North American Species of Cerambycid Beetles in the Genus *Neoclytus* Share a Common Hydroxyhexanone-Hexanediol Pheromone Structural Motif

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ABSTRACT Many species of cerambycid beetles in the subfamily Cerambycinae are known to use male-produced pheromones composed of one or a few components such as 3-hydroxyalkan-2-ones and the related 2,3-alkanediols. Here, we show that this pheromone structure is characteristic of the cerambycine genus *Neoclytus* Thomson, based on laboratory and field studies of 10 species and subspecies. Males of seven taxa produced pheromones composed of (*R*)-3-hydroxyhexan-2-one as a single component, and the synthetic pheromone attracted adults of both sexes in field bioassays, including the eastern North American taxa *Neoclytus caprea* (Say), *Neoclytus mucronatus mucronatus* (F.), and *Neoclytus scutellaris* (Olivier), and the western taxa *Neoclytus conjunctus* (LeConte), *Neoclytus irroratus* (LeConte), and *Neoclytus modestus modestus* Fall. Males of the eastern *Neoclytus acuminatus acuminatus* (F.) and the western *Neoclytus tenuiscriptus* Fall produced (2*S*,3*S*)-2,3-hexanediol as their dominant or sole pheromone component. Preliminary data also revealed that males of the western *Neoclytus balteatus* LeConte produced a blend of (*R*)-3-hydroxyhexan-2-one and (2*S*,3*S*)-2,3-hexanediol but also (2*S*,3*S*)-2,3-octanediol as a minor component. The fact that the hydroxyketone-hexanediol structural motif is consistent among these North American species provides further evidence of the high degree of conservation of pheromone structures among species in the subfamily Cerambycinae.

KEY WORDS chemical ecology, 3-hydroxyhexan-2-one, 2,3-hexanediol, wood-borer

Research over the last decade has revealed that many species of cerambycid beetles in the subfamily Cerambycinae use male-produced pheromones composed of 3-hydroxyalkan-2-ones, the isomeric 2-hydroxyalkan-3-ones, and the related 2,3-alkanediols (e.g., Hanks and Millar 2013). In many cases, congeners, and even more distantly related species (e.g., in different tribes) appear to produce similar or even identical pheromones (e.g., Hanks et al. 2007; Lacey et al. 2007a, 2009; Ray et al. 2009b). For sympatric species, deleterious interspecific attraction may be averted by seasonal or circadian temporal segregation or by the synergistic or antagonistic effects of minor components of pheromone blends (Mitchell et al. 2015).

In this article, we describe the variability in pheromone chemistry among 10 species and subspecies of the cerambycine genus *Neoclytus* Thomson. The genus *Neoclytus* is restricted to the Western Hemisphere and comprises ~55 species, including 26 species that are native to North America (Turnbow and Thomas 2006, Bezark and Monné 2013). The genus is in the tribe Clytini, adults of which are diurnal, often mating in bright sunlight (Linsley 1959). Clytines are considered general Batesian mimics of aculeate Hymenoptera, with the adults of most species having a dark integument with contrasting markings of bright white or yellow pubescence (Linsley 1959). Larvae of most *Neoclytus* species feed within the wood of hardwood trees (Craighead 1923, Linsley 1964).

The first *Neoclytus* species for which a pheromone was identified was *Neoclytus acuminatus acuminatus* (F.) of eastern North America, males of which produce a single-component attractant pheromone composed of (2*S*,3*S*)-2,3-hexanediol, which attracts both sexes (Lacey et al. 2004). The pheromone is produced from glands in the prothorax, and males adopt a characteristic body position when calling, with the front legs extended and the prothorax elevated (Lacey et al. 2007b). Pheromones subsequently were identified from another eastern species, the congener *Neoclytus mucronatus mucronatus* (F.), and partially identified from the western *Neoclytus modestus modestus* Fall, with males of both species producing only (*R*)-3-hydroxyhexan-2-one (Hanks et al. 2007, Lacey et al. 2007a).

The fact that these three North American *Neoclytus* taxa have such similar pheromones suggested that hydroxyketone-hexanediol structures might be

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characteristic of the genus. Here, we provide support for this hypothesis by providing information on the pheromone chemistry of another seven North American *Neoclytus* species and subspecies. We also present partial data on pheromone identifications of two additional species, *Neoclytus approximatus* (LeConte) and *Neoclytus jouteli jouteli* Davis, to guide future research efforts to confirm their pheromone chemistry.

Materials and Methods

Study Species. Our study focused on 10 species and subspecies of *Neoclytus* native to North America (Table 1). Four of these taxa are primarily eastern in distribution, including *N. a. acuminatus*, *N. m. mucronatus*, *Neoclytus caprea* (Say), and *Neoclytus scutellaris* (Olivier). *N. a. acuminatus* and *N. m. mucronatus* are among the most common cerambycids of eastern North America (Hanks et al. 2014). The remaining taxa are native to western North America and include *N. m. modestus*, *Neoclytus balteatus* LeConte, *Neoclytus conjunctus* (LeConte), *Neoclytus irroratus* (LeConte), *Neoclytus tenuiscriptus* Fall, and *Neoclytus mucronatus vogti* Linsley, a southwestern subspecies of the primarily eastern *N. mucronatus*. Of the western taxa, *N. conjunctus* and *N. irroratus* are considered common, whereas *N. balteatus* and *N. tenuiscriptus* are considered rare (Linsley 1964).

Some of the study species cause economic damage by infesting trees felled for lumber, most notably *N. a. acuminatus*, *N. caprea*, and *N. conjunctus* (Craighead 1923, Linsley 1964, Solomon 1995). *N. a. acuminatus* also appears to be highly invasive, as individuals of this species have been intercepted in international quarantine facilities outside of North America (D. M. Suckling, personal communication), and the species has become established in Argentina, Europe, and other parts of the world (e.g., Di Iorio 2004, Cocquempot and Lindelöw 2010).

Study Sites. Research on the eastern *Neoclytus* species was conducted at several sites in east-central Illinois that were wooded primarily with hardwoods (Table 2; see Hanks et al. 2014 for additional information on IL field sites). Most of the western study sites were in California (more variable in elevation, dominated by hardwoods at some sites and conifers at others) but also included sites in Arizona and Texas (Table 2).

Sources of Chemicals. Synthetic candidate pheromones were synthesized as described in the following publications: a blend of all four isomers of 2,3-hexanediol (henceforth "generic hexanediol"; e.g., Hanks et al. 2007); racemic *syn*-2,3-hexanediol and (2*S*,3*S*)-2,3-hexanediol (Lacey et al. 2004); racemic 3-hydroxyhexan-2-one and (*R*)-3-hydroxyhexan-2-one (Lacey et al. 2007a). (2*S*,3*S*)-Octanediol was synthesized by asymmetric dihydroxylation as described

Table 1. Natural history of study species in the genus *Neoclytus* that were targeted for collection of volatiles and field bioassays of synthetic candidate pheromones in Illinois, Arizona, California, and Texas during 2006–2014 (Craighead 1923, Linsley 1964, Solomon 1995, Yanega 1996, Lingafelter 2007, MacRae and Rice 2007, Hanks et al. 2014)

Scientific name	Distribution	Larvae diet breadth	Relative abundance
N. acuminatus acuminatus (F.)	Eastern	Broad (hardwoods)	Common
N. balteatus LeConte	Western (coastal)	*Ceanothus* spp.	Rare
N. caprea (Say)	Eastern	Narrow (hardwoods)	Common
N. conjunctus (LeConte)	Western (coastal)	Narrow (hardwoods)	Common
N. irroratus (LeConte)	Western	*Quercus* spp.	Common
N. modestus modestus Fall	Western (CA)	*Quercus* spp.	Not known
N. mucronatus mucronatus (F.)	Eastern	Narrow (hardwoods)	Common
N. mucronatus vogti Linsley	Southwestern	Narrow (hardwoods)	Not known
N. scutellaris (Olivier)	Eastern to TX	Narrow (hardwoods)	Common
N. tenuiscriptus Fall	Southwestern	*Baccharis* spp.	Rare

Table 2. Study sites in Illinois, Arizona, California, and Texas where adults of *Neoclytus* species were collected live for collection of headspace volatiles and where field bioassays of candidate pheromones were conducted during 2006–2014

State	Name	GPS coordinates	Elev. (m)	Tree community
IL	Allerton Park, Piatt Co.	39.985, −88.650	202	Hardwoods[a]
IL	Forest Glen Preserve, Vermilion Co.	40.015, −87.567	202	Hardwoods
IL	Private residence, Urbana, Champaign Co.	40.097, −88.203	227	Hardwoods, conifers
IL	Trelease Woods, Champaign Co.	40.134, −88.142	213	Hardwoods
IL	Univ. Illinois Operations and Maintenance, Champaign Co.	40.085, −88.216	225	Hardwood, conifers
AZ	Dry wash near Gleeson, Cochise Co.	31.731, −109.824	1,484	Sonoran desert[b]
CA	Barton Flats, San Bernardino Natl. Forest, San Bernardino Co.	34.162, −116.859	2,132	Hardwoods, conifers[c]
CA	Los Vaqueros Reservoir and Watershed, Contra Costa Co.	37.827, −121.766	176	Hardwoods
CA	Mt. Pinos, Los Padres Natl. Forest, Kern Co.	34.848, −19.092	1,731	Conifers
CA	Santa Rosa Plateau Reserve, Riverside Co.	33.511, −117.249	540	Hardwoods
TX	Texas AgriLife Research and Extension Center, Erath Co.	32.241, −98.1886	396	Hardwoods

[a] Illinois hardwoods primarily species of *Quercus*, *Carya*, *Fraxinus*, *Acer*; conifers primarily species of *Pinus*.
[b] Dominant plants were species of *Acacia*, *Prosopis*, and Cactaceae.
[c] California and Texas hardwoods primarily species of *Quercus*; California conifers primarily species of *Abies*, *Pseudotsuga*, and *Pinus*.

for the analog (2*S*,3*S*)-2,3-hexanediol, substituting (*E*)-2-octene for (*E*)-2-hexene as the starting material. Beginning in 2009, 3-hydroxyhexan-2-one was purchased from Bedoukian Research (Danbury, CN). 1-Hexanol was purchased from Aldrich Chemical Co. (Milwaukee, WI).

General Methods of Trapping. Experiments that targeted the various species of *Neoclytus* spanned several years, and though methods were improved over time, generally followed those of Graham et al. (2010). Beetles were captured live for collection of headspace odors using flight intercept traps (cross-vane, black corrugated plastic; Advanced Pheromone Technologies, Marylhurst, OR, or Alpha Scents, Portland, OR), replacing the supplied trap basins with funnels that directed insects into glass jars or plastic jars vented with aluminum window screen. Beginning in 2010, traps were coated with Fluon (Northern Products, Woonsocket, RI) to improve capture efficiency. Traps were suspended from L-shaped frames of polyvinyl chloride pipe mounted on steel bars that were driven partway into the ground or were hung from lower branches of trees (within a few meters of the ground).

Traps were baited with synthetic pheromone in emitters that consisted of uncapped 3.7 ml glass vials (in 2006), capped 1.8 ml glass vials with pipe cleaner wicks passing through the cap (in 2007; see Hanks et al. 2007), or clear polyethylene sachets (in 2008–2014; 5 by 7.5 cm press seal bags; 018161A, Fisher Scientific, Pittsburgh, PA, or Bagettes model 14770, Cousin Corp., Largo, FL). Emitters were loaded with solutions of synthetic pheromones in ethanol (2006–2010) or isopropanol (2011–2014) with 25 mg of an enantiomer or 50 mg of a racemic mixture in 1 ml of solvent. Control emitters contained 1 ml of solvent. Emitters were hung in the central open area of traps.

Sources of Specimens for Collection of Headspace Odors. For most of the study species, adult specimens were first captured in small numbers as by-catch during field bioassays that targeted other cerambycid species or in planned experiments that screened known pheromones of cerambycids so as to attract new species that then could be targeted for pheromone identification (e.g., Hanks et al. 2012, Hanks and Millar 2013). Although a variety of different pheromones were tested in these trials, adults of *Neoclytus* species invariably were attracted in greatest numbers to traps baited with 3-hydroxyhexan-2-one, generic hexanediol, or racemic *syn*-2,3-hexanediol. In many cases, candidate pheromones identified during the first aerations subsequently were used to trap more specimens for aeration. Sexes of captured beetles were determined using sexually dimorphic characters in the shape and length of antennae, abdominal sternites, and hind femora (Linsley 1964).

Intensive screening bioassays at several study sites in Illinois (e.g., see Hanks et al. 2014) yielded specimens of *N. caprea* for aeration, as well as specimens of *N. a. acuminatus* and *N. m. mucronatus* that were aerated after formal identification of their pheromones (Table 3). Two male *N. scutellaris* were captured for aeration during screening trials at the Texas AgriLife Research and Extension Center (Table 3) during 30 May–2 June 2010. Captured beetles were held under laboratory conditions in wire screen cages and provided 10% aqueous sucrose solution as moisture and nutrition (in a glass vial stopped with a cotton wick).

Specimens of the western species that were first captured during screening trials included (study sites in Table 2): 1) *N. conjunctus* at the Los Vaqueros site during March–May 2010; 2) *N. irroratus* and *N. m. modestus* at Barton Flats in May 2006; and 3) *N. tenuiscriptus* at the Santa Rosa Plateau Reserve in June–July 2006. One adult male *N. m. vogti* was collected from the trunk of a standing *Sideroxylon lanuginosum* Michx. (Sapotaceae) in a desert area of southern Arizona on 28 July 2006. Adult *N. balteatus* were reared from branches of *Ceanothus cordulatus* Kellogg (Rhamnaceae) that had been collected from a riparian habitat at the Mount Pinos site during early May 2006. The infested host material was sealed in plastic barrels, misted with water weekly, and held in a greenhouse at UC Riverside until adult beetles emerged. Captured beetles of western species usually were aerated shortly after they were captured or emerged.

Collection of Insect-Produced Volatiles. Volatiles produced by beetles of the eastern species were collected at the University of Illinois by aerating same-sex groups of beetles in Mason-style canning jars (~0.5 liter). Beetles of both sexes were aerated, with approximately equal numbers of aerations for each sex, if available. Jars were held under laboratory conditions (~20°C) near a north-facing window (natural photoperiod, ~14:10 [L:D] h) and were swept with clean air (1 liter/min) for 24 h, with headspace volatiles trapped on a collector of the adsorbent polymer HayeSep Q (150 mg; Sigma-Aldrich, St. Louis, MO). Aerations of empty jars were run simultaneously to check for system contaminants. Compounds were recovered from collectors by extraction with 1.5 ml of dichloromethane into silanized glass vials that were stored at −20°C. Beetles were returned to cages and allowed at least 24 h before being used again for collection of volatiles.

Specimens of western species were aerated at UC Riverside using similar methods. Beetles were placed

Table 3. Number of headspace collections from adult male beetles of *Neoclytus* species that contained volatiles in detectable quantities, and chemical composition of volatiles

Species	No. aerations	Mean % of volatiles		Reference
		(*R*)-3-hydroxy-hexan-2-one	(2*S*,3*S*)-2,3-hexanediol	
N. a. acuminatus	5[a]		100	Lacey et al. (2004)
N. balteatus	3	87	13[b]	Present study
N. caprea	19	100		Present study
N. conjunctus	1	100		Present study
N. irroratus	1	100		Present study
N. m. modestus	2[a]	100		Hanks et al. (2007)
N. m. mucronatus	8[a]	100		Lacey et al. (2007a)
N. m. vogti	1	100		Present study
N. scutellaris	3	100		Present study
N. tenuiscriptus	4		97[c]	Present study

[a] In addition to published data.
[b] Both C6 and C8 chain lengths (~6.5% each).
[c] Minor component 1-hexanol (3%).

singly or in same-sex pairs in glass aeration jars (263 ml), along with a water-filled 2 ml vial stoppered with a cotton wick to provide moisture. Jars were held at 25°C under a photoperiod of 16:8 (L:D) h cycle and swept with clean air (300 ml/min) for 1–4 d, with headspace volatiles trapped on a collector of activated charcoal. Collectors were eluted with 3 by 200 µl dichloromethane (see Hanks et al. 2007).

Analysis of Insect-Produced Volatiles. Aeration extracts from eastern species were analyzed at the University of Illinois with a gas chromatograph (GC) interfaced to a mass selective detector (Models 6809 and 5973, Hewlett-Packard, Palo Alto, CA; AT-5 ms column [30 m by 0.25 mm i.d., 0.25 µm film], Alltech Associates, Inc., Deerfield, IL). The GC oven was programmed from 40°C/1 min, 10°C/min to 210°C, hold 3 min. Injections were made in splitless mode, with an injector temperature of 250°C, and helium carrier gas. Sex-specific peaks were identified by comparing spectra and retention times with those of authentic standards.

Aeration extracts from adult beetles of western species were analyzed at UC Riverside using similar methods and with the same models of GC and mass selective detector, but the GC was fitted with a DB-5 column (30 m by 0.25 mm i.d., 0.25 µm film; J&W Scientific, Folsom, CA). The GC oven was programmed from 40°C/1 min, 10°C/min to 250°C. Injections were made in splitless mode, with an injector temperature of 250°C, with helium carrier gas.

For both eastern and western species, absolute configurations of the insect-produced chemicals were determined at UC Riverside by analyzing aliquots of extracts with an HP 5890 GC equipped with a chiral stationary phase Cyclodex B column (30 m by 0.25 mm ID, J&W Scientific), with the oven temperature programmed from 50°C/0 min, 5°C/min to 150°C, and an injector temperature of 100°C to minimize isomerization. Absolute configurations were confirmed by coinjection of an aliquot of aeration extract with the mixture of all stereoisomers of a particular synthetic chemical (Millar et al. 2009). Beginning in 2012, absolute configurations of chemicals produced by eastern species were determined at the University of Illinois using the same methods and with a similar GC setup.

Field Bioassays of Candidate Pheromones. Attraction of individual *Neoclytus* species to candidate pheromones identified in analyses of insect-produced volatiles was tested in field bioassays using traps similar to those already described but with supplied basins containing propylene glycol or saturated brine to kill and preserve captured beetles. Treatments included both chiral and racemic compounds so as to test for inhibition by nonnatural stereoisomers and solvent controls. For each bioassay, traps baited with different treatments were positioned 10–20 m apart in linear transects, with one treatment per transect randomly assigned to traps on day one. When multiple transects were used, they were separated by at least 100 m. Traps were serviced every 2–10 d (depending on travel distance to study sites), at which time treatments were rotated within transects to control for positional effects. Lures were replaced as needed (usually after no more than 2 wk).

Among the eastern species, *N. caprea* was targeted with three field bioassays in the yard of a private residence in Champaign Co., IL (Table 2; 1 transect) during 10 April–10 May 2011, 14 March–3 April 2012, and 11 April–6 May 2014. Attraction of *N. scutellaris* to synthetic pheromone was tested with a bioassay conducted at Allerton Park during 19 July–10 September 2012 (1 transect).

Western species were targeted for bioassays as follows (study sites in Table 2): 1) *N. balteatus* at the Mount Pinos site during June–July of 2007 and May–August 2008 (3 transects); 2) *N. conjunctus* at the Los Vaqueros site during 26 March–1 May 2010 (2 transects); 3) *N. irroratus* and *N. m. modestus* at Barton Flats during 6 June–22 June 2006 and 4 July–20 July 2006 (1 transect); 4) *N. m. modestus* also at Santa Rosa Plateau Reserve during 1–13 July 2009 (3 transects); 5) *N. tenuiscriptus* at Santa Rosa Plateau Reserve during 28 June–4 July 2008 (4 transects) and 25 June–14 August 2009 (5 transects), and 6) *N. m. vogti* at the Cochise Co., AZ site during July 2007 (2 transects).

Bioassay data were analyzed separately for each species and bioassay so as to provide independent assessments of treatment effects for each experiment. Differences between treatments in mean numbers of beetles captured were tested with the nonparametric Friedman's test (PROC FREQ with CMH option; SAS Institute 2011) because assumptions of analysis of variance were violated by heteroscedasticity (Sokal and Rohlf 1995). Pairs of treatment means were compared with the nonparametric Nemenyi multiple comparison test (Elliot and Hynan 2011, Zar 2010). Replicates that captured no beetles of the species in question were dropped from analyses.

Voucher specimens of *N. conjunctus*, *N. irroratus*, *N. m. modestus*, and *N. tenuiscriptus* have been deposited at the Entomology Research Museum at the University of California, Riverside (*N. conjunctus* UCRC ENT 406715-406724, *N. irroratus* UCRC ENT 256585-256589, *N. m. modestus* UCRC ENT 256595-256600, *N. tenuiscriptus* UCRC ENT 407229-406234). Voucher specimens of the eastern species are available from the laboratory collection of L. M. H., and the voucher of *N. m. vogti* is retained in the laboratory collection of A. M. R.

Results

Analysis of Insect-Produced Volatiles. Males of the *Neoclytus* taxa produced primarily (*R*)-3-hydroxyhexan-2-one or (2*S*,3*S*)-2,3-hexanediol (Table 3). These compounds were absent in all aeration extracts from females or system controls. Along with these compounds, 2,3-hexanedione appeared sporadically, probably as an artifact from degradation of (*R*)-3-hydroxyhexan-2-one (Millar et al. 2009).

Males of the eastern species *N. a. acuminatus* previously had been reported to produce only (2*S*,3*S*)-2,3-hexanediol (Lacey et al. 2004), and this was confirmed with another five aerations in this study (Table 3). The

same compound was produced by males of the western *N. tenuiscriptus*, along with small amounts of 1-hexanol. The only other species that produced alkanediols was the western *N. balteatus*, in which the male-produced volatiles contained (*R*)-3-hydroxyhexan-2-one as the major component, and both (2*S*,3*S*)-2,3-hexanediol and (2*S*,3*S*)-2,3-octanediol as minor components. (*R*)-3-Hydroxyhexan-2-one was the only component detected in headspace collections from males of the remaining seven taxa (confirmed for *N. m. mucronatus* with an additional eight aerations; Table 3).

Field Bioassays of Candidate Pheromones. Field bioassays confirmed attraction of adult beetles of all the study species to the dominant compounds that were produced by their males, with the exception of *N. balteatus* and *N. m. vogti* (no beetles captured during bioassays). Adults of the eastern *N. a. acuminatus* already were known to be attracted by (2*S*,3*S*)-2,3-hexanediol, and attraction was not affected by the presence of the (2*R*,3*R*)-enantiomer when the racemic *syn*-2,3-hexanediol was tested as a trap bait (Lacey et al. 2004).

The western species *N. tenuiscriptus* also was attracted to (2*S*,3*S*)-2,3-hexanediol during field trials in 2008 ($N = 36$ beetles total; sex ratio 83% female), with treatment means (±1 SE) of 1.44 ± 0.34, 1.89 ± 0.56, 0, and 0 beetles per replicate for (2*S*,3*S*)-2,3-hexanediol, (2*S*,3*S*)-2,3-hexanediol + 1-hexanol, 1-hexanol, and the control, respectively (means significantly different; Friedman's $Q_{3,36} = 17.6$, $P = 0.0005$). Treatment means for the diol alone, and blended with 1-hexanol, were significantly greater than the control (Nemenyi test, $P < 0.05$) but not significantly different from each other. These results suggest that attraction to the diol was not synergized by the 1-hexanol present as a minor component in the volatiles collected from males of this species.

These findings were supported by further field bioassays during 2009 in which 17 *N. tenuiscriptus* were caught (means of 1.0 ± 0.27, 0.45 ± 0.21, 0.09 ± 0.09, and 0 beetles per replicate for racemic *syn*-2,3-hexanediol, *syn*-2,3-hexanediol + 1-hexanol, generic hexanediol, and the control, respectively) (means significantly different; $Q_{3,44} = 12.5$, $P = 0.0059$). Only the mean for the *syn*-2,3-hexanediol treatment was significantly greater than the control ($P < 0.05$). These findings also suggest that attraction to the stereochemically pure (2*S*,3*S*)-2,3-hexanediol was antagonized by one or both of the enantiomers of the *anti*-diastereomer in the generic hexanediol, as was the case with *N. a. acuminatus* (Lacey et al. 2004).

Racemic 3-hydroxyhexan-2-one attracted significant numbers of beetles of all of the species that use (*R*)-3-hydroxyhexan-2-one as their pheromone (Table 4). The nonnatural (*S*)- enantiomer appeared to antagonize attraction to (*R*)-3-hydroxyhexan-2-one for *N. irroratus* and *N. m. modestus*, with both species being significantly less attracted to the racemate than to the pure (*R*)-3-hydroxyhexan-2-one. In contrast, there was no difference in attraction of *N. caprea* and *N. conjunctus* to either the racemate or pure (*R*)-3-hydroxyhexan-2-one. Few if any beetles were captured in control traps, which resulted in statistical significance despite small sample sizes in bioassays for *N. irroratus* and *N. m. modestus*.

Field bioassays confirmed that beetles of both sexes were attracted to pheromone-baited traps for all of the study species, although sex ratios were skewed in some bioassays, favoring either males or females (Table 4, and see preceding paragraphs of this section.). However, these results must be interpreted with caution because we had no independent measure of the actual sex ratios within the populations from which captured beetles had been drawn.

Discussion

Production of (*R*)-3-hydroxyhexan-2-one and (2*S*,3*S*)-2,3-hexanediol by males of all 10 of the *Neoclytus* species and subspecies described here supports our hypothesis that the hydroxyketone-hexanediol structural motif is characteristic of the genus in North America. Nevertheless, there remain both eastern and western *Neoclytus* species that have not yet been captured in any of our field bioassays, raising the possibility that these species might be using structures other than (*R*)-3-hydroxyhexan-2-one and (2*S*,3*S*)-2,3-hexanediol in their pheromone blends. The absent western taxa, however, are not known to occur in the

Table 4. Mean (±1 SE) number of beetles captured during field bioassays for species of *Neoclytus*, the males of which produce (*R*)-3-hydroxyhexan-2-one as a single-component pheromone

Species	State	Year	Total no. beetles	Sex ratio (% female)	Treatments			Friedman's Q[a]
					(*R*)-3-ketone	Racemic ketone	Control	
N. caprea	IL	2011	74	58.3	3.8 ± 1.2 a	3.4 ± 0.90 a	0.2 ± 0.13 b	$Q_{2,30} = 10.1$**
		2012	61	42.2	2.75 ± 1.1 ab	4.8 ± 0.8 a	0.13 ± 0.12 b	$Q_{2,24} = 12.6$**
		2014	55	58.9	3.4 ± 1.3 a	1.9 ± 0.7 a	0.20 ± 0.13 b	$Q_{2,30} = 8.7$*
N. conjunctus	CA	2010-A	132	44.4	3.55 ± 1.1 a	3.6 ± 1.0 a	0 b	$Q_{2,60} = 26.8$***
		2010-B	64	24.2		7.3 ± 2.1 a	0 b	$Q_{1,18} = 13.1$**
N. irroratus	CA	2006	12	83.3	3.0 ± 1.0 a	0.50 ± 0.29 b	0 b	$Q_{2,12} = 9.3$**
N. m. modestus	CA	2006-A	15	86.7	2.6 ± 0.87 a	1.4 ± 0.24 b	0 c	$Q_{2,15} = 8.7$*
		2006-B	9	77.8		2.0 ± 0.63 a	0 b	$Q_{1,10} = 8.0$**
		2009	13	71.0		1.1 ± 0.08 a	0 b	$Q_{1,19} = 14.4$***
N. scutellaris	IL	2012	30	93.3		3.33 ± 0.7 a	0.22 ± 0.15 b	$Q_{1,18} = 12.4$***

Sex ratio (% females of total) is from beetles in treatments with means significantly greater than controls. Empty cells indicate treatments that were not included in bioassays. Means within species and year with the same letters are not significantly different (Nemenyi test, $P < 0.05$).

[a] Asterisks indicate significance level of Q: *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.

areas where our studies were conducted in southern California (Table 2), being either more northern in distribution (e.g., *N. modestus zebratus* Van Dyke, *N. muricatulus infans* Casey, *Neoclytus nubilus* Linsley, *Neoclytus provoanus* Casey, *Neoclytus resplendens* Linsley) or more coastal (*Neoclytus interruptus* LeConte) (Linsley 1964). Also, the western *Neoclytus angelicus* Van Dyke is known only from the type specimen and may be a pale form of *N. balteatus* (Linsley 1964). Thus, our five western taxa may indeed represent a full accounting of the *Neoclytus* species that were likely to be present at our study sites.

Eastern taxa of *Neoclytus* that were missing from our study include *N. approximatus*, *Neoclytus horridus* (LeConte), *N. j. jouteli*, and *N. muricatulus muricatulus* (Kirby). Although native to the US central states, *N. approximatus* reportedly does not range as far east as Illinois (Yanega 1996). However, preliminary data from Texas suggest that adults are attracted to traps baited with *syn*-2,3-hexanediol (R.F.M. and L.M.H., unpublished data). The same racemic blend has attracted adult *N. j. jouteli* during field trials in Illinois (L.M.H., unpublished data). However, we have not yet found hexanediols or any other insect-produced compound in collections of volatiles from live *N. j. jouteli*.

Little is known of the very rare *N. horridus*, but it is morphologically similar to, and may be synonymous with *N. muricatulus muricatulus* (Linsley 1964). Our preliminary data for *N. muricatulus muricatulus* suggest that adults are attracted to traps baited with α-pinene and ethanol, as has been reported previously (Costello et al. 2008) and as is true for many other species of cerambycids whose larvae feed in conifers (e.g., Miller 2006). It should be noted that both *N. j. jouteli* and *N. muricatulus muricatulus* have long been considered uncommon, and it appears neither were recorded from Illinois prior to our field studies (Linsley 1964).

The fact that there are multiple sympatric species of *Neoclytus* that have apparently identical pheromones (i.e., [*R*]-3-hydroxyhexan-2-one as a single component) raises the question of how interspecific attraction is minimized. In a recent study, Mitchell et al. (2015), reported that *N. caprea* is one of the first cerambycid species to fly in spring, and so it is temporally isolated from *N. m. mucronatus* and *N. scutellaris*, both of which emerge more than 1 month later. It is not yet known how the latter two species segregate themselves. Moreover, the potential for interspecific attraction is not limited to interactions among the eastern species of *Neoclytus*, because (*R*)-3-hydroxyhexan-2-one is the primary or sole pheromone component for at least another 10 sympatric species in other cerambycine genera and tribes (Mitchell et al. 2015). Interspecific attraction among these species is in some cases averted by seasonal or circadian temporal segregation or by minor pheromone components that synergize the main component, or conversely, that inhibit attraction of heterospecifics that also produce (*R*)-3-hydroxyhexan-2-one as a major component of their pheromones (Hanks and Millar 2013, Mitchell et al. 2015). Segregation of species that share similar pheromones by phenology of adults, or by the use of pheromone blends rather than single-component pheromones, provides evidence that interspecific attraction is maladaptive and that there is strong selection for traits that minimize interspecific attraction.

The western species in our study that use (*R*)-3-hydroxyhexan-2-one as their pheromone also would seem to risk interspecific attraction. However, *N. conjunctus* may be reproductively isolated due to its early spring flight period (Craighead 1950), as is the case with *N. caprea* in the eastern community. However, there remain *N. irroratus* and *N. m. modestus*, adults of which were trapped during the same field trials in the mountains of California, as well as *N. balteatus*. The latter species may minimize interspecific attraction if its minor components are important synergists, or perhaps by differing from the other species in habitat preference. It also is possible that adult *N. irroratus* and *N. m. modestus* fly at different times of the day to minimize interspecific attraction. To our knowledge, the daily activity patterns of these two species have not been determined.

As with the eastern community, there are many western cerambycines in other genera and tribes that use (*R*)-3-hydroxyhexan-2-one as their primary or sole pheromone component, including at least nine species in the genera *Anelaphus*, *Brothylus*, *Phymatodes*, *Tragidion*, and *Xylotrechus* (Hanks et al. 2007, Ray et al. 2009b, A.M.R., L.M.H., and J.G.M., unpublished data). At least some of these species are unlikely to be cross attracted simply because they are not sympatric, such as *Neoclytus* species of this study that are confined to oak woodlands versus those endemic to desert habitats (Ray et al. 2009b).

Our studies also provide further evidence that attraction of cerambycid beetles to synthetic pheromones is a reliable predictor of their pheromone chemistry (e.g., Hanks et al. 2007). Moreover, screening trials of synthetic pheromones provide a convenient means of targeting cerambycine species for pheromone identification, because both sexes are attracted and therefore are available for comparative study of the volatiles they produce. For example, unanticipated attraction of adult *N. caprea* and *N. scutellaris* to traps baited with 3-hydroxyhexan-2-one in an earlier study (Lacey et al. 2009) inspired subsequent research that confirmed the pheromone chemistry reported here. That same earlier study also showed that the cerambycines *Anelaphus pumilus* (Newman) (tribe Elaphidiini) and *Cyrtophorus verrucosus* (Olivier) (Anaglyptini) were attracted to 3-hydroxyhexan-2-one, which later was confirmed to be the dominant pheromone component of both species (Mitchell et al. 2013, 2015).

Pheromones with the hydroxyketone-hexanediol structural motif have now been identified for species in several tribes of the Cerambycinae (e.g., Iwabuchi et al. 1986, Schroeder 1996, Lacey et al. 2009, Ray et al. 2009b, Mitchell et al. 2015) and attraction to synthetic 3-hydroxyhexan-2-one by adults of many other cerambycine species native to North America or to other parts of the world further attests to the global use of these pheromone structures (e.g., Hanks and Millar 2013, Imrei et al. 2013, Sweeney et al. 2014, Wickham

et al. 2014). Among cerambycines, the hydroxyketones and 2,3-alkanediols produced by males serve as aggregation-sex pheromones (sensu Cardé 2014), attracting both sexes, but with the primary purpose of attraction likely to be mating. However, 2,3-hexanediol isomers also serve as female-produced sex pheromones for some cerambycid species in the subfamily Prioninae, attracting only males (Ray et al. 2012).

The blend of compounds produced by male *N. balteatus* appears to be unusual for a North American cerambycid in having an eight-carbon component, (2*S*,3*S*)-octanediol, in addition to the six-carbon analog. The pheromone components of most North American cerambycines studied to date have been identified as six carbon hydroxyketones and alkanediols. Exceptions include (*E*)-2-hydroxyoct-4-en-3-one and related compounds, which are produced by males of the eastern species *Tylonotus bimaculatus* Haldeman (Zou et al. 2015) and (Z)-3-decenyl (*E*)-2-hexenoate produced by males of the western *Rosalia funebris* Motschulsky (Ray et al. 2009a). Another apparent exception is the cerambycine *Megacyllene caryae* (Gahan), males of which produce isomers of 2,3-hexanediols as minor components but also at least eight other chemicals that are better known as floral and wood volatiles (Lacey et al. 2008; R.F.M. and L.M.H., unpublished data). In contrast, hydroxyketones and 2,3-alkanediols of 8 and 10 carbon chain lengths have been identified from Eurasian cerambycines in the tribes Clytini and Anaglyptini (Iwabuchi et al. 1986, 1987, Leal et al. 1995, Hall et al. 2006, Narai et al. 2015).

Traps baited with synthetic pheromones can be effective in detecting species of cerambycids that otherwise are rarely encountered. For example, *N. tenuiscriptus* has long been considered rare (Hovore 1983). In fact, prior to our studies, there were no specimens of this species in the extensive holdings of the Entomology Research Museum at UC Riverside, even though the university is within the natural range of *N. tenuiscriptus*. Nevertheless, with pheromone-baited traps, we captured 36 adult *N. tenuiscriptus* in 5 d. Another example is our detection of species in Illinois that were outside their recorded ranges, including *N. j. jouteli* during the present studies, and the southeastern species *Curius dentatus* Newman in an earlier study (Lacey et al. 2004). Similar results were obtained in a study of the cerambycid fauna present in fragmented woodland habitats in Delaware (Handley et al. 2015). Thus, pheromone-baited traps could be used as sensitive probes for delimiting geographic distributions and assessing population sizes of rare, threatened, or endangered species of cerambycids (e.g., Ray et al. 2014). Moreover, the parsimony in pheromone chemistry across cerambycine species may be exploited by screening known cerambycid pheromones in different areas of the world, as a way of obtaining clues about the possible pheromone chemistry of potentially invasive species (e.g., Teale et al. 2011, Pajares et al. 2013, Wickham et al. 2014), so that the pheromones can be identified and developed as tools for detecting incursions of these species. Detection at the earliest possible moment is crucial to provide the best possible chance of eradicating the invader while populations are low and limited in distribution.

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