Demonstration of Sex Pheromones in *Molanna Uniophila* (Trichoptera: Molannidae), *Platycentropus Radiatus, Pycnopsyche Indiana, and P. Subfasciata* (Trichoptera: Limnephilidae), With an Assessment of Interspecific Attraction Between Four Sympatric *Pycnopsyche* Species

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Abstract

Evidence for sex pheromones in the caddisflies *Molanna uniophila* Vorhies, *Platycentropus radiatus* (Say), *Pycnopsyche indiana* (Ross), and *P. subfasciata* (Say) is presented here for the first time based on field studies conducted in southern Michigan. For all species, numbers of males caught in pheromone traps baited with conspecific female extracts were higher than those caught in traps with male extracts, or those without extract. Four sympatric species of *Pycnopsyche*: *P. guttifer* (Walker), *P. indiana*, *P. lepida* (Hagen), and *P. subfasciata* exhibited no interspecific pheromonal attraction despite a common mating season and, in the case of *P. indiana* and *P. lepida*, very similar genitalic morphology. Our results suggest the importance of pheromones in maintaining reproductive isolation between closely related species. Likewise, pheromone biology can help delineate morphologically cryptic species.

Pheromones are known to be a part of the mating systems of 8 insect orders (Pherobase 2009), and were first demonstrated experimentally within the Trichoptera in 1984 (Wood and Resh 1984). Since then, the attraction of conspecific males by a semiochemical extracted from females has been documented in 40 caddisfly species within 15 families (Table 1). Aggregation pheromones have been demonstrated in *Hydropsyche angustipennis* (Curtis) (Hydropsychidae), and male pheromones have been suggested in several species of Limnephilidae (Löfstedt et al. 1994, Bergmann 2002).

Ivanov and Löfstedt (1999) reviewed the status of caddisfly pheromone research. Much of the recent work involves physiological and biochemical studies (Bergmann et al. 2001, 2002, 2004; Löfstedt et al. 2008). Basic pheromone biology, however, is still in its relative infancy, as their presence has been demonstrated in less than 0.5% of the described caddisfly fauna. It is likely that pheromone use in the Trichoptera is much more prevalent, especially when considering the common and widespread use in their sister taxon, the Lepidoptera. Thus, basic caddisfly pheromone demonstration studies remain important.

Because pheromones are important in mating systems, they have the potential to influence selection and speciation, phenomena that have been studied extensively in the Lepidoptera. For example, the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), contains two morphologically identical strains that appear to be reproductively isolated from each other due to relatively subtle chemical differences in their pheromones (e.g., Klun et al. 1973). Within the heliothine moths (Lepidoptera: Noctuidae), species-specific pheromone compounds help to maintain reproductive isolation

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Table 1. The 15 families of Trichoptera recognized to employ female-excreted sex pheromones as part of their mating systems, with each suborder and the number of known pheromone-using species within each family.

Family	Suborder	# of species	Reference(s)
Apataniidae	Integripalpia	1	Solem and Solem 1991, Bergmann 2002
Glossosomatidae	Spicipalpia	1	Bergmann 2002
Helicopsychidae	Integripalpia	1	Resh et al. 1984
Hydrobiosidae	Spicipalpia	1	Bergmann 2002
Hydropsychidae	Annulipalpia	4	Bergmann 2002
Hydroptilidae	Spicipalpia	1	Bergmann 2002
Limnephilidae	Integripalpia	17	Bjostad et al. 1996, Jewett et al. 1996, Bergmann 2002, Houghton 2002, current paper
Molannidae	Integripalpia	2	Solem and Petersson 1987, Löftstedt et al. 2008, current paper
Philopotamidae	Annulipalpia	1	Bergmann 2002
Philorheithridae	Integripalpia	1	Bergmann 2002
Phryganeidae	Integripalpia	1	Ansteeg & Dettner 1991
Polycentropodidae	Annulipalpia	1	Ansteeg & Dettner 1991
Psychomyiidae	Annulipalpia	3	Bergmann 2002
Rhyacophilidae	Spicipalpia	4	Duffield 1981, Solem 1985, Ansteeg and Dettner 1991, Larsson and Hansson 1998, Bergmann 2002
Sericostomatidae	Integripalpia	1	Resh and Wood 1985

between sympatric congeners (e.g., Baker et al. 1998). Such phenomena may also occur in the Trichoptera. For example, the genus *Pycnopsyche* (Limnephilidae) frequently contains several congeners within a single habitat. All *Pycnopsyche* species exhibit a synchronous fall emergence and, thus, occur simultaneously in the riparian vegetation during mating. It is possible that chemical differences within the pheromones of these sympatric populations may keep them reproductively isolated. The purposes of this study were to demonstrate pheromone use in additional caddisfly species, and to assess cross-attraction between sympatric *Pycnopsyche* species.

Materials and Methods

Our field trials were conducted at Little Hog Creek in Allen County, MI (N 41º55’, W 84º 49’). At least four species of *Pycnopsyche* are known to occur at this site: *P. guttifer* (Walker), *P. lepida* (Hagen), *P. indiana* (Ross), and *P. subfasciata* (Say). The former two species have been previously demonstrated to use pheromones (Houghton 2002), the latter two have not. Two other species common at this site: *Molanna uniophila* Vorhies (Molannidae) and *Platycentropus radiatus* (Say) (Limnephilidae) were also tested for pheromone use.

To obtain pheromones, individual adult specimens of all six species were collected at Little Hog Creek and transported alive back to the laboratory. We did not determine if females had already oviposited, as Houghton (2002) found that there was no significant difference between virgin and post-oviposition females in their ability to attract males. Following the methods first used for Trichoptera by Wood and Resh (1984), pheromones were extracted by placing one living specimen into a 12 ml glass vial with 2–3 ml of HPLC-grade dichloromethane (Fisher Scientific, Pittsburg, PA) for 1–2 hours. Extracts not studied the next morning were stored at -80ºC until use. Foam *Drosophila* vial plugs (Carolina Biological, Burlington, NC) were infused with each extract and placed into a Trécé Pherocon 1C wing trap (Gempler’s, Belleville, WI).

Pheromone trapping occurred at Little Hog Creek during 2006–2008. In all trials, traps were hung in random order from riparian vegetation approximately 1–2 m above the water, and 10 m apart from each other. Each experiment began at dusk and lasted 24 hours. Voucher specimens of all tested species have been deposited in the Hillsdale College insect collection.

To assess sex pheromone use in the six species, extracts of females, males, and dichloromethane controls were simultaneously tested for their ability to attract conspecific males. Each species was tested in an individual experiment. After each experiment, differences in the mean number of males caught by each extract were assessed using a one-way analysis of variance (ANOVA) with *post-hoc* Tukey test using JMP for Windows® Software (JMP 2002).

To assess interspecific attraction between the sympatric *Pycnopsyche* species, extracts of females of all four were tested simultaneously along with a series of dichloromethane controls. Thus, the same female extracts concurrently tested the response of both conspecific and interspecific males. If the mean number of males caught by an extract was >0, it was then compared to that of the control treatment with an independent *t*-test.

Results

Pheromone use was demonstrated in all six species: *M. uniophila, P. radiatus*, *P. guttifer*, *P. indiana*, *P. lepida*, and *P. subfasciata*. For all species, the number of males caught was significantly higher in traps baited with conspecific female extracts than with male extracts or control extracts (Table 2). In the assessment of *Pycnopsyche* interspecific attraction, the mean number of males caught in traps baited with conspecific female extracts was,

Table 2. Mean (±SD) number of conspecific males caught in traps baited with various extracts from a series of field trials conducted in southern Michigan. Each series of three extracts constitutes a separate experiment. Superscript numbers denote statistically distinct groups of means (One-way Analysis of Variance with *post-hoc* Tukey test). The field trial of *M. uniophila* was repeated due to a low catch rate during the first trial.

Date	**Extract**	***n***	**# caught**	**df**	***F***	***P***
May 2006	*Molanna uniophila* males	3	0.0 (±0.0)^{A}			
May 2006	Dichloromethane only	9	0.0 (±0.0)^{A}			
May 2006	*Molanna uniophila* females	12	1.6 (±0.8)^{B}			
			Summary data	22	20.8	<0.001
May 2007	Dichloromethane only	4	0.0 (±0.0)^{A}			
May 2007	*Molanna uniophila* males	6	0.0 (±0.0)^{A}			
May 2007	*Molanna uniophila* females	10	2.7 (±1.2)^{B}			
			Summary data	21	22.1	<0.001
May 2007	Dichloromethane only	4	0.0 (±0.0)^{A}			
May 2007	*Platycentropus radiatus* males	3	0.0 (±0.0)^{A}			
May 2007	*Platycentropus radiatus* females	5	4.2 (±1.6)^{B}			
			Summary data	11	21.4	<0.001
September 2007	Dichloromethane only	5	0.0 (±0.0)^{A}			
September 2007	*Pycnopsyche indiana* males	8	0.0 (±0.0)^{A}			
September 2007	*Pycnopsyche indiana* females	10	4.0 (±1.2)^{B}			
			Summary data	22	64.6	<0.001
September 2007	Dichloromethane only	6	0.0 (±0.0)^{A}			
September 2007	*Pycnopsyche subfasciata* males	5	0.0 (±0.0)^{A}			
September 2007	*Pycnopsyche subfasciata* females	8	6.5 (±1.2)^{B}			
			Summary data	16	125.2	<0.001

likewise, significantly higher than that of control or interspecific extracts. In fact, no males or females were caught in traps baited with control extracts, or with extracts of females of a different species (Table 3).

Discussion

Data from this experiment support the use of sex pheromones in four species not previously documented. *Platycentropus radiatus* is the first species within its genus shown to use pheromones; however, several other species and genera in its tribe, the Limnephilini, have been previously shown to use them (Bjostad et al. 1996, Jewett et al 1996, Bergmann 2002). The chemical structure and electroantennogram response of *Hesperophylax occidentalis* (Banks) (Limnephilidae) pheromones have been particularly well studied; they consist of short-chained alcohols and ketones (Bjostad et al. 1996, Jewett et al 1996). *Molanna uniophila* is the second species in its genus demonstrated to use pheromones. *Molanna angustata* Curtis has been studied extensively in Europe; its pheromones are produced in glands within the 5^{th} sternite and are likewise composed of alcohols and ketones (Löfstedt et al. 2008). *Pycnopsyche indiana* and *P. subfasciata* are the third and fourth species within their genus shown to use pheromones. *Pycnopsyche guttifer* and *P. lepida* were previously studied in Minnesota (Houghton 2002). Based on field trials, allopatric populations of the latter species appear to exhibit pheromonal "dialects"; female extracts from a geographically isolated population were able to attract males, but at a lower rate than extracts from the local population.

With the demonstration of four more species in this study, pheromone use within the Trichoptera has now been documented in all three suborders: Integripalpia, Annulipalpia, and Spicipalpia (Table 1). Within the Annulipalpia, pheromone use has been found in both the Hydropsycoidea and the Philopotamoidea. Within the Integripalpia, it has been documented in both the Brevitentoria and Plenitentoria (Holzenthal et al. 2007a, b). Although not

Table 3. Results of a single *Pycnopsyche* cross-attraction experiment conducted during September 2008 in southern Michigan. A total of 41 traps were set: 10 dichloromethane controls and 31 extracted females. Since a female extract was simultaneously testing for the attraction of conspecific males, interspecific males, and females the sample size is listed separately for all three treatments. If the mean of individuals caught was >0 for a treatment, then it was compared to the control treatment with an Independent T-test.

Treatment	*n*	Mean (±SD)	*P*
Males caught with control extract	10	0	N/A
Females caught with any extract	31	0	N/A
Males caught with conspecific female extract			
P. guttifer	7	9.4 (±2.1)	<0.001
P. indiana	9	4.4 (±1.6)	<0.001
P. lepida	10	6.8 (±2.0)	<0.001
P. subfasciata	5	2.4 (±1.5)	<0.001
Males caught with interspecific female extract			
P. guttifer	7	0	N/A
P. indiana	9	0	N/A
P. lepida	10	0	N/A
P. subfasciata	5	0	N/A

yet extensively studied, pheromone use in the order is probably widespread. Furthermore, the chemical composition of leptoceroid pheromones is similar to that of the Annulipalpia—two widely divergent taxa—suggesting minimal evolutionary change within the order (Löfstedt et al. 2008).

The four *Pycnopsyche* congeners that occur at our field site exhibited no interspecific pheromonal attraction despite a similar morphology and mating period. *Pycnopsyche lepida, P. indiana*, and *P. subfasciata*, in particular, have very similar genitalic morphology and are all within the same species group (Wojtowicz 1982). The former two species are likely sister taxa. In fact, Wojtowicz (1982) has suggested that *P. indiana* may not even be a distinct species due to its similarity with *P. lepida* and its variable morphology. At our study site, *P. indiana* was smaller than *P. lepida*, and the two species had some subtle yet consistent genitalic differences. Despite their similarity, there was still no cross attraction between them, confirming *P. indiana's* status as a distinct species, reproductively isolated from *P. lepida*.

Pheromones can have a significant influence on selection and speciation due to their importance in mating systems. Moreover, pheromones are frequently proposed as important elements to maintaining reproductive isolation between closely related congeners, especially those with minimal morphological differences. For example, in Minnesota two morphologically identical strains of the European corn borer do not interbreed due to the differences in the ratios of (Z)- and (E)-11-tetradecenyl acetate isomers present in their pheromones (Klun et al. 1973). Changing the Z:E ratio from 98:2 to 95:5 caused a significant decline in Z-strain capture (Bartels et al. 1997). Although this phenomenon has not been as well-studied in the Trichoptera, Löfstedt et al. (2008) did find that increasing the ketone to alcohol ratio in *Molanna angustata* pheromones decreased field capture rates. These studies demonstrate the effects on attraction of minor differences in pheromone composition. A similar phenomenon is likely occurring in the sympatric populations of the four *Pycnopsyche* species.

Considerable future research is necessary to further elucidate the pheromonal interactions between species of Trichoptera. Most importantly, quantifying the chemical composition of the *Pycnopsyche* pheromones will allow for more comprehensive field experiments of interspecific attraction. Furthermore, because pheromones relate directly to mating, they may constitute an important character set for phylogenetic analysis, especially for deciphering cryptic species such as *P. indiana* and *P. lepida*.

Acknowledgments

We thank T. McDevitt and J. McDevitt for allowing us access to our field site, and M.A. Nussbaum and R.M. Miller for laboratory assistance. Research costs were paid by Hillsdale College biology department funds. The valuable comments of A.J. Pytel and two anonymous reviewers improved earlier versions of the manuscript.

Literature Cited

Ansteeg, O., and K. Dettner. 1991. Chemistry and possible biological significance of secretions from a gland discharging at the 5th abdominal sternit [sic] of adult caddisflies (Trichoptera). Entomol. Gen. 15: 303-312.

Baker, T. C., H. Y. Fadamiro, and A. A. Cosse. 1998. Moth uses fine tuning for odour resolution. Nature 393: 530.

Bartels, D. W., W. D. Hutchison, and S. Udayagiri. 1997. Pheromone trap monitoring of Z-strain European corn borer (Lepidoptera: Crambidae): optimum pheromone blend, comparison with blacklight traps, and trap number requirements. J. Econ. Entomol. 90: 449–457.

Bergmann, J. 2002. Identifizierung und synthese flüchtiger inhaltsstoffe aus insekten. PhD Thesis, University of Hamburg, Deutschland. 1-201.

Bergmann, J., C. Löfstedt, V. D. Ivanov, and W. Francke. 2001. Identification and assignment of the absolute configuration of biologically active methyl-branched ketones from limnephilid caddis flies. Eur. J. Org. Chem. 16: 3175-3179.

Bergmann, J., C. Löfstedt, V. D. Ivanov, and W. Francke. 2002. Electrophysiologically active volatile compounds from six species of caddisflies. Nova Supplement. Entomologia 15: 37–46.

Bergmann, J., C. Löfstedt, V. D. Ivanov, and W. Francke. 2004. Identification and synthesis of new bicyclic acetals from caddisflies (Trichoptera). Tetrahedron Letters 45: 3669-3672.

Bjostad, L. B., D. K. Jewett, and D. L. Brigham. 1996. Sex pheromone of caddisfly *Hesperophylax occidentalis* (Banks) (Trichoptera: Limnephilidae). J. Chem. Ecol. 22: **103-121.**

Duffield, R. M. 1981. 2-nonanol in the exocrine secretion of the nearctic caddisfly, *Rhyacophila fuscula* (Walker). Proc. Entomol. Soc. Wash. 83: 60-63.

Holzenthal, R. W., R. J. Blahnik, K. M. Kjer, and A. L. Prather. 2007a. An update on the phylogeny of caddisflies (Trichoptera). pp. 143-153. *In*, J. Bueno-Soria, R. Barba-Alvarez, and B.J. Armitage (editors). Proceedings of the 12th International Symposium on Trichoptera. The Caddis Press. Columbus, Ohio.

Holzenthal R. W., R. J. Blahnik, A. L. Prather, and K. M. Kjer. 2007b. Order Trichoptera Kirby 1813 (Insecta), Caddisflies. *In*, Z.-Q. Zhang and W.A. Shear (editors). Linneaus Tercentenary: Progress in Invertebrate Taxonomy. Zootaxa 1668: 639-701.

Houghton, D. C. 2002. Pheromone use in *Pycnopsyche guttifer* (Walker) and *P. lepida* (Hagen) (Trichoptera: Limnephilidae): evidence for pheromonal dialects, pp. 47–54. *In* W. Mey (ed) Proceedings of the 10th International Symposium on Trichoptera. Nova Suppl. Entomol. 15.

Ivanov, V. D., and C. Löfstedt. 1999. Pheromones in caddis flies, pp. 149–156. *In*: H. Malicky and P. Chantarmongkol (eds): Proccedings of the 9th International Symposium on Trichoptera, Chiang Mai, 1998. Faculty of Science, University of Chiang Mai.

Jewett, D. K., D.L. Brigham, and L. N. Bjostad. 1996. *Hesperophylaxc occidentalis* (Trichoptera: Limnephilidae): electroantennogram structure-activity of sex pheromone component 6-methylnonan-3-one. J. Chem. Ecol. 22: 123–137.

JMP. 2002. JMP, The Statistical Discovery Software. User's Manual, Version 5. SAS Institute, Cary, NC.

Klun, J. A., O. L. Chapman, K. C. Mattes, P. W. Wojitkowski, M. Berzoa, and P. E. Sonnet. 1973. Insect sex pheromones: minor amount of opposite geometric isomer critical to attraction. Science 181: 661–663.

Larsson, M. C., and B. S. Hansson. 1998. Receptor neuron responses to potential sex pheromone components in the caddisfly *Rhyacophila nubila* (Trichoptera: Rhyacophilidae). J. Insect Physiol. 44: 189-196.

Löfstedt, C., B. S. Hansson, E. Petersson, P. Valeur, and A. Richards. 1994. Pheromonal secretions from glands on the 5th abdominal sternite of hydropsychid and rhyacophilid caddisflies (Trichoptera). J. Chem. Ecol. 20: 153-170.

Löfstedt, C., J. Bergmann, W. Francke, E. Jirle, B. S. Hansson, and V. D. Ivanov. 2008. Identification of a sex pheromone produced by sternal glands in females of the caddisfly *Molanna angustata* Curtis. J. Chem. Ecol. 34: 220–228.

Pherobase 2009. The pherobase: database of pheromones and semiochemicals. (http://www.pherobase.com/). Accessed 10 October 2009.

Resh, V. H., and J. R. Wood. 1985. Site of pheromone production in three species of Trichoptera. Aquat. Insects 7: 65–71.

Re**sh, V. H., G. A. Lamberti, and J. R. Wood. 1984.** Summary of biological studies with *Helicopsyche borealis* (Hagen) in a coastal California stream, pp. 315–319. *In*: J. C. Morse (ed): Proceedings of the 4th International Symposium on Trichoptera, Clemson, SC, 1983. Dr. W. Junk, The Hague.

Solem, J. O. 1985. Female sex pheromones in *Rhyacophila nubila* (Zetterstedt) (Trichoptera, Rhyacophilidae) and arrival pattern to sticky traps. Fauna Norvegica, Series A, 32: 80–82.

Solem, J. O., and E. Petersson. 1987. Demonstration of female sex pheromones and adult behavior in *Molanna angustata* (Trichoptera: Molannidae). Entomol. Gen. 12: 115–118.

Solem, J. O., and T. Solem. 1991. Mate location and pre-mating in *Apatania fimbiata* Pictet (Trichoptera: Limnephilidae). Aquat. Insects 13: 1–8.

Wojtowicz, J. A. 1982. A review of the adults and larvae of the genus *Pycnopsyche* (Trichoptera: Limnephilidae) with a revision of the *Pycnopsyche scabripennis* (Rambur) and *Pycnopsyche lepida* (Hagen) complexes. PhD thesis, University of Tenessee.

Wood, J. R., and V. H. Resh. 1984. Demonstration of sex pheromones in caddisflies (Trichoptera). J. Chem. Ecol. 10: 171–175.