Mate Recognition and Sex Differences in Cuticular Hydrocarbons of the Diurnal Firefly *Ellychnia corrusca* (Coleoptera: Lampyridae)

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ABSTRACT There are several genera of diurnally active fireflies (Coleoptera: Lampyridae), and these adults generally do not produce bioluminescent signals. We investigated whether contact sex pheromones play a role in mate recognition in the diurnal firefly *Ellychnia corrusca* (L.). In laboratory behavioral assays, after antennal contact >70% of males attempted copulation with freeze-killed females, whereas no copulation attempts occurred when freeze-killed females had been washed with hexane. This shows that *E. corrusca* mate recognition relies on contact chemoreception and suggests that male mating responses are mediated by contact sex pheromones on the female cuticle. Using direct contact solid phase microextraction (SPME) and solvent extraction, we sampled cuticular hydrocarbons (CHCs) from both sexes of *E. corrusca* adults. Gas chromatography-mass spectrometry showed that SPME was more effective than solvent extraction in detecting CHCs (a two-fold increase in the number of compounds detected). Although *E. corrusca* males and females showed similar CHCs profiles, we documented several quantitative and qualitative differences between the sexes that may play a role in mate recognition. This report provides the first behavioral evidence for the existence of contact sex pheromones in any diurnal firefly.

KEY WORDS *Ellychnia corrusca*, mating behavior, contact pheromones, cuticular hydrocarbons, solid phase microextraction

Cuticular hydrocarbons (CHCs) on insect exoskeletons play a primary role in preventing desiccation and providing a barrier against microorganisms (Howard and Blomquist 2005). Because of their relatively low volatility, CHCs also may serve as contact chemical signals for species and mate recognition in many insects (Blomquist et al. 1996). In Coleoptera, particularly Cerambycidae, CHCs' role as contact sex pheromones has been extensively investigated, and in several species the chemical structure of these pheromones has been identified (Fukaya et al. 1996, 2000; Ginzel et al. 2003a,b, 2006; Lacey et al. 2008).

In nocturnally active fireflies, mate attraction generally relies on bioluminescent signals (Lewis and Cratsley 2008). In most diurnally active fireflies, however, adults are incapable of bioluminescence, and these species are generally assumed to rely on sex pheromones for mate attraction. For several diurnally active fireflies, field or laboratory bioassay studies have provided behavioral evidence that mate attraction is based on long-range volatile pheromonal signals (Lloyd 1972, Ohba 2004, De Cock and Matthysen 2005). In addition, two previous studies have found greater CHCs diversity and abundance in diurnal compared with nocturnal firefly species (Shibue et al. 2004, South et al. 2008). Therefore, much remains to be learned about chemical signals in this insect group, and cuticular hydrocarbons may potentially play an important role in mate recognition in diurnally active fireflies.

Diurnal fireflies in the genus *Ellychnia* (Coleoptera: Lampyridae) are widely distributed across the United States (Lloyd 2002). Adults lack light production although larvae are bioluminescent (Williams 1917). *Ellychnia corrusca* (L.) adults overwinter on tree trunks, and during their early spring mating season these beetles crawl along the tree surface to locate mates (Rooney and Lewis 2000). Previous field observations of *E. corrusca* gave no indication that mate finding involved volatile pheromones (Rooney and Lewis 2000), but it was noted that males first contact females with their antennae or palps before they mount females dorsally to initiate copulation. Also, in laboratory bioassays, *E. corrusca* males spent more time in contact with extracts from conspecific females compared with female extracts from *Lucidota atra* (Olivier) (Coleoptera: Lampyridae), another diurnal firefly (South et al. 2008). Thus, previous work suggests that male *E. corrusca* may use contact sex pheromones for mate recognition.

In the current study, we first conducted experiments comparing behavioral responses of *E. corrusca*

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males to freeze-killed females with and without CHCs. Second, we used gas chromatography-mass spectrometry (GC-MS) analysis to characterize and compare CHCs profiles from both sexes and different body parts of *E. corrusca* by using two sampling methods: traditional solvent extraction and solid phase microextraction (SPME). Last, we tested the bioactivity of one female-specific compound that we detected with the aim of identifying potential contact sex pheromones in this species.

Materials and Methods

Source of Fireflies. *E. corrusca* adults were collected during their mating season in Belmont, MA (42° 39′N, 71° 17′W) from April to May 2009, and in Holderness, NH (43° 74′N, 71° 59′W) during June 2009. Adults were kept in single-sex groups in mesh cages at room temperature and on a natural light cycle and provided with 10% maple syrup solution.

Behavioral Assays. To test the hypothesis that male *E. corrusca* use contact pheromones to recognize females, we conducted bioassays in the laboratory that compared male behavioral responses to control freeze-killed females versus those that had been hexane-washed to remove CHCs, following previously established methods (Ginzel and Hanks 2003; Ginzel et al. 2003a,b). Control females were freeze-killed at −20°C and then allowed 30 min to warm to room temperature. Another group of freeze-killed females were immersed in 0.5 ml of hexane for 30 min during which the solvent was replaced three times; these hexane-washed females were then allowed to air dry for 30 min. Two size-matched females (one from each treatment) were simultaneously presented under lab lighting to a single male *E. corrusca* in a covered plastic arena (9 cm in diameter by 1.5 cm in height), lined with P8 filter paper (Thermo Fisher Scientific, Waltham, MA). Females were placed on opposite sides of the arena, with their positions on left or right randomized to control for location effects.

We described mate recognition of *E. corrusca* males by using three phases of behavioral responses: 1) contact—the male touches a female with his antennae or mouthparts; 2) mounting—the male climbs onto the female's dorsal surface; and 3) copulation—the male extrudes his genitalia, recurves his abdomen and attempts to copulate. Behavioral bioassays were run using seven *E. corrusca* males (two from Massachusetts, five from New Hampshire). During 10-min observation periods, we recorded the number of contacts, mounts and copulations, as well as copulation duration. We compared differences in the average male behavioral response to hexane-washed and control females by using a nonparametric Mann–Whitney *U* test. Fisher exact test for homogeneity of proportions was used to compare the percentages of males exhibiting each behavioral response (contact, mounting, and copulation) between hexane-washed and control females. Summary statistics are reported as means ± SE.

Chemical Extraction and Analysis of CHCs. *Direct Contact (DC)-SPME.* Females and males were freeze-killed (−20°C) for 30 min, allowed to warm to room temperature for 30 min. A polydimethylsiloxane (PDMS) SPME fiber (100 μm; Supelco, Bellefonte, PA) with a manual SPME holder was placed in contact with the cuticular surface for 30 min (pressure was sufficient to maintain contact without bending the fiber); during this time, the fiber was also manually wiped across the cuticular surface 10 times every 10 min, rotating the fiber slightly between wipings. Fibers were conditioned according to the manufacturer's instructions before use and systematically reconditioned before each sampling. We separately sampled CHCs from the elytra and pronotum for both males ($n = 7$ for elytra, $n = 4$ for pronotum) and females ($n = 5$ for elytra, $n = 3$ for pronotum).

Samples were analyzed by coupled GC-MS with electron impact ionization (70 eV) by using a QP 5050 GC-MS (Shimadzu, Kyoto, Japan) equipped with a Rtx-XLB capillary column (30 m × 0.25 mm × 0.25 μm; Restek, Bellefonte, PA) in splitless mode, with helium as carrier gas. The column was programmed from 50°C for 1 min, 20°C/min to 280°C, and then held for 10 min. Injector temperature was 270°C, and transfer line temperature was 270°C. SPME fiber was desorbed in the injection port of the GC at 270°C for 2 min, and a narrow-bore Supelco 0.75-mm i.d. GC inlet liner was used for SPME analyses.

Solvent Extraction and Analysis. Cuticular chemicals were solvent-extracted from female and male *E. corrusca* ($n = 3$ for each sex) by removing both elytra and immersing them together in 300 μl of hexane for 30 min. Extractions were concentrated to ≈50 μl under a stream of nitrogen. For each sample, 4 μl of extract was injected for GC-MS analysis using the same temperature settings as given above.

For both SPME and solvent extraction, we calculated relative peak areas (as a percentage of total peak area) for individual peaks, and compared the average relative peak areas between males and females using two-tailed *t*-tests with unequal variances.

Testing Activity of Female-Specific Compound. We found one abundant, female-specific compound, which we identified by comparison with the retention time and mass spectrum of a commercially obtained standard (Sigma-Aldrich, St. Louis, MO). To test the activity of this female-specific compound, we pipetted ≈1 female equivalent (1 FE, consisting of 40 μl of standard in hexane at 27.4 ng/μl) onto freeze-killed, hexane-washed females; for control females, we pi-

Table 1. Percentage of *E. corrusca* males ($n = 7$) exhibiting mating behavioral responses to freeze-killed control versus hexane-washed females

Treatment	Contact		Mounting		Copulation	
Control females	100	$P = 0.070$	86	$P = 0.029$	71	$P = 0.021$
Hexane-washed females	43		14		0	

Differences between treatments were tested using Fisher's exact test.

Table 2. Behavioral responses (mean ± SE) by *E. corrusca* males ($n = 7$) to females during 10-min observation periods

Treatment	No. contacts		No. mounts		No. copulations	
Control females	5.3 ± 1.9	$P = 0.136$	2.3 ± 1.0	$P = 0.009$	1.4 ± 0.5	$P = 0.009$
Hexane-washed females	2.1 ± 1.1		0.1 ± 0.1		0	

Differences between treatments were tested using Mann–Whitney *U* tests.

petted 40 μl of hexane only onto freeze-killed, hexane-washed females. Females in both treatments were allowed to air dry for 5 min to allow hexane to evaporate. Two females (one from each treatment) were simultaneously presented on opposite sides of a plastic dish lined with filter paper, as described above for the behavioral assay. We recorded the behavioral response of five *E. corrusca* males, which were scored as responding if the male mounted and attempted copulation with either female within 10 min after first contact.

Results

Behavioral Assays. Most *E. corrusca* males contacted and mounted the control freeze-killed females, and >70% of males completed the entire courtship sequence with these females (Table 1). However, significantly fewer males mounted, and none copulated, with freeze-killed females that had been hexane-washed to remove their contact pheromones. We found no significant difference between control versus hexane-washed females in the mean number of

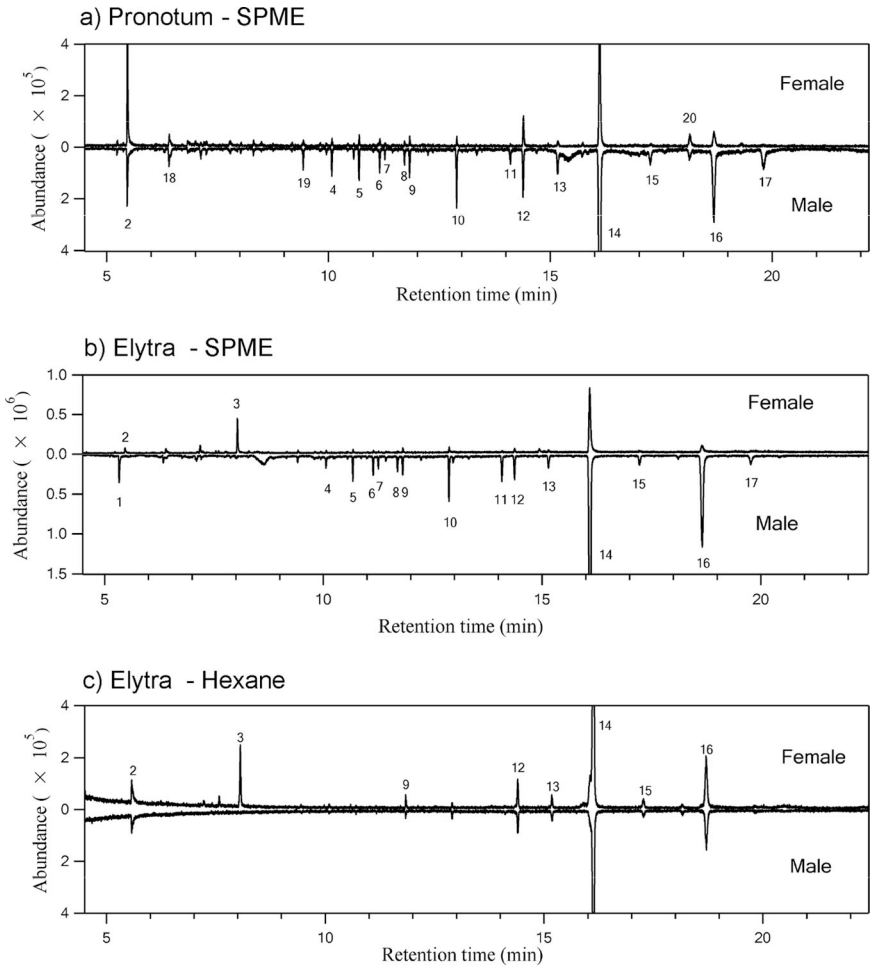

Fig. 1. Representative total ion chromatograms of cuticular hydrocarbons compared between *E. corrusca* adult females and males using different sampling techniques: (a) direct contact SPME sample collected from the pronotum, (b) direct contact SPME sample collected from the elytra, and (c) solvent extraction of elytra with hexane. Peak numbers correspond with Table 3.

Table 3. Relative abundances of cuticular hydrocarbons (mean percentage of total peak area ± SE) in female and male *E. corrusca* sampled using three different methods

Peak no.	Retention time (min)	SPME from pronotum			SPME from elytra			Hexane extracts of elytra		
		Females	Males	*P*	Females	Males	*P*	Females	Males	*P*
1	5.33	nd	nd		nd	1.67 ± 0.44		nd	nd	
2	5.47	11.71 ± 6.00	2.38 ± 0.42	0.260	1.97 ± 0.20	1.83 ± 0.33	0.720	2.78 ± 0.08	3.76 ± 1.11	0.265
3	8.04	nd	nd		26.60 ± 4.96	nd		8.71 ± 1.24	nd	
4	10.07	1.80 ± 0.37	1.73 ± 0.23	0.870	1.09 ± 0.15	0.90 ± 0.11	0.343	nd	nd	
5	10.68	1.58 ± 0.08	1.93 ± 0.20	0.170	1.17 ± 0.19	1.17 ± 0.17	0.968	nd	nd	
6	**11.14**	1.09 ± 0.10	1.37 ± 0.15	0.203	**1.51 ± 0.04**	**0.94 ± 0.10**	**0.001**	nd	nd	
7	11.26	nd	nd		nd	0.71 ± 0.10		nd	nd	
8	11.70	1.01 ± 0.04	nd		1.19 ± 0.02	0.86 ± 0.15	0.120	nd	nd	
9	**11.81**	2.81 ± 0.73	2.18 ± 0.36	0.494	**1.88 ± 0.27**	**1.16 ± 0.09**	**0.043**	**0.95 ± 0.03**	**0.72 ± 0.02**	**0.004**
10	12.87	2.80 ± 0.69	5.46 ± 0.87	0.062	2.51 ± 0.55	3.25 ± 0.44	0.320	nd	nd	
11	14.08	nd	1.73 ± 0.37		1.42 ± 0.09	1.40 ± 0.27	0.953	nd	nd	
12	14.37	5.04 ± 0.93	2.73 ± 0.40	0.114	2.11 ± 0.35	3.35 ± 0.72	0.168	2.71 ± 0.04	2.79 ± 0.24	0.793
13	15.15	nd	2.08 ± 0.05		1.55 ± 0.32	1.65 ± 0.15	0.784	1.16 ± 0.06	1.38 ± 0.16	0.292
14	16.10	54.34 ± 4.26	55.94 ± 3.41	0.783	53.50 ± 3.19	57.86 ± 1.69	0.284	58.08 ± 2.48	60.40 ± 3.47	0.618
15	17.23	nd	1.80 ± 0.12		1.29 ± 0.03	1.54 ± 0.14	0.133	**1.14 ± 0.07**	**1.46 ± 0.04**	**0.023**
16	**18.66**	**8.18 ± 1.09**	**13.35 ± 1.09**	**0.021**	**6.69 ± 0.23**	**14.40 ± 2.11**	**0.014**	**9.97 ± 0.13**	**11.16 ± 0.17**	**0.006**
17	19.78	nd	2.83 ± 0.05		nd	1.19 ± 0.03		nd	nd	
18	6.41	1.98 ± 0.41	1.43 ± 0.20	0.311	nd	nd		nd	nd	
19	9.43	1.12 ± 0.13	1.55 ± 0.41	0.406	nd	nd		nd	nd	
20	18.15	**3.96 ± 0.05**	**2.19 ± 0.03**	**<0.001**	nd	nd		nd	nd	

Peaks numbers correspond to labels shown in Fig. 1 (nd, not detected). *P* values are given for two-tailed, unequal variances *t*-tests of differences in mean relative abundance between sexes, with significant differences indicated in bold.

contacts made by *E. corrusca* males (Table 2), but males showed significantly more mounts and attempted copulation significantly more often with control females. These *E. corrusca* males attempted to copulate with control females for an average duration of 4.2 ± 1.6 min ($n = 5$ males).

Chemical Analysis of CHCs. Representative chromatograms obtained from SPME and hexane extraction of *E. corrusca* females and males are shown in Fig. 1. Although most of the peaks represented a low percentage of the total peak area, all analysis methods showed a single compound (peak 14) that was dominant in both sexes, representing >50% of the total peak area (Table 3).

SPME sampling from either the pronotum (Fig. 1a) or elytra (Fig. 1b) show similar CHC profiles, although some qualitative differences were observed (Table 3, peaks 1, 3, 18, 19, and 20). In contrast, hexane extracts of elytra yielded markedly different profiles (Fig. 1c) as these showed fewer than half the compounds detected from elytra using SPME (Fig. 1b; Table 3).

Both sexes of *E. corrusca* showed similar CHC profiles (Fig. 1), but there were both quantitative and qualitative differences between males and females (Table 3). Relative peak areas of several compounds showed significant differences between sexes (Table 3, peaks 6, 9, and 16 in SPME elytral samples; peaks 16 and 20 in SPME pronotum samples; and peaks 9, 15, and 16 in hexane extracts from elytra). In addition, several compounds were present in one sex but were not detected in the other (Table 3, peaks 1, 3, 7, 8, 13, 15, and 17). In particular, peak 3 (retention time, 8.04 min) constituted 26.6% of total peak area in SPME samples from female elytra (8.7% in hexane extracts) but was absent from males (Fig. 1b and c; Table 3). This compound was identified as exo, exo-2,3-camphanediol by comparisons of mass spectrum and retention time with a commercially available standard. However, when we applied 1 FE of this female-specific compound to hexane-washed, freeze-killed females, only one of the five tested males showed mounting behavior after contacting these females, and none attempted copulation.

Discussion

The insect wax layer contains a complex mixture of compounds that mainly include straight-chain saturated and unsaturated hydrocarbons, and methyl-branched hydrocarbons (Gibbs 1998, Howard and Blomquist 2005). Because CHCs are species-specific, these compounds may serve as cues in conspecific discrimination; for some species, it also has been suggested that they might play a role during courtship and mating (Howard 1993). Our behavioral assay results show that *E. corrusca* males readily attempted to mate with freeze-killed females, but none responded to solvent-washed females (Table 1). This demonstrates that mate recognition in this species probably occurs through contact chemoreception and that male mating responses are mediated by contact sex pheromones in the CHCs of females. Behavioral studies of several other diurnal firefly species, including *L. atra*, *Pyropyga nigricans* (Say), and *Photinus indictus* (LeConte) (Lloyd 1972), *Phosphaenus hemipterus* Fourcroy (De Cock and Matthysen 2005), and *Lucidina biplagiata* (Motschulsky) (Ohba 2004), have shown that their mate attraction depends on the production of volatile sex pheromones by females. The current finding provides the first behavioral evidence of contact sex pheromones in any diurnal firefly species within the Lampyridae. However, mate recognition by contact sex pheromones has been found to be very common among Cerambycinae beetles (Hanks et

al. 1996; Fukaya et al. 1996, 2000; Ginzel and Hanks 2003; Ginzel et al. 2003a,b, 2006; Lacey et al. 2008).

We used two sampling methods to extract CHCs from *E. corrusca:* the traditional solvent extraction and DC-SPME wipe sampling. Our results showed that SPME methods yielded more representative CHCs profiles than solvent extraction, which detected fewer than half the compounds from elytra that were detected using SPME (Fig. 1b and c; Table 3). Because SPME sample is directly desorbed into the injection port, potential analyte losses are greatly reduced compared with solvent extracts. Such differences in CHC profiles also may be due to the differences in the polarities and chemical affinities of the PDMS fiber and hexane. In addition, unlike the solvent, the SPME fiber is applied directly to the targeted body parts, and thus is not subject to contamination from adjacent tissues remaining after dissection. Therefore, our results confirm that the SPME technique is a very suitable alternative to solvent extraction for insect CHCs, as has been suggested by other studies (Moneti et al. 1997; Turillazzi et al. 1998; Sledge et al. 2000; Bland et al. 2001; Ginzel et al. 2003b, 2006).

Although insect cuticle often contains a complex mixture of hydrocarbons, only a few may comprise the contact pheromone of a species (Howard 1993). Extracts from the elytra of male and female *E. corrusca* were qualitatively different (Fig. 1b and c; Table 3), and we found a female-specific compound (peak 3) that we identified as exo, exo-2,3-camphanediol. We speculate that this female-specific compound may be used as a contact sex pheromone in *E. corrusca.* However, contrary to expectation, *E. corrusca* males did not respond to this female-specific compound; this lack of bioactivity suggests the possibility of a multicomponent sex pheromone, where this female-specific compound needs to be present with other compounds. It is also possible that the cuticular hydrocarbons that serve as contact pheromones are present in both sexes, but differ quantitatively between them, as found in other insects, e.g., the fly *Musca autumnalis* De Geer (Uebel et al. 1975) and beetles *Xylotrechus colonus* (F.) (Ginzel et al. 2003a) and *Megacyllene robiniae* (Forster) (Ginzel et al. 2003b). In our study of *E. corrusca*, we found several compounds (peaks 6, 9, 15, 16, and 20) that differed in their relative abundance between males and females (Table 3), and these may represent potential contact pheromones. Future studies should focus on the identification of sex pheromones used by nonluminescent, diurnal fireflies.

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References Cited

Bland, J. M., W.L.A. Osbrink, M. L. Cornelius, A. R. Lax, and C. B. Vigo. 2001. Solid phase microextraction for the detection of termite cuticular hydrocarbons. J. Chromatogr. A 932: 119–127.

Blomquist, G. J., J. A. Tillman-Wall, L. Guo, D. R. Quilici, P. Gu, and C. Schal. 1996. Hydrocarbon and hydrocarbon derived sex pheromones in insects: biochemistry and endocrine regulation, pp. 317–351. *In* D. W. Stanley-Samuelson and D. R. Nelson [eds.], Insect lipids: chemistry, biochemistry and biology. University of Nebraska Press, Lincoln, NE.

De Cock, R., and E. Matthysen. 2005. Sexual communication by pheromones in a firefly, *Phosphaenus hemipterus* (Coleoptera: Lampyridae). Anim. Behav. 70: 807–818.

Fukaya, M., T. Yasuda, S. Wakamura, and H. Honda. 1996. Reproductive biology of the yellow-spotted longicorn beetle, *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae). III. Identification of contact sex pheromone on female body surface. J. Chem. Ecol. 22: 259–270.

Fukaya, M., T. Akino, T. Yasuda, S. Wakamura, S. Satoda, and S. Senda. 2000. Hydrocarbon components in contact sex pheromone of the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae), and pheromonal activity of synthetic hydrocarbons. Entomol. Sci. 3: 211–218.

Gibbs, A. G. 1998. Water-proofing properties of cuticular lipids. Am. Zool. 38: 471–482.

Ginzel, M. D., and L. M. Hanks. 2003. Contact pheromones as mate recognition cues of four species of longhorned beetles (Coleoptera: Cerambycidae). J. Insect Behav. 16: 181–187.

Ginzel, M. D., G. J. Blomquist, F. G. Millar, and L. M. Hanks. 2003a. Role of contact pheromones in mate recognition in *Xylotrechus colonus.* J. Chem. Ecol. 29: 533–545.

Ginzel, M. D., J. G. Millar, and L. M. Hanks. 2003b. (Z)-9-Pentacosene-contact sex pheromone of the locust borer, *Megacyllene robiniae.* Chemoecology 13: 135–141.

Ginzel, M. D., J. A. Moreira, A. M. Ray, J. G. Millar, and L. M. Hanks. 2006. (Z)-9-Nonacosene-major component of the contact sex pheromone of the beetle *Megacyllene caryae.* J. Chem. Ecol. 32: 435–451.

Hanks, L. M., J. G. Millar, and T. D. Paine. 1996. Mating behavior of the eucalyptus longhorned borer (Coleoptera: Cerambycidae) and the adaptive significance of long "horns." J. Insect Behav. 9: 383–393.

Howard, R. W. 1993. Cuticular hydrocarbons and chemical communication, pp. 179–226. *In* D. W. Stanley-Samuelson and D. R. Nelson [eds.], Insect lipids: chemistry, biochemistry and biology. University of Nebraska Press, Lincoln, NE.

Howard, R. W., and G. J. Blomquist. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu. Rev. Entomol. 50: 371–393.

Lacey, E. S., M. D. Ginzel, J. G. Millar, and L. M. Hanks. 2008. 7-methylheptacosane is a major component of the contact sex pheromone of the cerambycid beetle *Neoclytus acuminatus acuminatus.* Physiol. Entomol. 33: 209–216.

Lewis, S. M., and C. K. Cratsley. 2008. Flash signal evolution, mate choice and predation in fireflies. Annu. Rev. Entomol. 53: 293–321.

Lloyd, J. E. 1972. Chemical communication in fireflies. Environ. Entomol. 1: 265–266.

Lloyd, J. E. 2002. Lampyridae, pp. 187–196. *In* R. Arnett, M. C. Thomas, P. Skelley, and J. H. Frank [eds.], American beetles, volume 2: Polyphaga. CRC, Boca Raton, FL.

Moneti, G., F. R. Dani, G. Pieraccini, and S. Turillazzi. 1997. Solid-phase microextraction of insect epicuticular hydrocarbons for gas chromatographic/mass spectrometric analysis. Rapid Commun. Mass Spectrom. 11: 857–862.

Ohba, N. 2004. Flash communication systems of Japanese fireflies. Integr. Comp. Biol. 44: 225–233.

Rooney, J., and S. M. Lewis. 2000. Notes on the life history and mating behavior of *Ellychnia corrusca* (Coleoptera: Lamypridae). Fla. Entomol. 83: 324–334.

Shibue, K., Y. Goto, I. Kawashima, and T. Shibue. 2004. Chemical analysis of surface hydrocarbons in fireflies by direct contact extraction and gas chromatography-mass spectrometry. Anal. Sci. 20: 1729–1731.

Sledge, M. F., G. Moneti, G. Pieraccini, and S. Turillazzi. 2000. Use of solid phase microextraction in the investigation of chemical communication in social wasps. J. Chromatogr. A 873: 73–77.

South, A., K. LeVan, L. Leombruni, C. Orians, and S. M. Lewis. 2008. Examining the role of cuticular hydrocarbons in firefly species recognition. Ethology 114: 916–924.

Turillazzi, S., M. F. Sledge, and G. Moneti. 1998. Use of a simple method for sampling cuticular hydrocarbons from live social wasps. Ethol. Ecol. Evol. 10: 293–297.

Uebel, E. C., P. E. Sonnet, R. W. Miller, and M. Beroza. 1975. Sex pheromone of the face fly, *Musea autumnalis* De Geer (Diptera: Muscidae). J. Chem. Ecol. 1: 195–202.

Williams, F. X. 1917. Notes on the life-history of some North-American Lampyridae. J. N.Y. Entomol. Soc. 25: 11–33.

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