141:71–77.

Mitchell, R. F., Hughes, D. T., Luetje, C. W., Millar, J. G., Soriano-Agatór, F., Hanks, L. M., and Robertson, H. M. 2012. Sequencing and characterizing odorant receptors of the cerambycid beetle *Megacyllene caryae*. *Insect Biochem. Mol. Biol.* 42:499–505.

Pajares, J. A., Álvarez, G., Ibeas, F., Gallego, D., Hall, D. R., and Farman, D. I. 2010. Identification and field activity of a

male-produced aggregation pheromone in the pine sawyer beetle, *Monochamus galloprovincialis*. *J. Chem. Ecol.* 36:570–583.

RAY, A. M., LACEY, E. S., and HANKS, L. M. 2006. Predicted taxonomic patterns in pheromone production by longhorned beetles. *Naturwissenschaften* 93:543–550.

RAY, A. M., SWIFT, I. P., MOREIRA, J. A., MILLAR, J. G., and HANKS, L. M. 2009. (*R*)-3-hydroxyhexan-2-one is a major pheromone component of *Anelaphus inflaticollis* (Coleoptera: Cerambycidae. *Environ. Entomol.* 38:1462–1466.

RAY, A. M., ŽUNIČ, A., ALTEN, R. L., MCELFRESH, J. S., HANKS, L. M., and MILLAR, J. G. 2011. *cis*-Vaccenyl acetate, a sex attractant pheromone of *Ortholeptura valida*, a longhorned beetle in the subfamily Lepturinae. *J. Chem. Ecol.* 37:172–178.

RODSTEIN, J., MCELFRESH, J. S., BARBOUR, J. D., RAY, A. M., HANKS, L. M., and MILLAR, J. G. 2009. Identification and synthesis of a female-produced sex pheromone for the cerambycid beetle *Prionus californicus*. *J. Chem. Ecol.* 35:590–600.

RODSTEIN, J., MILLAR, J. G., BARBOUR, J. D., MCELFRESH, J. S., WRIGHT, I. M., BARBOUR, K. S., RAY, A. M., and HANKS, L. M. 2011. Determination of the relative and absolute configurations of the female-produced sex pheromone of the cerambycid beetle *Prionus californicus*. *J. Chem. Ecol.* 37:114–124.

SAS INSTITUTE 2001. SAS/STAT User's Guide for Personal Computers, Release 8.01. SAS Institute, Cary, NC, USA.

SILK, P. J., SWEENEY, J., WU, J., PRICE, J., GUTOWSKI, J. M., and KETTELA, E. G. 2007. Evidence for a male-produced pheromone in *Tetropium fuscum* (F.) and *Tetropium cinnamopterum* (Kirby) (Coleoptera: Cerambycidae). *Naturwissenschaften* 94:697–701.

SYKOROVA, M. 2008. Molecular phylogeny of the subfamilies Spondylidinae and Lepturinae (Coleoptera: Cerambycidae) based on mitochondrial 16S rDNA. Bachelor's thesis, Faculty of Natural Sciences, South Bohemian University, Ceske Budejovice.

TEALE, S. A., WICKHAM, J. D., ZHANG, F., SU, J., CHEN, Y., XIAO, W., HANKS, L. M., and MILLAR, J. G. 2011. A male-produced aggregation pheromone of *Monochamus alternatus* (Coleoptera: Cerambycidae), a major vector of pine wood nematode. *J. Econ. Entomol.* 104:1592–1598.