Examining the Role of Cuticular Hydrocarbons in Firefly Species Recognition

Adam South, Katherine LeVan, Lisa Leombruni, Colin M. Orians & Sara M. Lewis

Department of Biology, Tufts University, Medford, MA, USA

Correspondence
Sara M. Lewis, Department of Biology, Tufts University, Medford, MA 02155, USA.
E-mail: sara.lewis@tufts.edu

Received: March 19, 2008
Initial acceptance: April 2, 2008
Final acceptance: May 1, 2008
(D. Zeh)

doi: 10.1111/j.1439-0310.2008.01535.x

Abstract

During animal courtship, multiple signals transmitted in different sensory modalities may be used to recognize potential mates. In fireflies (Coleoptera: Lampyridae), nocturnally active species rely on long-range bioluminescent signals for species, sex, and mate recognition, while several diurnally active species rely on pheromonal signals. Although in many insects non-volatile cuticular hydrocarbons (CHC) also function in species and sex discrimination, little is known about the potential role of CHC in fireflies. Here, we used gas chromatography to characterize species and sex differences in the CHC profiles of several North American fireflies, including three nocturnal and two diurnal species. Additionally, we conducted behavioral bioassays to determine whether firefly males (the searching sex) were differentially attracted to extracts from conspecific vs. heterospecific females. Gas chromatography revealed that nocturnal *Photinus* fireflies had low or undetectable CHC levels in both sexes, while diurnal fireflies showed higher CHC levels. No major sex differences in CHC profiles were observed for any firefly species. Behavioral bioassays demonstrated that males of the diurnal firefly *Ellychnia corrusca* were preferentially attracted to chemical extracts from conspecific vs. heterospecific females, while males of the remaining species showed no discrimination. These results suggest that while CHC may function as species recognition signals for some diurnal fireflies, these compounds are unlikely to be important contact signals in nocturnal *Photinus* fireflies.

Introduction

Animal courtship signals and responses play a key role in species, sex, and mate-quality recognition. Courtship interactions potentially involve multiple signals transmitted in several different sensory modalities (Candolin 2003; Hebets & Papaj 2005; Partan & Marler 2005). Signal generation can involve multiple organs, and complex signaling behaviors can evolve together as a unit (Hebets & Papaj 2005). For example, courtship by male *Schizocosa* wolf spiders depends on both visual and vibratory signals (Uetz & Roberts 2002; Hebets 2005), and males in the Hawaiian *Drosophila* species complex solicit copulations using chemical, tactile, visual, and vibrational signals (Greenspan & Ferveur 2000; Boake 2005). Thus, a comprehensive understanding of animal communication requires examining the potential for information to be transmitted across multiple signaling modalities.

Insect cuticular hydrocarbons (CHC) are secreted from a variety of glands and accumulate on the external surface of the exoskeleton. These low-volatility lipids function in protection against desiccation (Gibbs 1998; Singer 1998; Howard & Blomquist 2005; Barbour et al. 2007), and have also been shown to act as contact signals for species, kin, and mate recognition in numerous social insects (reviewed by Howard

Ethology **114** (2008) 916–924 © 2008 The Authors
Journal compilation © 2008 Blackwell Verlag, Berlin

1993; Howard & Blomquist 2005; Dani 2006) as well as in many solitary insects including *Drosophila* (Ferveur 2005), *Laupala* (Mullen et al. 2007), *Gryllus* crickets (Tregenza & Wedell 1997), *Cataglyphis niger* ants (Lahav et al. 1998), *Glossina* tsetse flies (Carlson et al. 2005), and many beetles (Peschke 1987; Page et al. 1990; Johansson & Jones 2007; Stoeffler et al. 2007). Recent work on *Laupala* crickets has demonstrated both interspecific and sex differences in CHC composition, and suggests that acoustic signals are used for long-range mate attraction while CHC function in close-range species discrimination (Mullen et al. 2007).

Within the family Lampyridae, firefly species differ considerably in the signal modalities used during courtship (Lloyd 1979; Ohba 2004; Lewis & Cratsley 2008). In many nocturnally active species, photic signals consisting of bioluminescent flashes or glows are used to attract mates, while mate attraction in some diurnally active species has been shown to involve volatile pheromonal signals (Lloyd 1972; De Cock & Matthysen 2005). Most North American *Photinus* fireflies are nocturnal, and males broadcast precisely timed bioluminescent signals to elicit female flash responses. *Photinus* males fly in search of perched females, making their final approach by walking to contact the stationary females. Temporal characteristics of *Photinus* courtship flash signals convey information concerning species identity, sex, and mate quality (Lloyd 1966; reviewed by Lewis & Cratsley 2008). There is no evidence that volatile pheromones are important in nocturnal *Photinus* fireflies, as flash responses from females in airtight containers readily attracted conspecific males (Lloyd 1966). The possibility that contact chemical cues may function in *Photinus* pre-mating reproductive isolation was first suggested by Lloyd (1966). In two sympatric *Photinus* species with similar flash signals, Lloyd noted that males were attracted to flash responses from heterospecific females, yet males subsequently rejected such females after contact. Further behavioral observations indicate that after initial contact with a female, *Photinus* males vigorously antennate the female and pass their maxillary palps over the female's pronotum and elytra (S.M. Lewis, unpublished data). Insect maxillary palps and antennae function as olfactory and gustatory receptors capable of distinguishing chemosensory signals (Chapman 1998). These observations suggest the possibility that while nocturnal *Photinus* fireflies rely on flash signals for long-range mate location, they may also use contact chemical signals for close-range species and/or sex discrimination.

Although diurnal fireflies are generally presumed to use chemicals for mate attraction, considerably less is known about their courtship signals (but see Shibue et al. 2000). Field experiments have shown that in several diurnal fireflies including *Lucidota atra*, females produce volatile pheromones that attract males (Lloyd 1972; De Cock & Matthysen 2005). In another diurnal firefly, *Ellychnia corrusca*, males use their maxillary palps to examine females before copulation (Rooney & Lewis 2000). Shibue et al. (2004) characterized CHC profiles for several firefly species, and found greater CHC diversity in a diurnal species compared to nocturnal species, although few individuals were examined and *Photinus* fireflies were not included in this study. No studies have explored the potential role of CHC for species recognition in diurnal fireflies.

This study was conducted to assess whether CHC may play a role in species or sex recognition in fireflies. We conducted behavioral bioassays to determine whether firefly males (the searching sex) were differentially attracted to chemical extracts from conspecific vs. heterospecific females. Additionally, we used gas chromatography (GC) to characterize species differences in CHC profiles between three nocturnal and two diurnal North American firefly species, as well as to examine possible sex differences and individual variation in CHC profiles.

Methods

Beetle Collection and Maintenance

The nocturnal fireflies *Photinus greeni* and *P. obscurellus* were collected in Lincoln, MA (42°26′N, 71°18′W), and *P. ignitus* were collected in Lancaster, MA (42°46′N, 71°67′W). Diurnal fireflies *L. atra* and *E. corrusca* were collected in Holderness, NH (43°74′N, 71°59′W) and in Belmont, MA (42°39′N, 71°17′W) respectively. All *Photinus* fireflies were kept individually in plastic cups with moist paper towel, while *L. atra* and *E. corrusca* beetles were kept in single-sex groups in mesh cages. All beetles were kept at room temperature and on a natural light cycle.

CHC Extraction for Bioassays and Gas Chromotography

To collect CHC from *P. greeni* and *P. obscurellus,* we rubbed cotton held by a toothpick (both washed in hexane and then autoclaved) 75 times over each beetle's pronotum and elytra, and CHC were isolated using standard techniques (Turillazzi et al. 1998;

Sumana et al. 2005). The cotton was placed in 500 μl dichloromethane and sonicated for 20 min, after which the cotton was removed and the sample allowed to evaporate. Samples were resuspended in 15 μl pentane, and test extracts were made by pooling extracts from several individuals to control for individual variation. To collect CHC for bioassays with *E. corrusca* and *L. atra* beetles, we switched over to the more efficient method of removing both elytra from each firefly and soaking them in 500 μl dichloromethane (CH_2Cl_2) for 20 min. Samples were evaporated and re-suspended in 100 μl GC capillary grade heptane and vortexed for 10 s. This more efficient method was used to extract CHC for GC characterization. Gas chromatographs comparing samples collected with both methods were similar.

Gas Chromatography Analysis of CHC Profiles

Cuticular hydrocarbon composition was analyzed with a Hewlett Packard 6980 gas chromatograph with a flame-ionization detector using standard techniques modified from Shibue et al. (2004). An Agilent HP-5 column (30 m × 0.319 mm × 0.25 μm) coated with non-polar (5% phenyl)-methylpolysiloxane was used with the following temperature program: 50°C initial temperature, a 20°C/min ramp, 280°C final temperature with a 10-min hold, and a 3-min post-run. Helium was the carrier gas and the program was performed in splitless mode. Final detector temperature was set at 300°C. Four microliters of each sample was injected, and we analyzed multiple individuals of each species and sex (*P. greeni* – 4 males, 15 females; *P. obscurellus* – 8 males, 5 females; *P. ignitus* – 9 males, 12 females; *L. atra* – 3 males, 2 females; *E. corrusca* – 39 males, 39 females). In each group of samples, we included at least one quality-control extract (following the identical protocol without adding elytra); this allowed us to identify and disregard any non-beetle contaminants. Multiple GC runs of the same extract confirmed that CHC profiles were highly repeatable. Insect CHC, including those previously identified from fireflies, have been shown to be between 21 and 35 carbons in length (Stanley-Samuelson & Nelson 1993; Shibue et al. 2004), and were identified as compounds with a retention time >11 min based on a heneicosane ($C_{21}H_{44}$) standard. To concisely summarize the differences in CHC profiles between the two diurnal firefly species, *L. atra* and *E. corrusca*, we used principal component analysis on the correlation matrix of relative peak areas (for this statistical analysis, we included compounds only with relative peak areas >6, as these could be readily distinguished from background peaks that were also present in quality controls). Nocturnal *Photinus* fireflies were not included in this analysis due to their low/undetectable levels of CHC. Principal component analysis was conducted using JMP 5.0 (SAS Inc., Cary, NC, USA).

Behavioral Bioassays

To examine whether chemical signals may be used by firefly males for species recognition, we conducted behavioral assays in which we measured male response to extracts from conspecific vs. heterospecific females. We focused on male discriminatory abilities because in all of these firefly species, males approach females, make contact, and initiate copulation. For nocturnal fireflies, we conducted bioassay trials to determine whether males of *P. greeni* (n = 11 males) and *P. obscurellus* (n = 8 males) were differentially responded to extracts from *P. greeni* compared with *P. obscurellus* females. These two locally sympatric *Photinus* species are morphologically indistinguishable in terms of body size, shape, and coloration; they differ only in male genitalic structure and flash behavior (Green 1956; Lloyd 1969). Therefore, our bioassays were designed to test whether males could distinguish between conspecific and heterospecific females solely on the basis of chemical cues. Chemical extracts (prepared as described above) from females were applied to filter paper; presenting chemical extracts on filter paper is a well-established method that has been used successfully to examine discriminatory abilities of many insects, including *Argas* ticks (Leahy et al. 1973), *Lariophagus* parasitic wasps (Steidle & Ruther 2000), *Ixodes* deer ticks (Allan & Sonenshine 2002), *Tenebrio* beetles (Bryning et al. 2005), *Piezodorous* stink bugs (Borges et al. 2007), *Callosobruchus* weevils (Nojima et al. 2007), and *Lonomia* moths (Zarbin et al. 2007). For each firefly species, bioassays were conducted during the appropriate mating period in either the field or the laboratory (at 24–26°C).

Bioassays for nocturnal firefly species were conducted by placing each male in a 200 cm^3 transparent chamber containing two 1 cm^2 squares of Whatman filter paper placed 7 cm apart on the chamber floor. Males were allowed a 5-min acclimation period, after which 15 μl (representing one female equivalent) of pooled extracts from either conspecific or heterospecific females was pipetted onto the filter paper. Male behaviors (walking, flying, antennation) were monitored continuously for 10 min during which we recorded how long each

Ethology 114 (2008) 916–924 © 2008 The Authors
Journal compilation © 2008 Blackwell Verlag, Berlin

male spent in the vicinity (within 2 cm) of each filter paper square. Bioassays for diurnal fireflies were similar, except chambers consisted of a 9 cm Petri dish, as these males generally approach females by walking; trials were conducted on eight *L. atra* males and nine *E. corrusca* males.

For each species, we compared the duration of time males spent associated with the conspecific and the heterospecific extracts; because these paired data were not normally distributed, we used a non-parametric Wilcoxon signed-rank test (two-tailed) to determine if beetles showed a preference for either stimulus. Because differences of zero between paired stimuli are excluded in this analysis, sample sizes were reduced for some species.

Results

Gas chromatography analysis revealed major differences in CHC profiles among firefly species. In the nocturnal *P. greeni*, no detectable CHC were found in any females (Fig. 1a) or males (Fig. 1b). Similarly, both sexes of *P. ignitus* as well as all *P. obscurellus* females and most *P. obscurellus* males exhibited no detectable CHC (data not shown; two of eight *P. obscurellus* males showed measurable but low CHC abundance).

In contrast, both diurnal firefly species exhibited a much greater abundance and diversity of CHC (Fig. 2). *Lucidota atra* showed the highest CHC abundance, with both females (Fig. 2a) and males (Fig. 2b) showing the same eight CHC peaks. In *E. corrusca* (Fig. 2c,d), overall CHC abundance was lower than in *L. atra*, and again, similar peaks were present in both sexes. Principal component analysis of relative peak areas for these diurnal fireflies (Fig. 3) showed that these two species separated mainly along principal component 1, with a fairly wide range of individual variation within species.

In bioassay tests of nocturnal fireflies, there were no significant differences in the time that focal males spent in close proximity to extracts from conspecific

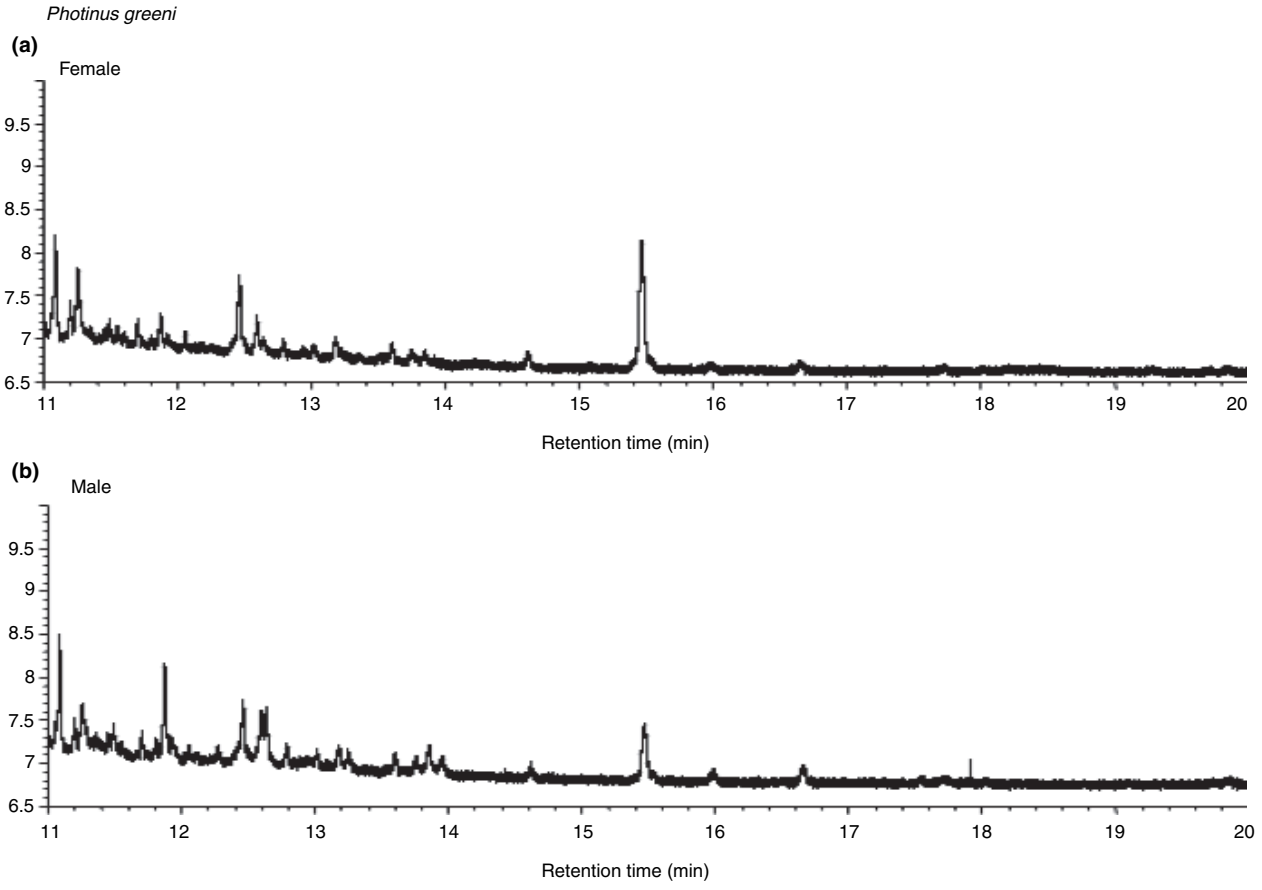

Fig. 1: Representative gas chromatography profiles of cuticular hydrocarbons from nocturnal fireflies, *Photinus greeni* – (a) female, (b) male. All peaks seen in both chromatograms were also present in quality control samples.

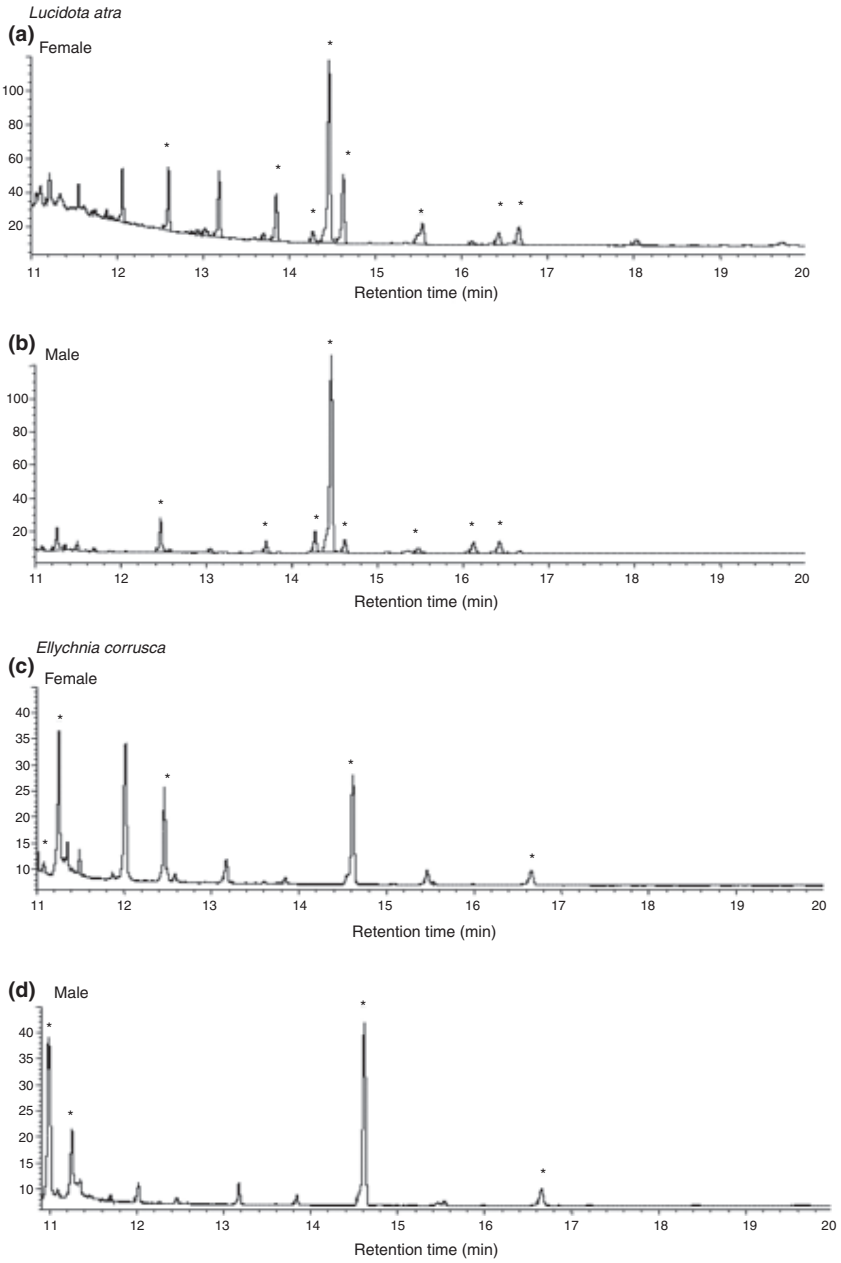

Fig. 2: Representative gas chromatography profiles of cuticular hydrocarbons from diurnal fireflies (note change in Y-axis scale from Fig. 1): *Lucidota atra* – (a) female, (b) male; *Ellychnia corrusca* – (c) female, (d) male. Peaks with asterisks represent cuticular hydrocarbons based on retention times and absence in quality controls.

vs. heterospecific females for either *P. greeni* (Fig. 4a; n = 11 males, Wilcoxon signed ranks test $W_s = 48$, p = 0.182) or for *P. obscurellus* (Fig. 4b; n = 8, $W_s = 18$, p = 1.0). Nocturnal firefly behavior during the bioassays was characterized by periods of inactivity interspersed with brief periods of flight or walking.

For diurnal fireflies, *E. corrusca* males spent significantly more time in contact with extracts from conspecific compared to heterospecific females (Fig. 4c: n = 6, $W_s = 21$, p = 0.028), while *L. atra* males showed no significant difference (Fig. 4d: n = 7, $W_s = 17$, p = 0.612). Diurnal fireflies showed a marked increase in walking and antennation when they encountered filter paper containing the extracts.

Discussion

A communication role for CHC has been well-documented in many social and solitary insect species; contact CHC signals have been shown to function in species, sex, and nestmate recognition, as well as in mate choice (Howard 1993; Singer

Ethology **114** (2008) 916–924 © 2008 The Authors
Journal compilation © 2008 Blackwell Verlag, Berlin

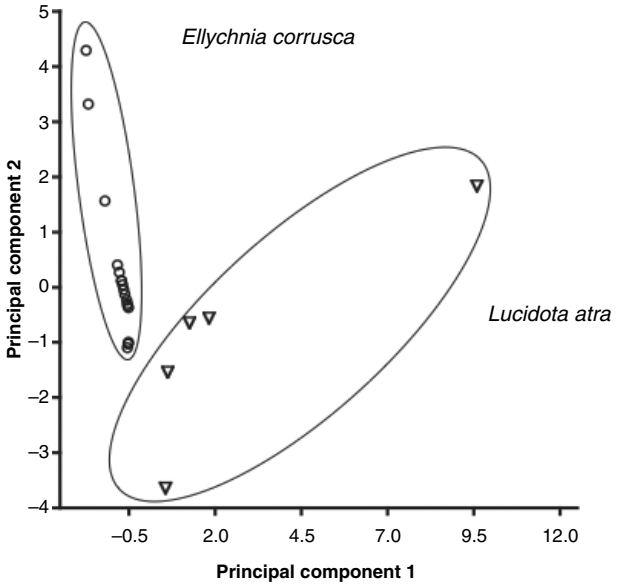

Fig. 3: Principal component analysis summarizing species differences and individual variation in cuticular hydrocarbons relative peak areas for two diurnal firefly species (18 *E. corrusca* individuals, 5 *L. atra* individuals).

1998; Ginzel & Hanks 2003; Ginzel et al. 2003; Sumana et al. 2005; Dani 2006; Barbour et al. 2007; Johansson & Jones 2007; Mullen et al. 2007). Sex-specific differences in CHC presence and abundance have been found in several coleopterans (Ginzel et al. 2003; Barbour et al. 2007; Peterson et al. 2007). Our study is the first to provide a comprehensive assessment of species and sex differences in CHC profiles across several firefly species using multiple individuals. CHC were abundant in two diurnal fireflies, *E. corrusca* and *L. atra*. However, CHC were not detected in the nocturnal fireflies *P. greeni*, *P. ignitus*, or in *P. obscurellus* females and CHC were found in very low abundance in a few *P. obscurellus* males. These low CHC levels found in nocturnal fireflies are unlikely to reflect methodological artifacts, as we readily detected CHC in diurnal fireflies using identical procedures. Although new techniques may allow detection of higher molecular weight CHC excluded by the standard GC approach used here (Cvacka et al. 2006), it is possible that lower dessication risk in nocturnal fireflies precludes the need for high CHC abundance as CHC function to prevent

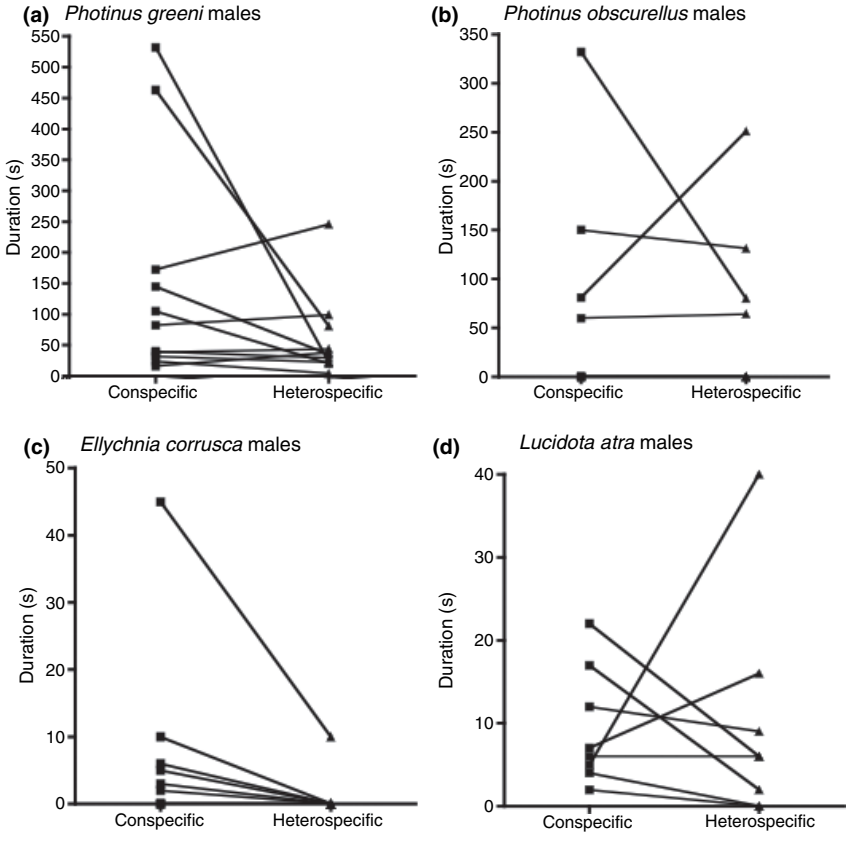

Fig. 4: Behavioral bioassay results for male fireflies responding to extracts from conspecific vs. heterospecific females. Duration of time (s) spent in proximity to extracts on filter paper was recorded during 10-min observation periods. Each line represents the behavioral responses of a single male: (a) *Photinus greeni* (n = 11 males), (b) *Photinus obscurellus* (n = 8 males), (c) *Ellychnia corrusca* (n = 9), (d) *Lucidota atra* (n = 8).

dehydration in many insects (Howard & Blomquist 2005). Our results are consistent with those obtained by Shibue et al. (2004), who also found greater CHC diversity and abundance in diurnal compared with nocturnal fireflies, and identified eight CHC peaks in two *L. atra* females. The present study also revealed major differences in CHC profiles between two sympatric diurnal fireflies, suggesting that these contact signals might play a role in species recognition and pre-mating reproductive isolation in this group.

Results from the behavioral bioassays also provide support for the idea that species recognition by *E. corrusca* males may involve contact chemical signals, as these males preferentially responded to extracts from conspecific females compared with *L. atra* females. Based on the lack of discrimination by *L. atra* males in bioassays, however, contact chemicals appear less important as recognition signals in *L. atra*. *Lucidota atra* females produce volatile pheromonal signals that attract males (Lloyd 1972), although the active compounds have yet to be identified.

Bioassay results for the two nocturnal *Photinus* species, *P. greeni* and *P. obscurellus,* indicated that males did not discriminate between contact chemicals from conspecific vs. heterospecific females. Unfortunately, in the absence of any known chemical attractants for these beetles, positive controls could not be incorporated into the bioassay design. However, evidence supports an interpretation of our bioassay results as indicating *Photinus* males do not use chemical cues for close-range species discrimination. First, these bioassay results are consistent with our gas chromatographic analyses, which showed CHC were below detection limits in these nocturnal fireflies. Second, it is not likely that the observed lack of discrimination exhibited during behavioral bioassays reflected a lack of motivation to mate, as all bioassays were conducted during the appropriate evening mating period for each species. Additionally, previous studies have used similar extraction and bioassay methods to show behavioral discrimination in many different insects (Ginzel et al. 2003; Bryning et al. 2005; Barbour et al. 2007), suggesting that these methods should also have been sufficient to detect any use of contact chemical signals by fireflies. Finally, it seems unlikely that lack of discrimination by *Photinus* males reflects insufficient statistical power, as a significant preference for conspecific female extracts was demonstrated for *E. corrusca* males using similar sample sizes. Taken together, these findings suggest that contact chemical signals do not play a major role in species recognition for nocturnal *Photinus* fireflies.

In conclusion, CHC seem unlikely to play a major role in species or sex recognition in nocturnal firefly species. Current evidence suggests that species, sex, and mate recognition in nocturnal fireflies relies primarily on long-range bioluminescent visual signals. However, CHC may be important species recognition signals for at least one diurnal firefly, and may replace or act in conjunction with volatile pheromones shown to be important in other diurnally active fireflies. Future studies of multimodal signaling in fireflies might focus on close-range tactile signals associated with pre-mating behavioral interactions.

Acknowledgements

We thank K. Shibue and M.P. Scott for helpful advice, and P.T.B. Starks and two anonymous reviewers for comments on the manuscript. This research was supported by the Tufts Biology NSF Research Experiences for Undergraduates Program and by NSF award #I0B-0543738.

Literature Cited

Allan, S. A. & Sonenshine, D. E. 2002: Evidence of an assembly pheromone in the black-legged deer tick *Ixodes scapularis*. J. Chem. Ecol. **28**, 15—27.

Barbour, J. D., Lacey, E. S. & Hanks, L. M. 2007: Cuticular hydrocarbons mediate mate recognition in a species of longhorned beetle (Coleptera: Cerambycidae) of the primitive subfamily Prioninae. Ann. Entomol. Soc. Am. **100**, 333—338.

Boake, C. R. B. 2005: Sexual selection and speciation in Hawaiian *Drosophila*. Behav. Genet. **35**, 297—303.

Borges, M., Millar, J. G., Laumann, R. A. & Moraes, M. C. B. 2007: A male-produced sex pheromone from the Neotropical redbanded stink bug, *Piezodorus guildinii* (W.). J. Chem. Ecol. **33**, 1235—1248.

Bryning, G. P., Chambers, J. & Wakefield, M. E. 2005: Identification of a sex pheromone from male yellow mealworm beetles *Tenebrio molitor*. J. Chem. Ecol. **31**, 2721—2730.

Candolin, U. 2003: The use of multiple cues in mate choice. Biol. Rev. **78**, 575—595.

Carlson, D. A., Mramba, F., Sutton, B. D., Bernier, U. R., Geden, C. J. & Mori, K. 2005: Sex pheromone of the tsetse species, *Glossina austeni*: isolation and identification of natural hydrocarbons, and bioassay of synthesized compounds. Med. Vet. Entomol. **19**, 470—479.

Chapman, R. F. 1998: The Insects, 4th edn. Cambridge University Press, Cambridge.

Cvacka, J., Jiros, P., Sobotnik, J., Hanus, R. & Svatos, A. 2006: Analysis of insect cuticular hydrocarbons using

Ethology **114** (2008) 916–924 © 2008 The Authors
Journal compilation © 2008 Blackwell Verlag, Berlin

matrix-assisted laser desorption/ionization mass spectrometry. J. Chem. Ecol. **32**, 409—434.

Dani, F. R. 2006: Cuticular lipids as semiochemicals in paper wasps and other social insects. Ann. Zool. Fenn. **43**, 500—514.

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

Ferveur, J. F. 2005: Cuticular hydrocarbons: their evolution and roles in *Drosophila* pheromonal communication. Behav. Genet. **35**, 279—295.

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

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

Ginzel, M. D., Blomquist, G. J., Millar, J. G. & Hanks, L. M. 2003: Role of contact pheromones in mate recogntion in *Xylotrechus colonus*. J. Chem. Ecol. **29**, 533—545.

Green, J. W. 1956: Revision of the nearctic species of *Photinus* (Lampyridae: Coleoptera). Proceedings Calif. Acad. Sci. **28**, 561—613.

Greenspan, R. J. & Ferveur, J. F. 2000: Courtship in *Drosophila*. Annu. Rev. Genet. **34**, 205—232.

Hebets, E. A. 2005: Attention-altering signal interactions in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. Behav. Ecol. **16**, 75—82.

Hebets, E. A. & Papaj, D. R. 2005: Complex signal function: developing a framework of testable hypotheses. Behav. Ecol. Sociobiol. **57**, 197—214.

Howard, R. W. 1993: Cuticular hydrocarbons and chemical communication. In: Insect Lipids: Chemistry, Biochemistry and Biology (Stanley-Samuelson, D. W. & Nelson, D. R., eds). University of Nebraska Press, Lincoln, pp. 179—226.

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

Johansson, B. G. & Jones, T. M. 2007: The role of chemical communication in mate choice. Biol. Rev. **82**, 265—289.

Lahav, S., Soroker, V., Hefetz, A. & Vander Meer, R. K. 1998: Nestmate recognition in the ant *Cataglyphis niger*: do queens matter? Behav. Ecol. Sociobiol. **43**, 203—212.

Leahy, M. G., Vandehey, R. & Galun, R. 1973: Assembly pheromone(s) in the soft tick *Argus persicus* (Oken). Nature **246**, 515—517.

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

Lloyd, J. E. 1966: Studies on the flash communication system in *Photinus* fireflies. Univ. Mich. Misc. Publ. **130**, 1—95.

Lloyd, J. E. 1969: Flashes, behavior and additional species of Nearctic *Photinus* fireflies (Coleoptera: Lampyridae). Coleopt. Bull. **23**, 29—40.

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

Lloyd, J. E. 1979: Sexual selection in luminescent beetles. In: Sexual Selection and Reproductive Competition in Insects (Blum, M. S. & Blum, N. A., eds). Academic Press, New York, pp. 293—342.

Mullen, S. P., Mendelson, T. C., Schal, C. & Shaw, K. L. 2007: Rapid evolution of cuticular hydrocarbons in a species radiation of acoustically diverse Hawaiian crickets (Gryllidae: Trigonidiinae: *Laupala*). Evolution **61**, 223—231.

Nojima, S., Shimomura, K., Honda, H., Yamamoto, I. & Ohsawa, K. 2007: Contact sex pheromone components of the cowpea weevil *Callosobruchus maculatus*. J. Chem. Ecol. **33**, 923—933.

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

Page, M., Nelson, L. J., Haverty, M. I. & Blomquist, G. J. 1990: Cuticular hydrocarbons of eight species of North American cone beetles, *Conophthorus* Hopkins. J. Chem. Ecol. **16**, 1173—1198.

Partan, S. R. & Marler, P. 2005: Issues in the classification on multimodal communication signals. Am. Nat. **166**, 231—245.

Peschke, K. 1987: Cuticular hydrocarbons regulate mate recognition, male aggression, and female choice of the rove beetle, *Aleochara curtula*. J. Chem. Ecol. **13**, 1993—2008.

Peterson, M. A., Dobler, S., Larson, E. L., Juarez, D., Schlarbaum, T., Monsen, K. J. & Francke, W. 2007: Profiles of cuticular hydrocarbons mediate male mate choice and sexual isolation between hybridising *Chrysochus* (Coleoptera: Chrysomelidae). Chemoecology **17**, 87—96.

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

Shibue, K., Goto, Y., Shibue, T. & Ohba, N. 2000: Analysis of sex-attractant pheromones of firefly *Pyrocoelia oshimana* by gas chromatography mass spectrometry. Anal. Sci. **16**, 995—996.

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

Singer, T. 1998: Role of hydrocarbons in the recognition system of insects. Am. Zool. **38**, 394—405.

Stanley-Samuelson, D. W. & Nelson, D. R. 1993: Insect Lipids: Chemistry, Biochemistry, and Biology. University of Nebraska Press, Lincoln, Nebraska.

Steidle, J. L. M. & Ruther, J. 2000: Chemicals used for host recognition by the granary weevil parasitoid *Lariophagus distinguendus*. J. Chem. Ecol. **26**, 2665—2675.

Stoeffler, M., Maier, T. S., Tolasch, T. & Steidle, J. L. M. 2007: Foreign-language skills in rove-beetles? Evidence for chemical mimicry of ant alarm pheromones in myrmecophilous Pella beetles (Coleoptera: Staphylinidae). J. Chem. Ecol. **33**, 1382—1392.

Sumana, A., Liebert, A. E., Berry, A. S., Seitz, G. T., Orians, C. M. & Starks, P. T. 2005: Nest hydrocarbons as cues for philopatry in a paper wasp. Ethology **111**, 469—477.

Tregenza, T. & Wedell, N. 1997: Definitive evidence for cuticular pheromones in a cricket. Anim. Behav. **54**, 979—984.

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

Uetz, G. W. & Roberts, J. A. 2002: Multisensory cues and multimodal communication in spiders: insights from video/audio playback playback studies. Brain Behav. Evol. **59**, 222—230.

Zarbin, P. H. G., Lorini, L. M., Ambrogi, B. G., Vidal, D. M. & Lima, E. R. 2007: Sex pheromone of *Lonomia obliqua*: daily rhythm of productions, identification, and synthesis. J. Chem. Ecol. **33**, 555—565.

Ethology **114** (2008) 916–924 © 2008 The Authors
Journal compilation © 2008 Blackwell Verlag, Berlin