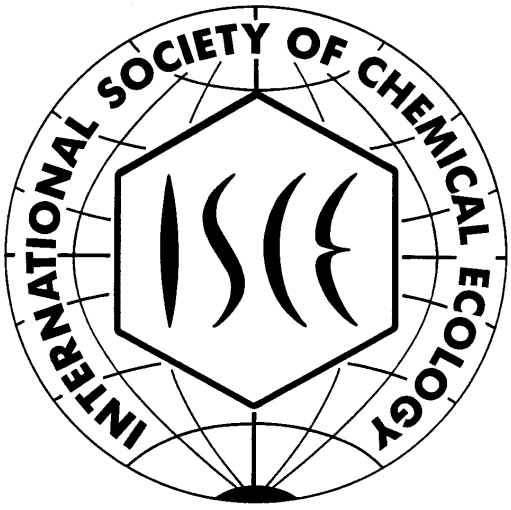

International Society of Chemical Ecology

18th Annual Meeting

Granlibakken Resort, Lake Tahoe

July 7-12, 2001

SCIENTIFIC PROGRAM AND ABSTRACTS

Meeting Host:	Gary J. Blomquist
Organizing Committee:	Jocelyn Millar
	Claus Tittiger
	John Hildebrand
ISCE President:	Walter S. Leal

Saturday, July 7

1:00-6:00 p.m. **Registration**
6:00 p.m. **Opening ceremony**

6:05-7:00 p.m. **Silver Medal Lecture sponsored by Fuji Flavor Co. Ltd.**
(Introduced by ISCE President: **Dr. Walter Leal**)

Dr. David Wood
Selection and Colonization Behavior of Tree-Killing Bark Beetles (Coleoptera: Scolytidae)- A Brief Progress Report

7:00-8:00 p.m. **Welcome reception** (cocktails, beer, wine)
7:30-9:00 p.m. **Dinner**

Sunday, July 8

7:30-8:30 a.m. **Breakfast**

SYMPOSIUM: FRONTIERS OF OLFACTORY RECEPTION, INFORMATION PROCESSING, AND NEUROETHOLOGY IN INSECTS

Organized and Chaired by **Dr. John Hildebrand**

8:30-8:45 **Dr. John G. Hildebrand** Introduction

8:45-9:10 **Dr. Ring T. Cardé** Chemical signals in the environment: influences of patterns of encounter on insects' behavioral responses

9:10-9:35 **Dr. R. Alexander Steinbrecht** Smelling proteins - localization of olfactory transduction proteins in moth and fly

9:35-10:00 **Dr. Bill S. Hansson** Function and central nervous projection patterns in insect olfactory receptor neurons

10:00-10:20 **Coffee Break**

10:20-10:45 **Dr. Giovanni Galizia** How are odors represented in the insect brain? Results from optical imaging studies

10:45-11:10 **Dr. Hanna Mustaparta** Imaging of glomerular activity and correlation with cellular recordings in moths

11:10-11:35 **Dr. Thomas A. Christensen** Into the minds of moths recording patterns of brain activity with multichannel silicon microelectrode arrays

11:35-12:00 **Dr. Neil J. Vickers** Poking a nose in where it doesn't belong: effects of interspecific antennal transplants on olfaction

12:00-12:10 **Dr. John G. Hildebrand** Summary and conclusions

12:10-1:30 **Lunch**

PHEROMONE BINDING PROTEINS/RECEPTION - Contributed papers

Chairperson: **Dr. Thomas Christensen**

1:30-1:45 **Dr. Erika Plettner** Structure and function of gypsy moth pheromone binding proteins

1:45-2:00 **Dr. Atle Wibe** Methyl-anisole, a non-host volatile, elicits large electroantennograms in the pine weevil (*Hylobius abietis*) and prevails over α-pinene in a laboratory bioassay

PRACTICAL USE OF PHEROMONES - Contributed papers

Chairperson: **Dr. Tom Baker**

2:00-2:15 **Dr. Rob van Tol** Application of insect-repellent odours in agriculture

2:15-2:30 **Dr. David R. Britton** Developing an attracticide for male cotton bollworm *Helicoverpa armigera* (Hübner)(Lepidoptera: Noctuidae): Visual and chemical cues

2:30-2:45 **Dr. Alan Cork** Development of an odor-baited trap for use in control of a vector of Chagas disease, *Triatoma infestans*

2:45-3:00 **Dr. John A. Pickett** New opportunities for commercial exploitation of insect olfactory reception

3:00:3:15 **Dr. Lara Maistrello** Repellency and toxicity of nootkatone to formosan subterraneaan termite *Coptotermes formosanus.*

3:15-3:30 **Coffee Break**

INSECT PHEROMONES -Contributed papers

Chairperson: **Dr. Jenny Fäldt**

3:30-3:45 **Dr. Karsten Seidelmann** The role of phenylacetonitrile in the olfactory system of the desert locust*, Schistocerca gregaria*

3:45-4:00 **Dr. Jocelyn Millar** Scents and songs: Sexual signaling in stink bugs (Hemiptera: Pentatomidae)

4:00-4:15 **Dr. Joachim Ruther** Make love not war: a common arthropod defense compound and a sexual kairomone mediate mate finding in the forest cockchafer *Melolontha hippocastani* Fabr. (Coleoptera: Scarabaeidae)

4:15-4:30 **Dr. Stefan Schulz** The semiochemistry of springtails

4:30-4:45 **Dr. John A. Byers** Moth pheromone components and constraints on their evolution

4:45-5:00 **Dr. Johan Andersson** Pierid communication systems: a theme with variations

5:00-5:15 **Dr. Stephen Teale** Semiochemical attractants for the Asian longhorn beetle

5:15-5:30 **Dr. Wittko Franke** Chemical signals at phylogenetic branching points

5:30-7:00 **Dinner**

7:00-8:00 **Silverstein-Simeone lecture sponsored by Kluwer Academic/Plenum Publishers**

Chairperson: **Dr. Jim Tumlinson**

Dr. Glenn Prestwich Molecular Motivation

Student Travel Awardees Presentations: Sponsored by Trécé and the USDA-NRI

Chairperson: **Dr. Walter S. Leal**

8:00-8:15 **Dr. Michael LeMaster** Evidence for a female sex pheromone mediating male mate choice in red-sided garter snakes (*Thamnophis sirtalis parietalis*)

8:15-8:30 **Dr. Florence Vincent** Mammalian odorant and pheromone binding proteins

8:30-8:45 **Dr. Valérie Campanacci** Insect pheromone binding proteins and chemosensory proteins: Functional and structural studies

8:45-9:00 **Dr. Raphaël Boulay** The source of recognition pheromone in the ant *Camponotus fellah*

9:00-9:15 **Dr. Dieter Spiteller** Gut bacteria are involved in the synthesis of elicitor-active compounds from lepidopterans

Monday, July 9

7:00-8:00 **Breakfast**

AQUATIC CHEMICAL ECOLOGY SYMPOSIUM

Organized and Chaired by **Dr. Niels Lindquist** and **Dr. Peter Sorenson**

8:00-8:05 **Dr. Niels Lindquist** Introduction

8:05-8:35 **Dr. Richard K. Zimmer** Chemical information flow in aquatic environments

8:35-8:40 **Dr. Peter Sorenson** The ecology of fish pheromones – an introduction

8:40-9:10 **Dr. Peter Sorenson** Fish migratory pheromones: A case study of the bile acid derived migratory pheromone in sea lamprey

9:10-9:40 **Dr. Norman Stacey** Fish sex pheromones: A case study of goldfish hormonal sex pheromones

9:40-10:10 **Dr. John J. Stachowicz** Chemical mediation of complex interactions among seaweeds, invertebrates, and their predators: consequences for marine communities

10:10-10:30 **Coffee Break**

10:30-11:00 **Dr. Erik Edward Sotka** The phylogeography of herbivore tolerance for chemically rich seaweeds

11:00-11:30 **Dr. Raymond M. Newman** Herbivore attractants and deterrents from aquatic macrophytes: Examples from watercress and watermilfoil

11:30-12:00 **Dr. Mark Hay** Chemical defenses of freshwater macrophytes: Contrasts with marine patterns

12:00-1:00 **Lunch**

1:00-1:30 **Dr. Elisabeth Maria Gross** Do macronutrients interfere with allelopathic interactions of submersed macrophytes with other primary producers?

1:30-2:00 **Dr. John Faulkner** The role of symbionts in the production of compounds found in marine invertebrates

2:00-2:30 **Dr. Niels Lindquist** Consequences of consuming chemically rich prey

2:30-3:00 **Coffee Break**

MARINE - Contributed Papers

Chairperson: **Dr. J. Jay Stachowicz**

3:00-3:15 **Dr. Ole B. Stabell** Latent alarm signals: a new approach to the study of chemical communication in aquatic environments

3:15-3:30 **Dr. Charles D. Amsler** Macroalgal defenses in the nitrogen replete, carbon limited Southern Ocean: No evidence for nitrogenous secondary metabolites

3:30-3:45 **Dr. Verena Jung** Chemical defense of the invasive green alga *Caulerpa taxifolia*

3:45-4:00 **Dr. Channing R. Jones** Hydroid defenses against predators: Importance of secondary metabolites versus nematocysts

4:00-4:15 **Dr. Valerie J. Paul** Chemical ecology and biodiversity of *Phestilla* nudibranchs

4:15-4:30 **Dr. A. Jennifer Mordue (Luntz)** Behavioral and electrophysiological responses of sea lice, *Lepeophtheiros salmonis* (Copepoda: Caligidae) to semiochemical stimuli

4:30-4:45 **Dr. K. Håkan Olsén** Salmon siblings school during downstream smolt migration

4:45-5:00 **Dr. Julia Kubanek** A new defense strategy against marine pathogens and saprophytes

5:00-7:00 **Dinner**

7:00-7:45 Social Lecture: **Dr. Wendell Roelofs** (Introduced by Tom Baker)
Wendell Roelofs: Scientist or Coach???

7:45-10:30 **Posters** with refreshments (cocktails, beer, wine)

Tuesday, July 10

7:00-8:00 **Breakfast**

SYMPOSIUM: SEMIOCHEMICAL TECHNIQUES

Organized and Chaired by **Dr. Jocelyn Millar**

8:00-8:30 **Dr. Wilhelm Boland** Thioglycosides: valuable molecular probes for the analysis of sequestration processes in leaf beetle larvae

8:30-9:00 **Dr. Claus Tittiger** Molecular approaches towards understanding insect pheromone biosynthesis and regulation

9:00-9:30 **Dr. Allard A. Cossé** From fly to flea beetle: Identifying non-lepidopteran semiochemicals with the aid of GC-EAD

9:30-10:00 **Dr. Robert J. Bartelt** Practical use of SPME for qualitative and quantitative analysis

10:00-10:30 **Dr. Ring Cardé** Video methods for behavioral bioassays and analyses

10:30-10:50 **Coffee Break**

Contributed papers

Chairperson: **Dr. Jeremey McNeil**

10:50-11:05 **Dr. Jeffrey R. Aldrich** Coevolved and non-coevolved *Telenomus* parasitoids of *Euschistus* spp. Stink Bugs: combining the best of North and South American Strains

11:05-11:20 **Dr. Stefano Colazza** Cues mediating host location in *Trissolcus basalis*: the role of volatiles induced by host eggs-plant interaction

11:20-11:35 **Dr. Emma Conquest** Novel techniques for unraveling the complex pheromone mediated biology of *Dermestes maculatus* Degeer

11:35-11:50 **Dr. Robin M. Crewe** Analysis of honeybee mandibular gland pheromones using silicone tubing

11:50-12:30 Special Lecture: **DR. CHARLES R. GOLDMAN**
Lake Tahoe: Moving beyond the conflicts through four decades of change

12:30-1:30 **Lunch**

1:30 Leave for boat tour of Lake Tahoe

2:00-4:00 **Tahoe Gal boat tour**

6:30-8:00 **Dinner**

8:00-9:30 Informal Workshop organized by:

Tom Baker and Christer Löfstedt
Practical Use of Pheromones

Wednesday, July 11

7:00-8:00 **Breakfast**

SYMPOSIUM: PHEROMONE PRODUCTION AND REGULATION

Organized and chaired by **Dr. Coby Schal**

8:00-8:30 **Dr. Wendell Roelofs** Pheromone biosynthesis: Desaturase genes

8:30-9:00 **Dr. Ada Rafaeli** PBAN regulation of lepidopteran pheromone production

9:00-9:30 **Dr. Russell Jurenka** Pheromone biosynthesis in moths

9:30-10:00 **Dr. Gary Blomquist** Regulation of pheromone production by ecdysteroids and JH

10:00-10:20 **Coffee break**

10:20-10:50 **Dr. Claude Wicker-Thomas** Production of cuticular pheromones of *Drosophila melanogaster* and related species

10:50-11:20 **Dr. Coby Schal** Synthesis and hemolymph transport of hydrocarbons and sex pheromones

11:20-11:50 **Dr. Desiree Vanderwel** Pheromone biosynthesis in the Yellow Mealworm Beetle

11:50-12:20 **Dr. Walter Soares Leal** Regulation of sex pheromone biosynthesis in scarab beetles

12:20-1:30 **Lunch**

Pheromone Production - Contributed Papers

Chairperson: **Dr. Erika Plettner**

1:30-1:45 **Dr. Stephen Foster** Two routes for pheromone biosynthesis in the moth, *Epiphyas postivittana* (Walker): Possible consequences for regulation

1:45-2:00 **Dr. Cheng-hua Zhao** PBAN regulation of sex pheromone biosynthesis in two moth species, *Dendrolimus punctatus* and *Operophtera brumata*

2:00-2:15 **Dr. Gregory M. Hall** *De novo* isoprenoid pheromone production occurs in midgut tissue in bark beetles

2:15-2:30 **Dr. Steven J. Seybold** A novel sex-specific and inducible monoterpene synthase activity associated with the bark beetle, *Ips pini* (Say)

2:30-2:45 **Dr. Jörg Degenhardt** The terpene synthase gene family in *Zea mays* and its role in defense against herbivory

2:45-3:00 **Coffee Break**

Hydrocarbons and Waxes in Chemical Communication

Chairperson and Introduction: **Dr. Jean-Luc Clément**

3:00-3:15 **Dr. Matthew D. Ginzel** Role of cuticular hydrocarbons in mate recognition of long-horned beetles

3:15-3:30 **Dr. Michael J. Greene** Chemically mediated interactions between native ants and the invasive Argentine ant: the role of cuticular hydrocarbons in species recognition

3:30-3:45 **Dr. Michael D. Breed** Discrimination of blends of honey bee recognition pheromones

3:45-4:00 **Dr. Anne-Geneviève Bagnères** Identification and assay of chemical recognition signals for scorpions (Scorpionida: *Hadrurus arizonensis*)

4:00-4:15 **Dr. Dangsheng Liang** Hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*

4:15-4:30 **Dr. Reinhard Jetter** Probing the plant surface: the composition of leaf epicuticular waxes and their potential effects on insects and fungi

Insect-Plant Interactions

Chairperson: **Dr. Christer Löfstedt**

4:30-4:45 **Dr. Monika Hilker** Insect egg deposition induces *Pinus* to "call" for egg parasitoids

4:45-5:00 **Dr. Jenny Fäldt** Compositional changes of monoterpene enantiomers in response to fungal inoculation in Scotts Pine

5:00-5:15 **Dr. Jonathon Gershenzon** Testing the defensive function of terpenoid resin in Norway Spruce (*Picea abies*)

5:15-6:30 **Business Meeting**

6:30-7:30 **Reception** (cocktails, beer, wine)

7:30 **Banquet**

Thursday, July 12

7:30-9:00a.m. **Breakfast and Departure**

POSTER PRESENTATIONS

Student Travel Awardees

1. **Mitchell L. Wise, Gregory L. Rorrer, Jason J. Polzin and Rodney Croteau** Characterization of a myrcene synthase from bioreactor cultures of the marine red alga *Ochitodes secundiramea.*

2. **Yongliang Fan, Jody Chase, Veeresh Sevala and Coby Schal** Hydrocarbon synthesis by oenocytes and its delivery by lipophorin to oocytes and cuticle of the German cockroach.

3. **Raimondas Mozuraitis, Ilme Liblikas and Anna-Karin Borg-Karlson** Parthenogenesis, calling behaviour and potential sex pheromone of the leafminer moth *Phyllonorycter emberizaepenella.*

4. **César Gemeno, Walter S. Leal and Coby Schal** Behavioral and electrophysiological responses of *Supella longipalpa* to sex pheromone stereoisomers.

5. **Göde Schüler and Wilhelm Boland** *Coronatine analoga* as molecular probes of the secondary plant metabolism.

Contributed Posters

6. **Robert T. Mason and Michael P. LeMaster** Occurrence and significance of pheromone variation among denning populations of red-sided gartner snakes (*Thamnophis sirtalis parietalis).*

7. **Sirlei Dias Teixeira, Beatriz Helena L.Noronha Sales Maia, Sandra Bos Mikich, Gledson V. Bianconi, Ana L. Lordello and Maria E. Stefanello** Interaction between frugivorous bats and *Piper gaudichaudianum* fruits.

8. **Jason E. Co, Tappey H. Jones, Hector D. Douglas III and William E. Conner** Identification of the citrus odor of a seabird (crested auklet: *Aethia criatatella).*

9. **A. Bily, C Malouin, C Ashekian, T. Durst, L.M. Reid, C. Regnault-Roger, J.T.Arnason and B.J.R. Philogène** Is *Helicoverpa zeae* survival in corn ears affected by *Fusarium graminearum* toxins?

10. **Michel Guillon, Ramon Albajes, Basil Maxomenos, Brigitte Frérot and Olivier Etcheparre** Mating disruption of Mediterranean corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidea) using sprayable formulations of pheromone in France.

11. **Ashraf M. El-Sayed and Josef Gödde** Precision delivery of semichemicals/olfactory stimuli using a sprayer.

12. **Walter S Leal, J. Mauricio S. Bento, Yasuhiro Murata, Miikio Ono, José R. P. Parra and Evaldo F. Vilela** Identification, synthesis and field evaluation of the sex pheromone of the citrus fruit borer *Ecdytolopha aurantiana.*

13. **P.-O. Christian Olsson, Camilla Ryne, Peter G.Valeur, Mats Ekeberg and Christer Löfstedt** Water as an attractant for stored product moths revisited a comparative study including *Ephestia cautella* and *E. kuehniella.*

14. **Imen Said, Pamela Ramiez-Lucas, Rosa Aldana de la Torre, Rolletha Purba and Didier Rochat** Development of olfactometric tool to study behavioral response of large size insects.

15. **Geraldine A. Wright, Bethany D. Skinner and Brian H. Smith** How insects pollinators analyze floral odors.

16. **Neil J. Vickers** Heritable characteristics influence olfactory discrimination by hybrid moths.

17. **Ylva Hillbur and Jan Löfqvist** Male pea midge, *Contarinia pisi,* response to stereoisomers not produced by the females: behavioral and electrophysiological studies.

18. **Tadashi Nemoto, Tatsuya Nakano, Yuto Komeiji, Kazuo Kitaura, Uebayasi Masami, Yasuko Ishizuka, Kenji Kanazawa, Walter Soares Leal and Hiroshi Nakanishi** Computational approach for pheromone-pheromone binding protein interaction.

19. **Alexandre Quintana, Robert Faure, Judith Reinhard, Anne-Geneviève Bagnères and Jean-Luc Clément** New approach - new products : HPLC purification and micro quantity NMR analysis of termite defensive secretions.

20. **Judith Reinhard, Alexandre Quintana, Leam Sreng, Anne-Geneviève Bagnères and Jean-Luc Clément** Worker, soldiers, frontal glands: communication of alarm and defense in European termites of the genus *Reticulitermes.*

21. **Camilla Ryne, P.-O. Christian Olsson and Christer Löfstedt** Behaviour and possible production sites of male emitted sex pheromones in *Tribolium confusum*.

22. **Ellen M. Santangelo, Luciana G. B. Pereora, Kathrin Stein, Alvaro E. Eiras, C Rikard Unelius and Arlene G. Correa** Electrophysiological studies and identification of possible sex pheromone components of three different Brazilian populations of the sugar-cane borer *Diatraea saccharalis.*

23. **Brian T. Sullivan** Evidence for a sex pheromone in the bark beetle parasitoid *Roptrocerus xylophagorum* (Hymenoptera: Pteromalidae).

24. **Glen P. Svensson, Camilla Ryne and Christer Löfstedt** Heritable variation of sex pheromone production and the potential for resistance evolution to pheromone-based mating disruption of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae).

25. **Pavlína Vrkocová, Blanka Kalinová, Oldfiich Hovorka, Jifií Kindl, Irena Valterová and Bohumír Koutek** Absolute configuration of two active components from *Scolytus intricatus* abdominal extract.

26. **Oliver Walenciak, Andreas Brune and Elisabeth M. Gross** Gut conditions of Tannin-tolerant Larvae of *Acentria ephemerella* (Lepidoptera: Pralidae).

27. **Ilme Liblikas, Anna-Karin Borg-Karlson, Enno Mõttus** Specificity in host odour attraction of co-existing beetles in the genera *Phyllotreta* and *Meligethes.*

28. **Keiich Honda, Ken'ichiro Muta, Hisashi Ômura and Nanao Hayashi** Oviposition stimulants for the sulfur butterfly, *Colias erate:* D(+)-pinitol as a host recognition chemical.

29. **Thomas Hartmann and Jacques Pasteels** Independent evolution of pyrrolizidine-alkaloid-mediated exocrine defense in palaearctic and neotropical leaf beetles.

30. **Stephen Foster, Megan Noll and Shareon Grugel** Chemical basis for host selection in the banded sunflower moth, *Cochyis hospes* Wallsingham and the sunflower moth, *Homoeosoma electellum* Hulst.

31. **Lily Falach and Arnon Shani** Removal and restoring of sexual activity in the *Maladera matrida* beetle.

32. **Allard A. Cossé, Robert J. Bartelt and Bruce W.Zildowski** Identification of the male-specific aggregation pheromone of the ceral leaf beetle (*Oulema melanopa*, Coleoptera: Chrysomelidae).

33. **Robert J Bartelt, Allard A. Cossé, Bruce W.Zilkowski, David Weisleder and Frank A. Momany** Male-specific sesquiterpenes from *Aphthona* and *Phyllotreta* flea beetles.

34. **Jocelyn G. Millar and Diane Hinkens** Identification and synthesis of the sex pheromone of the vine mealybug , *Planococcus ficus.*

35. **Aijun Zhang and Miguel Borges** Identification of a species-specific male-produced pheromone blend for neotropical brown stink bug, *Euschistus heros.*

36. **Luciane G. B. Pereira, Kathrin Stein, André F. de Paula, Jardel A. Moreira, Ivan Cruz, José Perri Jr. and Arlene G. Correa** Sex pheromone studies for the Brazilian population of the fall armyworm *Spodoptera frugiperda.*

37. **G. S. Wheeler, L. M. Massey and I. A. Southwell** Anti-predator defense of the biological control agent *Oxyops vitiosa* is mediated by plant volatiles sequestered from their host plant *Melaleuca quinquenervia.*

38. **Stefan Schulz, Markus Müller and Anthony S. Clare** Configuration of Trioxilin A: the presumed egg-hatching factor of the barnacle, *Eliminus modestus*.

39. **Christine Loughrey and Peter Daniel** Chemical deterrents of lobster feeding behavior.

40. **Oliver Walenciak, Walter Zwisler and Elisabeth M. Gross** Influence of freshwater macrophytes on epiphytic bacterial communities.

41. **Peter W. Sorensen** Juvenile Hawaiian gobiid fish employ odor cues to locate freshwater streams from the ocean and to guide them up their terminal waterfalls.

42. **A. Sahakyan, G. Pirumyan , V. Marukhyan and G. Torosyan** The catalytic activity of the bottom sediment regarding H_2O_2 reactions.

43. **A. Sahakayan, G. Pirumyan, E. Pirumyan, V. Marukhyan, G. Torosyan** The water quality of Lake Sevan/ Armenia/.

44. **Kristen Whalen, Julia Kubanek, Sebastian Engle, Timothy P. Henkel and Joseph R. Pawlik** Optimizing resources in marine sponges: evidence for multiple defense roles of triterpene glycosides.

45. **Jerome Naar, Julia Kubanek, Andrea J. Bourdelais and Daniel G. Baden** Detoxification of brevetoxins by molluscs via sulfide-linked derivatives.

46. **Anna Luxová, Irena Valterová, Alex Svatos, Krel Stránsk and Oldrich Hovorka** Biosynthetic studies on marking pheromones of the bumblebee male.

47. **Andrea Eigenheer, Gary J. Blomquist and Claus Tittiger** Genomics of pheromone biosynthesis in *Ips pini.*

48. **Jeremy Bearfield, Gary J. Blomquist and Claus Tittiger** Determination of the *Dendroctonous jeffreyi* and *Ips pini* HMG-R promoter regions.

49. **A. Huma Nural, Claus Tittiger, William Welch and Gary Blomquist** Isolation and characterization of isoprenyl diphosphate synthase from cotton boll weevil.

50. **Julie A. Tillman, Fang Lu, Zoe Donaldson, Silver C Dwinell, Claus Tittiger, Gregory M. Hall, Gary J. Blomquist and Steven J. Seybold** Biochemical and molecular aspects of the regulation of pheromone biosynthesis in pine bark beetles (*Ips spp.*).

51. **Anna Young, Claus Tittiger, William Welch and Gary J. Blomquist** Monoterpenoid pheromone biosynthesis: fishing for the elusive geranyl diphosphate synthase in bark beetles.

52. **Judith X. Becerra, and Philip H. Evans** Chemical characterization of the genus *Bursera* (Burseraceae).

53. **Phillip H. Evans and William S. Bowers** Aroma constituents of the medicinal plant *Ligusticum porteri.*

54. **Monika Persson and Anna-Karin Borg-Karlson** Stress induced changes in monoterpene composition in *Pinus sylvestris*.

55. **Tadanobu Nakayama, Keiicho Honda, Hisashi Ômura and Namao Hayashi** A new oviposition stimulant for *Papilio polytes* from its major host pant, *Toddalia asiatica* (Rutaceae).

56. **Ursula S. R. Röse and Jonathan Gershenzon** Induction of terpenoid volatiles released in response to insect herbivory in cotton plants.

57. **Kelsey R. Downum, David Lee, Francis Hallé, J. E. Martin Quirke, Zyta Abramovsky, Sandrine Calvez, Emily Catonwine, Sabine Sabine and G.H. Neil Towers** Antimicrobials from tropical rain forest plants in Gabon.

58. **Chul-S Kim, Probal K. Datta, Tetsurou Hara, Martey J. Quaye, Shin-ichi Tebayashi and Michio Horiike** Insecticidal components in *Spiraea thunbergii* (Rosaceae).

59. **Kirsten A. Copren, Lori J. Nelson, Casey Delphia and Michael I. Haverty** The role of cuticular hydrocarbons in nestmate recognition of the subterranean termite, *Reticulitermes hesperus.*

60. **Michael I. Haverty, Gail M. Getty, Lori J. Nelson and Vernard R. Lewis** Reproductive isolation between sympatric cuticular hydrocarbons phenotypes of *Reticulitermes* in northern California.

61. **Dennis R. Nelson** Novel trimethyl-branched hydrocarbons in *Helicoverpa zea* pupae.

62. **Lori J. Nelson, Laurence G. Cool and Michael I. Haverty** Cuticular hydrocarbons and soldier defense secretions of *Reticulitermes* from southern California.

63. **Marion Page, Lori Nelson and Michael Haverty** Cuticular hydrocarbons of *Reticulitermes* from North America: analysis of character evolution in phylogenetic studies support three monopyletic lineages.

64. **Mitko Subchev and Russell Jurenka** Pheromone production in moths utilizing hydrocarbons.

65. **Carina Eriksson, Olof Smitt, Fredrik Schlyter, Kristina SjÖdin and Hans-Erik Högberg** Antifeedants for protection of pines and spruces against the pine weevil, *Hylobius abietis*.

66. **Nicole M. Kalberer, Heather L. Stein, Wendy L. Mechaber and John G. Hildebrand** Trans-sexual antennal transplants alter sex specific host plant-approach behavior in a moth.

67. **Martin Wilt, George T. Ferrell, William Otrosina, David L. Wood and Eugene Zavarin** Conifer moisture relations, genetics, and the production of defensive terpenoids in response to an insect-transmitted fungal pathogen.

68. **Jeremy N. McNeil and Joanne Delisle** Are there costs to delayed sexual maturation in response to habitat deterioration?

69. **Christer Löfstedt, D. Plepys, Fernando Ibarra, Bill S. Hansson and Wittko Francke** Odour-mediated nectar foraging in the Silvery moth, *Autographa gamma.*

70. **Claudia Naumann, Thomas Hartmann and Dietrich Ober** Senecionine N-oxygenase: a specific detoxifying enzyme of lepidopterans adapted to pyrrolizidine alkaloids: cloning, expression and characterization.

71. **Joachim Ruther, Lars Podsiadlowski and Monika Hilker** Sex attractants of cockchafers: quinone titers in adults and grubs, release rates and antimcrobial properties (Coleoptera: Scarabaeidae).

72. **Guihong Peng and Walter Soares Leal** Identification and cloning of a pheromone-binding protein from the oriental beetle, *Exomala orientalis*

73. **Hiromi Sasagawa, Y.-J. Hua and F.-L. Hu** Co-evolution of plant and insect: the oriental orchid (*Cymbidium floribundum* Lindl.*)* controls the social behavior of the Asian Honey bee?

Abstracts

Selection and Colonization Behavior of Tree-Killing Bark Beetles (Coleoptera: Scolytidae)- A Brief Progress Report

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Bark beetles colonize a great diversity of woody plants growing in the forests of the world. A small number of species are adapted to kill a living tree, while most are "opportunists" and colonize dead and dying trees or tree parts. Research over the past half-century gives considerable insight into the behavioral, physiological and biochemical processes that mediate host selection and colonization behavior by these bark beetles. Scolytids (Coleoptera) play an important role in recycling of nutrients from woody plants, as the adult can penetrate the tough outer bark and thereby introduce, either directly or indirectly, microorganisms that digest cell wall constituents that comprise the oldest living organisms on earth. All of the trees' woody tissues and structures are selectively colonized by bark beetles. For example, a ponderosa pine growing next to Granlibakken is colonized by the following taxa:

Cones: *Conophthorus ponderosae*
Twigs and shoots: *Pityophthorus confertus*
Trunk
Outer bark: *Dendroctonus brevicomis*
Phloem: *D. brevicomis, D. ponderosae, D. valens, Ips paraconfusus, I. pini*
Xylem: *Gnathotrichus sulcatus*
Roots: *D. valens, Hylastes macer*

The colonization behavior of tree-killing bark beetles, such as the western pine beetle (*D. brevicomis*), has been divided into four phases: dispersal, selection, concentration and establishment. All phases have been shown to be mediated by semiochemicals that function as pheromones, kairomones and allomones. As will become evident, this is a story with many contributors from many disciplines and organizations and is a story primarily about collaboration among students and colleagues. Behavioral work initiated in the 1950's resulted in the identification of ipsenol (2-methyl-6-methylene-7-octen-4-ol), ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), and *cis*-verbenol (*cis*-4,6,6-trimethyl bicyclo[3.1.1]-hept-3-en-2-ol) from the California five-spined ips in 1966 by Robert "Milt" Silverstein and colleagues. These terpene alcohols comprised a multi-component aggregation pheromone, all of which were required to elicit attraction to the tree under attack. Surprisingly, none of these compounds were active alone. This invasion by thousands of beetles inoculates the phloem and xylem with pathogenic fungi which results in the death of the tree. Following the death of the tree, the female excavates galleries in the phloem and deposits her eggs in niches in the gallery wall. Through serendipity we discovered that these compounds attracted a bark beetle predator (kairomone) and interrupted the response of a competing bark beetle species (allomone). Later research lead to discoveries about: 1) how the first arriving beetles ("pioneers") detect trees weakened by root disease (*D. valens, D. brevicomis*) and pitch canker (*Pityophthorus* spp); 2) the role of the vectored bluestain fungi in interrupting the flow of water in the sapwood, resulting in the death of the tree; 3) the role of feeding behavior and sound production (stridulation) in colonization behavior; 4) pheromone biosynthesis; and 5) pheromone composition of allopatric and sympactric *Ips* species.

Many exciting discoveries lie ahead for the next generation of chemical ecologists who undertake studies of this fascinating group of insects.

Chemical signals in the environment: influences of patterns of encounter on insects' behavioral responses.

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Odor molecules released into wind form a plume as they are carried away from the odor's source. Molecular and especially turbulent diffusion shapes the odor plume. As the plume meanders downwind, these forces create an expanding, filamentous structure within the plume. Gaps between filaments expand and the filaments are diluted. The effect is that an insect would encounter an odor as a series of discrete bursts varying in concentration and spacing. In flying moths, the pattern of moment-to-moment encounter with filaments of pheromone within the plume dictates the how the moth orients. Rates of 5 Hz promote a zigzag path upwind, and rates of 10 Hz or more can induce a rapid flight that is aimed nearly straight upwind. At the antennal level, rates in excess of 30 bursts/second may be detected, but such high rates do not seem to be preserved in subsequent levels of processing. The value of the plume's fine scale structure in orientation of moths to pheromone seems wide spread across long-diverged lineages, but the role of such information in other systems is not well explored. The fine-scale features of a plume of CO_2 seem important to upwind orientation of some female mosquitoes. The importance of plume structure in behavioral reactions, peripheral reception, and subsequent processing is an emerging area of inquiry.

SMELLING PROTEINS - LOCALIZATION OF OLFACTORY TRANSDUCTION PROTEINS IN MOTH AND FLY.

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Biochemistry has in the last two decades contributed enormously to our understanding of insect olfaction by the characterization of a great variety of transduction proteins which are thought to be involved either in perireceptor processes or intracellular transduction cascades. Finally also several putative olfactory receptor proteins belonging to the well-known family of G-protein coupled receptor proteins have been described in *Drosophila.*

Insect olfactory organs are highly compartmentalized and each sensillum is a separate miniature sensory organule. This special trait has been appreciated by the name "compound nose" in analogy to the insect compound eye and has been effectively exploited by combining electron microscopy and electrophysiology for the characterization of structural and functional sensillar subtypes. Biochemistry and molecular biology, by the very nature of their methodology, cannot directly take advantage of this high compartmentalization and identified proteins can only be very roughly localized, e.g., as antenna-specific. However, when antibodies against such proteins are available, localization is possible down to the subcellular level.

In this paper the present status of knowledge is reviewed as to the expression patterns of 4 OBPs in the silkmoth, *Bombyx mori,* and of 5 different OBPs in *Drosophila.* In both species the emerging mosaic, albeit highly complex, shows many features in common and allows for several generalizations: (i) OBP expression does correlate with morphological sensillum types and subtypes; (ii) several OBPs may be co-localized in the same sensillum; (iii) OBP localization is not necessarily restricted to olfactory sensilla.

The distribution pattern of proteins involved in intracellular signalling is much more uniform. Very recently also the first olfactory receptor protein has been successfully localized on the membrane of the sensory dendrites of a certain morphological sensillum subtype in *Drosophila.* Thus, immunoelectron microscopy eventually will build the bridge between morphologically and electrophysiologically characterized sensillum types on one side and the various protein components decyphered by biochemistry and molecular biology on the other.

FUNCTION AND CENTRAL NERVOUS PROJECTION PATTERNS IN INSECT OLFACTORY RECEPTOR NEURONS

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To perform odor-dependent behaviors, insects rely on a sensitive and selective detection apparatus. Olfactory receptor neurons perform the primary task of molecular detection and distribute the neural message to the glomerular array of the antennal lobe. Among neurons detecting sex pheromones a very high specificity has been demonstrated in most insect species investigated. This high specificity is sometimes also encoutered in neurons detecting behavioral antagonist and host plant odors. However, among the latter two classes the specificity has sometimes been sacrificed to allow a broader detection, which in some circumstances can be argued to be favorable. A broad spectrum can allow a single neuron to detect a number of odors having the same behavioral significance. The same broad spectrum can also allow for combinatorial coding of a higher number of odors, a coding type that has been identified in optical imaging of receptor neuron input into the antennal lobe.
Male receptor neurons detecting female sex pheromone components project to the male-specific macroglomerular complex. From experiments using activity-dependent staining of receptor neurons it was clear that neurons displaying different specificities distributed their axonal branches into the different glomeruli of the macroglomerular complex in an odotopic fashion. The odotopic pattern has now been corroborated in a number of species by the same technique, by optical imaging and recently also by molecular methods. Receptor neurons expressing a certain olfactory receptor type all project to the same glomerulus.

HOW ARE ODOURS REPRESENTED IN THE INSECT BRAIN? RESULTS FROM OPTICAL IMAGING STUDIES

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In insects the olfactory receptor axons project to the antennal lobes, where the synapses with the central neurons are confined in glomerular units. These can be identified on the basis of their shape and relative position. The glomeruli are interconnected by local interneurons, and from the glomeruli projection neurons lead to higher order brain centres. After labelling all neurons by bath-applying a calcium-sensitive dye, it is possible to measure odour-evoked glomerular activity patterns. In honeybees (*Apis mellifera*), these studies show that each odour elicits a mosaic of activated glomeruli, and that each glomerulus can take part in the mosaic of several odours. Furthermore, responses are graded: a glomerulus may be weakly activated by one odour, and strongly by another (or by a higher concentration of the same odour). Mapping physiological responses to morphologically identified olfactory glomeruli shows that these are genetically determined, allowing for the creation of a functional atlas. From these data, predictions can be made about the perceptual odour space in honeybees. We have validated these predictions using a behavioural assay: free-flying bees were trained to an odour, and then tested against a panel of 48 optically identical feeders which only differed in their odour. The results show that the only odours that are mistaken in the behavioural analysis are those with strongly overlapping glomerular patterns.

By selectively staining projection neurons with calcium-sensitive dyes we have measured the net outcome of the antennal lobe network. We have measured both single neurons and population-fills. The activity patterns in the projection neurons are spatially more restricted than those in all antennal lobe neurons, i.e. for a given odour they involve less glomeruli. Furthermore, they are temporally more complex, often involving alternating phases of inhibitory and exitatory responses. These response properties are equal for different individuals, and depend on the innervated glomerulus and the tested odour.

IMAGING OF GLOMERULAR ACTIVITY AND CORRELATION WITH CELLULAR RECORDINGS IN MOTHS.

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Information about insect and plant produced odours are processed in separate glomerular systems in the antennal lobe of moths. The male specific macroglomerular complex MGC in *Heliothis virescens,* receiveing information about insect produced signals, consists of four units. As demonstrated by electrophysiological recordings combined with stainings, each unit seems to receive projections of receptor neurons tuned to one compound; the cumulus and the dorso-medial compartment from neurons tuned to the two principal pheromone components and the two ventral compartments from neurons tuned to two interspecific signals. Receptor neurons tuned to specific plant produced compounds have been demonstrated. However, their projections in the antennal lobes are not yet investigated.

By the use of optical recordings (Ca++-green), we have studied the activity pattern of glomerular units in the antennal lobe during stimulation with the four insect produced and some plant produced odorants. The activity patterns elicited by the single insect produced compounds corresponded with the electrophysiological data at simmlar concentration range. Activity was mainly obtained in the area of cumulus and the dorso-medial compartment when stimulating with the major and the secondary pheromone component, respectively, and in the ventral part of MGC when stimulating with one of the interspecific components. Stimulation with plant odours elicited strong responses in the area of the ordinary glomeruli both in females and males, but not in the MGC. Different plant odours elicited activity patterns showing an across-glomerular code.

INTO THE MINDS OF MOTHS: RECORDING PATTERNS OF BRAIN ACTIVITY WITH MULTICHANNEL SILICON MICROELECTRODE ARRAYS

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The computations used in the brain to discriminate different odorants have been the subject of active inquiry for many years, but the details of these neural operations remain unknown. In this symposium we have seen how important new methods like optical imaging can reveal patterns of functional activation in the brain, and how different odorants often evoke distinct spatial activity patterns across an array of olfactory glomeruli. Sometimes, however, different odorants will trigger spatial patterns that are very similar. What mechanisms does the brain use to discriminate them? What specific neural interactions underly these activity patterns? We have developed a method using a 3-pronged silicon microelectrode array to record odor-evoked spike activity simultaneously from ensembles of up to several dozen neurons innervating multiple glomeruli in the moth antennal lobe. Our studies revealed a number of surprising findings: 1) a single odorant can evoke many different patterns of ensemble activity, and these patterns are context dependent; 2) As expected, different neurons innervating a particular glomerulus always display similar stimulus tuning, but *blends* of odorants sometimes evoke patterns that cannot be predicted from the responses to individual odorants; 3) While *spatial* patterns evoked by different odorants may show substantial overlap, our data show that the *timing* underlying these patterns is odor specific -- each odorant evokes synchronous firing in only a subset of neurons in the complete ensemble. Synchrony is furthermore very reproducible from one odor pulse to the next. These results lead to the following hypothesis: in order to recognize a single odorant embedded in a complex olfactory space with many scents blended together, the brain does not simply pay attention to *which* neurons in the glomeruli fire in response to that odorant. It also uses information about which ones fire *together* with each "sniff", thus further reinforcing subtle differences in the brain patterns evoked by the different odorants present.

POKING A NOSE IN WHERE IT DOESN'T BELONG: EFFECTS OF INTERSPECIFIC ANTENNAL TRANSPLANTS ON OLFACTION

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Information about the volatile, chemical world is first processed in the brain within olfactory glomeruli, structures common to widely divergent animal taxa. Male moths possess a sexually dimorphic subset of glomeruli (collectively known as the macroglomerular complex, MGC) that play a role in discriminating between conspecific and heterospecific female odor blends. In two species of heliothine moth, *Heliothis virescens* and *Helicoverpa zea*, the morphological arrangement and numbers of MGC glomeruli in adult males are distinct, reflecting important differences in physiological properties of sensory afferents, central interneurons and, ultimately, the behavioral preferences of these two species. The glomerular organization of the antennal lobe (AL) is established during metamorphosis by the interaction of in-growing sensory afferents derived from the larval antennal imaginal disk and central neuronal tissues. Transplantation of imaginal disks, first performed between male and female *Manduca sexta* (Schneiderman et al., 1984), provided a technique for challenging the 'host' brain with a novel array of 'donor' sensory fibers during adult development. We are transplanting imaginal disks between *H. virescens* and *H. zea* males to further our understanding of the functional role of glomeruli in olfactory processing and discrimination of odor (pheromone) blends in these two species. Cross species transplant males produced MGCs that appeared to bear the morphological and neurophysiological characteristics of a normal donor AL, yet the behavioral responses of such males often differed from both normal *H. zea* and *H. virescens* males.

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STRUCTURE AND FUNCTION OF GYPSY MOTH PHEROMONE BINDING PROTEINS

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The gypsy moth, *Lymantria dispar*, uses 7(*R*), 8(*S*)-*cis*-2-methyl-7,8-epoxyoctadecane, (+) disparlure, as the major component of its sex attractant. The enantiomer, (-) disparlure, is a powerful antagonist. Pheromone components and antagonists are detected by specialized sensory hairs on the antennae of male moths. Pheromone binding proteins (PBPs) are small soluble proteins found abundantly in the lymph of the pheromone sensory hairs. The gypsy moth has two PBPs, which have been shown to discriminate the enantiomers of disparlure in a subtle manner. The function of the PBPs is not well understood. They appear to be responsible for transport of the hydrophobic pheromone through the aqueous lymph. We have investigated in detail two aspects of this process: desorption of pheromone from hydrophobic surfaces and the effect of pH, ionic strength and disulfide reductants on pheromone binding. A second question that remains unanswered is whether the subtle odorant discrimination exhibited by PBPs is of significance in the overall olfactory process. The pH-dependence of pheromone binding and studies with pheromone analogs have revealed a number of important amino acid residues involved in chiral discrimination. Studies with pheromone analogs have revealed that PBPs compensate unfavourable protein/ligand interactions with favourable ones. This observation may explain the apparent flexibility of PBPs towards structurally related compounds: there is molecular discrimination, but it does not show in the dissociation constants.

METHYL-ANISOL, A NON-HOST VOLATILE, ELICIT LARGE ELECTROANTENNOGRAMS IN THE PINE WEEVIL (*Hylobius abietis*) AND PREVAIL OVER α-PINENE IN A LABORATORY BIOASSAY.

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The pine weevil (*Hylobius abietis*) feeds as adults on the bark of conifers, causing great damage on seedlings of spruce (*Piecea abies*) and pine (*Pinus sylvestris*) in reforestation areas in northern and middle Europe. Attraction caused by volatiles emitted from the host is well documented. In the search for which chemicals are influencing the behaviour of the pine weevil, it has been employed gas chromatography linked to electrophysiology as well as behavioural bioassays. These studies have shown that the pine weevil has at least 30 types of olfactory receptor neurons detecting 40-50 chemicals. Attraction has been shown to α-pinene and ethanol acting as synsergists in the field.

The present study includes gas chromatography linked to recordings of EAGs and behavioural tests in a simple laboratory bioassay. When testing on the pine weevil antennae the essential oil of the non-host Ylangilang via the gas chromatograph, a large EAG was recorded as response to the component *para*-methyl-anisol. This compound was not present in the principal host materials previously tested. The results were confirmed by stimulating the antennae with the three different isomers of methyl-anisol. In the bioassay, testing attraction to different chemicals, it was found that the methyl-anisol isomers had a relative strong attractive effect on the pine weevil. In many experiments designed to test the preference for the methyl-anisol isomers contra (+)-α-pinene, it was found a stronger attraction to methyl-anisol. The biological significance of methyl-anisol as a chemical cue for the pine weevil is not yet clear.

APPLICATION OF INSECT-REPELLENT ODOURS IN AGRICULTURE

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Phytophagous insects use plant odours to locate host plants and to assess the suitability of plants as food source. The importance of odours for insects to discriminate between host and non-host plants makes interference in the process of host-plant selection possible through using plant odours to mislead insects. Application of repellents, *e.g.,* non-host odours, on host plants can be used to avoid colonisation by pest insects and, consequently, protects the crop. For three pest species we developed different control strategies with repellent odours and tested this in field experiments.

Aphids (*Macrosiphum rosae* and *Macrosiphum euphorbiae*) in roses
Roses growing in the field, were sprayed weekly with slow-release formulations of repellents in mineral oil or in microspheres. With several repellents the level of aphid control was similar to the control with the insecticide imidacloprid.

Red bud borer (*Resseliella oculiperda*) in apple stock
This gall midge deposit eggs in graft slits or cuts in the bark of newly budded stock. The hatching larvae feed on the sap in the cambium between scion and stock for 2 – 3 weeks, thereby preventing taking of grafts or buds. Incorporation of one of the tested repellent odours in the budding tape reduced egg laying of the gall midges with 95% compared to a control.

Gladiolus thrips (*Thrips simplex*) in Gladiolus corms

This thrips species causes damage to gladiolus corms during storage. Dipping of the corms, before storage, in several formulations of an effective repellent prevented damage and strongly reduced the development of thrips populations. Pest control by the repellent was similar to the control with the insecticide imidacloprid.

DEVELOPING AN ATTRACTICIDE FOR MALE COTTON BOLLWORM *Helicoverpa armigera* (Hübner) (LEPIDOPTERA: NOCTUIDAE): VISUAL AND CHEMICAL CUES

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Attract and kill formulations are ideal tools for inclusion in IPM strategies to control insect pests, as they can be targeted at a single pest species without affecting beneficial insects, or creating secondary pest outbreaks. Initial field studies with attract and kill formulations using sex pheromone for *H. armigera* male moths indicate that development of a successful formulation will require consideration of a range of behavioural factors. The rate of contact with lures can be significantly increased by inclusion of a dead female *H. armigera* as a visual cue, but it is difficult to mimic these cues with the formulation. Observations in the field indicate that there are no deterrent effects caused by inclusion of a pyrethroid insecticide in the pheromone formulation. The possibility of missing components in the pheromone blend and the future prospects of using attracticides to control *H. armigera* are discussed.

DEVELOPMENT OF AN ODOUR-BAITED TRAP FOR USE IN CONTROL OF A VECTOR OF CHAGAS DISEASE, *TRIATOMA INFESTANS*.

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Chagas' disease is an endemic problem in South America that affects more than 16 million people. The disease causing flagellate, *Trypanosoma cruzi,* is mainly transmitted through feces deposited on potential hosts while vectors, such as *Triatoma infestans* (Hemiptera: Reduviidae), are blood feeding. Intradomiciliar infestations of *T. infestans* and other vectors in rural communities are currently controlled through coordinated fumigation programs with pyrethroid insecticides. The efficacy of such control campaigns is monitored using unbaited insect refuges and manual searching, both insensitive and time-consuming procedures when population densities are low. There is considerable evidence in the literature to suggest that *T. infestans* produce a range of semiochemicals that could be used as the basis for an odour-baited trap that would improve the sensitivity and efficiency of population survey work. We report the results of our research to identify compounds in feces that attract and arrest conspecifics, the sex pheromone produced in copula that attracts male *T. infestans* and compounds present in, and released from, Brindley's gland that are thought to elicit alarm and defensive responses from conspecifics and predators respectively. The results of this work, together with work on trap design and controlled release formulations have enabled the development of an effective odour-baited trap that is currently undergoing pre-field trials in Argentina as a prelude to large-scale trials in Paraguay. Ethical problems associated with the use of attractant baits for trapping disease vectors in domestic environments will also be briefly discussed.

NEW OPPORTUNITIES FOR COMMERCIAL EXPLOITATION OF INSECT OLFACTORY RECEPTION

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Insect olfactory receptor systems offer high sensitivity to specific molecular structures, even in the presence of homologous or analogous compounds at many orders of magnitude greater concentrations. This is a consequence both of the selectivity of individual olfactory neurons, even for ubiquitous compounds such as semiochemicals involved in host location, and of the neurophysiological integration of such responses within the insect central nervous system, resulting in motor neuronal responses. The wide repertoire of natural products, to which insects respond selectively, allows detection of commercially or legally significant phenomena such as onset of food deterioration, food flavour quality and the illegal presence of natural products, e.g. plant-derived drugs, and organisms such as human beings and other higher animals. Certain insects, particularly the Hymenoptera, can 'learn' to respond to unnatural stimuli, widening the detection spectrum to include synthetic molecular indicators, e.g. from explosives. Using specific examples with the fruit fly *Drosophila melanogaster* and the blowfly *Calliphora vomitoria*, we show the value of single olfactory neuron recordings in detecting the onset of deterioration of fresh tomatoes and frozen fish respectively, which is unobservable at the stage detected by currently available techniques. As another specific example, the pea aphid, *Acyrthosiphon pisum*, provides olfactory neurons for distinguishing between frozen peas with high and low value flavour.

The programme extends to use of whole insects, including exploitation of honeybee 'learning' in the odour conditioned proboscis extension assay, and molecular studies on the aphid olfactory receptor system, which may in the long term offer non-living systems embodying insect olfactory reception.

REPELLENCY AND TOXICITY OF NOOTKATONE TO FORMOSAN SUBTERRANEAN TERMITE *COPTOTERMES FORMOSANUS*.

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Nootkatone, one of over 300 components of vetiver oil, is a mildly pungent sesquiterpene ketone with a distinctive grapefruit flavor, largely responsible for vetiver oil unique aroma. As part of our research on natural products efficacy against Formosan subterranean termite, the major wood pest in the USA, previous experiments showed that vetiver oil and nootkatone disrupt normal termite behavior and significantly affect feeding ability and survival. A novel bioassay for the evaluation of nootkatone-treated wood and sand was designed to better understand how nootkatone affects the behavior and physiology of these termites. Daily counts on the number of feeding termites, weekly measurements of food consumption, abundance of symbiont protozoa present over time and termite survival were recorded. Our study proved that this essential oil component acts as a repellent, as a feeding deterrent and as a toxicant to Formosan subterranean termite. Nootkatone represents a promising natural alternative to synthetic termiticides for the management of termite control.

THE ROLE OF PHENYLACETONITRILE IN THE OLFACTORY SYSTEM OF THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*

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Phenylacetonitrile (PAN, syn.: Benzylcyanide) is supposed to be the major and most potent adult aggregation pheromone component in the desert locust. However, by studying the emission characteristics of PAN we found a very unusual pattern for an releaser aggregation pheromone: PAN was emitted exclusively by mature, gregarious males. The amount of PAN released depended only on the presence of mature males; mature females locusts did not stimulate the release of PAN. After physical isolation of previously grouped males a steadily reduction in PAN release was observed. However, the PAN emission was restored after regrouping the isolated males. In olfactometer bioassays PAN had a clear repellent effect on gregarious last instar hoppers, immature and mature adults of both sexes. Among the tested groups mature males showed the strongest avoidance of PAN. The repellent effect of PAN was not affected by other components of the mature male odor bouquet. Also the rest bouquet alone (without PAN) was not attractive to the locusts. From our observations we infer, that this semiochemical might serve as a scent flag that announces (together with the bright yellow color) the sexual identity of the bearer. PAN prevents as an “abstinon” homosexual mating attempts by other mature males. Furthermore we observed that PAN prevents other male competitors from approaching mating locust couples. Thus the substance acts also as a “courtship inhibiting pheromone”. In summary we conclude that PAN is not the releaser aggregation pheromone. Instead PAN has an important function in the reproductive behavior of crowded living locusts. This semiochemical is of advantage both for mate searching male locusts by avoiding unsuccessful courtship as well as for the copulating male by discouraging possible competitors ensuring its own reproductive success.

SCENTS AND SONGS: SEXUAL SIGNALING IN STINK BUGS (HEMIPTERA: PENTATOMIDAE).

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Phytophagous pentatomid bugs produce sex and/or aggregation pheromones, and the pheromones of a number of species have been identified. In laboratory and field trials, bugs appear to be weakly attracted to pheromone sources and traps. However, it has been noted that bugs are frequently found in significant numbers around pheromone-baited traps, even though few or no bugs were caught in the traps, suggesting an alternate form of communication at close range. We will describe the identification of pheromones for several economically important stink bug species, and the substrate-borne vibrational signals produced by adult bugs of both sexes for communication over shorter distances. Both the pheromones and the vibrational signals appear to be species-specific, providing unique channels for communication using two entirely different sensory modalities.

MAKE LOVE NOT WAR: A COMMON ARTHROPOD DEFENSE COMPOUND AND A SEXUAL KAIROMONE MEDIATE MATE FINDING IN THE FOREST COCKCHAFER *MELOLONTHA HIPPOCASTANI* FABR. (COLEOPTERA: SCARABAEIDAE)

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Mate location in *M. hippocastani* occurs during a swarming flight performed mainly by males at sunset. We studied the chemical cues involved. Field experiments during the swarming period demonstrated that males orientate towards volatiles emitted by damaged leaves in order to locate females that stay at this time within the host trees and continue to feed. Chemical analyses revealed the presence of typical so-called green leaf volatiles (GLV). Many of these compounds were shown to be electrophysiologically active. However, field tests demonstrated that only (*Z*)-3-hexen-1-ol and mixtures containing this compound attracted males.

Further experiments revealed that males of *M. hippocastani* also use a sex pheromone for mate finding. The active compound was identified as 1,4-benzoquinone, a well-known chemical defense compound of several arthropods. 1,4-benzoquinone was only slightly attractive when used alone but enhanced synergistically the response of males towards synthetic GLV. Hence, by responding towards 1,4-benzoquinone males are enabled to discriminate between leaf damage caused by eating females and non-specific leaf damage.

The fact that not only females but also males and grubs of *M. hippocastani* contained 1,4-benzoquinone, suggests that the sex pheromone of *M. hippocastani* might have evolved secondarily from a more primitive role as defense compound.

THE SEMIOCHEMISTRY OF SPRINGTAILS

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Springtails have so far not received much attention by chemical ecologists, despite their widespread occurrence and importance in many ecosystems. Previously we have shown that the large European species *Tetradontophora bielanensis* has an effective system of chemical defence against potential predators, using unique pyridopyrazines [1]. We therefore investigated additional species in order to identify other compounds used for defense. In addition, putative pheromones, which are described from some species, and the cuticular lipids of springtails were investigated. In some species, characteristic tetraterpenes are present on the cuticle. In the presentation we want to describe the results we have obtained so far. Species will include *Podura aquatica* and the snow-loving *Ceratophysella sigillata* among others.

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MOTH PHEROMONE COMPONENTS AND CONSTRAINTS ON THEIR EVOLUTION

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Of the estimated 185,000 moth species (Lepidoptera), about 528 pheromone components have been identified from 1683 species (Pherolist). These moth pheromone components are structurally related, the majority being acetate esters, alcohols, or aldehydes of unsaturated olefinic chains from 5 to 22 carbons (but mostly 12, 14, or 16 carbons). The question arises, how many different molecules (e.g. acetate esters) can be constructed from different unsaturated (carbon-carbon double bond) positions and configurations (*E* or *Z*) given a certain chain length? If the numbers are not large, then it is more probable that a species could be constrained in evolving multiple component pheromones (moving to another channel) since other species may have already evolved the use of these components. BASIC programs were developed that count and name all possible unsaturated structures given specific chain lengths with or without a functional group. *E*- and *Z*-configurations are assumed to occur at any position except next to a functional group, nor can two unsaturations be adjacent, while end unsaturations are delta. The results showed, for example, that there are 2,097,126 different geometrical isomers of olefinic acetate esters of chain lengths 5 to 22. For 14-carbon acetate esters, there are 1039 isomers with 1 to 3 unsaturations. Thus, there appears to be little constraint on evolution of new communication channels even for closely related moth species such as in *Yponomeuta*. When blends of 2 to 4 components are considered, the possibilities quickly extend into the millions and beyond. A discussion of evolution of moth pheromone components and speciation is presented.

PIERID COMMUNICATION SYSTEMS: A THEME WITH VARIATIONS!

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Sexual selection theory predicts that the different selection pressures on males and females result in sexual conflict. However, in the pierid butterflies males and females share a common interest, which could lead to sexual cooperation. The male and the recently mated female share a common interest to reduce female harassment by other males during egglaying after mating. We will present
the different communication systems in three Pierid species. They are closely related and use related chemical signals; however the theme seems to vary slightly, the Pieris brassicae male tries to cloak his mate with his own odour while P. napi and P. rapae uses one or several compound that indicate the females unreceptive state. The origin of the active compounds were elucidated thru 13C labeled amino acids administrated to the feeding larvae.

SEMIOCHEMICAL ATTRACTANTS FOR THE ASIAN LONGHORN BEETLE

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The Asian longhorn beetle, *Anoplophora glabripennis*, is a serious pest of hardwoods in its native range in northern China and has recently been introduced to North America. The eradication program in the U.S. has been hindered by the lack of monitoring and detection tools. Species of long horn beetles (Cerambycidae) have been reported to use a wide variety of semiochemicals: male, female, or host produced compounds that function over short, medium and long ranges or as contact pheromones. The majority of species use long range attractants. The objectives of this research are to identify long-range, chemical attractants for monitoring including host volatiles and female-produced pheromones. A combination of techniques including GC-EAD, GC-MS, and laboratory and field assays has identified a suite of potential attractants. Most of these are host produced; one is female produced. The female produced compound is structurally similar to two male produced compounds. Laboratory assays show that males are attracted to female-host extracts. An array of compound combinations has been field tested in China; two combinations appear to be active.

CHEMICAL SIGNALS AT PHYLOGENETIC BRANCHING POINTS

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Caddisflies (Trichoptera) are phylogenetically considered as the sister order of butterflies and moths (Lepidoptera). Recent investigations showed, that primitive moths of the family Eriocraniidae and caddisflies exhibit similarities in physiology, morphology, and systems of chemical communication. While more advanced lepidopteran species produce sex pheromones representing long-chain unsaturated acetates, aldehydes, and primary alcohols in glands located near the ovipositor, caddisflies and eriocraniid moths possess glands in the 4th and 5th abdominal segment where they mainly produce unbranched, short-chain methylketones and methylcarbinols. However, in the limnephilid caddisfly, *Hesperophylax occidentalis*, a branched ketone, 6-methyl-3-nonanone was reported to be the sex-pheromone. [i] Here we describe the structure assignment of (*S*)-4-methyl-3-heptanone (**1**), (4*S*,6*S*)-4,6-dimethyl-3-octanone (**2**), and (4*S*,6*S*)-4,6-dimethyl-3-nonanone (**3**) in three other species of the family Limnephilidae, *Potamophylax latipennis*, *Potamophylax cingulatus*, and *Glyphotaelius pellucidus*. Though the biological significance of the compounds is yet unknown, it is interesting to note that they elicit strong response in the antennae of males, suggesting a role in chemical communication of these species.

MOLECULAR MOTIVATION

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During the past fifty years of natural products chemistry and chemical ecology, we have progressed from compartmentalized disciplines to highly integrated interdisciplinary programs. Then, chemists would make the molecules and determine the structures; biologists would evaluate them in behavioral tests. Now, the boundaries are blurred and the excitement is at the interfaces. Molecular and cellular biochemists work with chemists and biologists to address the unsolved mechanistic questions at the level of how pheromones and hormones act as signal transducers. I have been privileged to be part of this transitional scientific generation. The thrill of discovery, the challenge of constantly learning new disciplines, and the fun of working with new people have been scientifically and personally rewarding. This lecture will be a scientific retrospective, providing a very brief history of our work with hormones, pheromones, and chemical defense. Featured will be my favorite subjects – termites, moths, elephants - and their amazing motivational chemicals.

EVIDENCE FOR A FEMALE SEX PHEROMONE MEDIATING MALE MATE CHOICE IN RED-SIDED GARTER SNAKES (*THAMNOPHIS SIRTALIS PARIETALIS*)

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Male garter snakes display a chemically mediated courtship preference for larger females during the breeding season. It has been suggested that the chemical cue utilized by males to discriminate among females of varying size is the sexual attractiveness pheromone, a previously characterized sex pheromone composed of a homologous series of saturated and ω-9 cis-unsaturated methyl ketones. We examined individual variation in the expression of the sexual attractiveness pheromone of the red-sided garter snake (*Thamnophis sirtalis parietalis*) to determine whether a relationship exists between pheromone expression and female body size. Female red-sided garter snakes of varying snout-vent length were collected during the breeding season and their pheromone profiles examined utilizing gas chromatography/mass spectroscopy. Overall quantities of pheromone expressed, numbers of unique methyl ketones composing the pheromone, and relative concentrations of saturated and unsaturated methyl ketones composing the pheromone were found to vary extensively among females. When examined in relation to female snout-vent length, we found that one of the three quantitative and qualitative measures, the relative concentration of saturated and unsaturated methyl ketones composing the pheromone, was strongly correlated with female body size; smaller females expressed pheromone profiles higher in saturated methyl ketones while larger females expressed pheromone profiles higher in unsaturated methyl ketones. These results demonstrate that the female sexual attractiveness pheromone of the red-sided garter snake contains the necessary variation for this pheromone to function as a reliable indicator to males of female body size.

MAMMALIAN ODORANT AND PHEROMONE BINDING PROTEINS

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Odorant binding proteins (OBPs) or subclass binding pheromones (PBPs), pertain to one of the most abundant classes of proteins found in the olfactory apparatus. OBPs are a subclass of lipocalines, defined by their property of reversibly binding volatile chemicals, 'odorants' or pheromones. Numerous sequences of OBPs are now available, derived from protein sequencing from nasal mucus material, or from DNA sequences. The structural knowledge of OBPs has been improved too in recent years, with the availability of two X-ray structures. Whether OBPs are involved in the perception of general odors or in the recognition of specific pheromones, the physiological role of OBPs remains hypothetical. In recent studies we have been able to assign a function to an OBP, with a possible role of Bovine OBP in the ecological relationships between bovine and insect species .The present knowledge on OBP/PBP biochemistry, sequence and structure will be examined here in relation to the different functional hypotheses proposed for OBPs.

INSECT PHEROMONE BINDING PROTEINS AND CHEMOSENSORY PROTEINS: FUNCTIONAL AND STRUCTURAL STUDIES

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Pheromone binding proteins (PBPs) and chemosensory proteins (CSPs) belong to 2 large families of insect proteins involved in chemical communication and perception. PBPs are located in the sensillum lymph of pheromone-responsive antennal hairs, and are thought to transport the hydrophobic pheromones to the membranes of olfactory neurons. CSPs are a group of ubiquitous small proteins believed to be involved in chemical communication and perception (olfaction or taste). We have expressed one of the antennal CSP from *Mamestra brassicae* (CSPMbraA6) in large quantities as a soluble recombinant protein in *E. coli* periplasm. This 112 residues protein is a highly soluble monomer of 13,072 Da with a pI of 5.5. NMR data (^{1}H and ^{15}N) indicate that CSPMbraA6 is well folded and contains 7 □-helices and 2 short strands. 29 amino acids are involved in □-turns and coiled segments and 6 amino acids are not assigned in the NMR spectra (N-terminus, internal residues), probably due to their mobility. Moreover, we have investigated the binding properties and specificity of PBPs from *Mamestra brassicae* (MbraPBP1), *Antheraea polyphemus* (ApolPBP1), *Bombyx mori* (BmorPBP) and a hexa-mutant of MbraPBP1 (Mbra1-M6), mutated at residues of the internal cavity to mimic that of BmorPBP, using the fluorescence probe 1-amino-anthracene (AMA). Several pheromones are able to displace AMA from the MbraPBP1 and ApolPBP1 binding sites, without, however, any evidence of specificity for their physiologically relevant pheromones. These findings bring into doubt the currently held belief that all PBPs are specifically tuned to distinct pheromonal compounds.

THE SOURCE OF RECOGNITION PHEROMONE IN THE ANT *CAMPONOTUS FELLAH*

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Eusocial insects developed very efficient behaviors to transmit information to their nestmates. Trophallaxis, allogrooming and cuticular contacts permit to exchange hydrocarbons, to date the only known nestmate recognition pheromone in ants. The cuticle of *Camponotus fellah* carry a blend of alkanes, methyl- and dimethyl alkanes from 25 to 33 carbons. Colonies kept in the lab under controlled conditions of temperature and food for 2 years still exhibit strong differences between the relative quantities of their cuticular hydrocarbons as well as strong nestmate recognition capabilities. This suggests that food is of minor importance as a discriminator. Queenright and queenless groups of workers don't show aggressiveness towards each other even after 3 months of separation. However workers individually isolated for only 3 weeks are strongly aggressed when introduced with their former nestmates. These violent aggression most of time lead to the death of the isolated disregarding they are introduced in a queenright or in a queenless group, suggesting that the queen does not label the workers. Workers individually isolated into single mesh cages (that allow cuticular contacts) and double mesh cages (that prevent contacts) are also aggressed but less than totally isolated workers. During dyadic reunion tests, double mesh caged workers also ask for more grooming and trophallaxis than single mesh caged workers, but less than totally isolated workers. These results suggest that in addition to a different hydrocarbon profile, isolated workers may be recognized because they are not submitted to volatile compounds emerging from the nest.

GUT BACTERIA ARE INVOLVED IN THE SYNTHESIS OF ELICITOR-ACTIVE COMPOUNDS FROM LEPIDOPTERANS

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Volicitin (17-Hydroxylinolenoylglutamine) - first isolated from regurgitate of *S. exigua* larvae - was claimed to elicit the *de novo* biosynthesis of plant volatiles.[1] Besides volicitin, a great variety of other lipid derived glutamine conjugates could be identified in oral secretion of lepidopteran larvae by LC-Ms/Ms.

Similar compounds are known as microbial metabolites since 30 years.[2] As shown previously several microorganisms from the gut of lepidopteran larvae were also able to produce acylglutamine conjugates *in vitro*.[3] *N*-acylglutamines are excellent surfactants. It is, therefore, assumed that the gut bacteria supply the lepidopteran larvae with these compounds to act as emulsifiers to facilitate digestion.

Volicitin is clearly not a general general elicitor of the plant volatiles. – In bioassays with excised plantlets of the Lima bean volicitin did not stimulate volatile biosynthesis. Antagonistic effects of enantiomeric mixtures of volicitin could not account for that observation as we determined the absolute configuration of the 17-hydroxy group of volicitin from 4 lepidopteran larvae (*S. exigua, S. frugiperda, S. littoralis, H. virescens*) as *S* in high enantiomeric eccess.[4]

Acylglutamine surfactants destabilise lipid membranes eventually resulting in membrane depolarisation or facilitating such effects synergistically with other compounds.

The involvement of bacteria in the biosynthesis of compounds which play a pivotal role in the interaction of plants, herbivores and their predators adds a new trophic level to this complex network of interactions.

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CHEMICAL INFORMATION FLOW IN TERRESTRIAL AND AQUATIC ENVIRONMENTS

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Understanding the mechanisms by which environmental chemical signals mediate various life-history processes can lead to important insights about the ecology and evolution of organisms. For chemical signals released into the environment, establishing the principles that control chemical production and transport is critical for interpreting biological responses to these stimuli within appropriate natural-historical contexts. The physics governing chemical transport are similar in air and water and can be described by essentially the same equations. Still, because the molecular viscosity of an aerosol is so much smaller than that of an aqueous solution, bulk transport (called 'advection') and turbulent mixing of signal molecules are generally much faster in air. Such similarities and differences between the properties of the fluid media lead to convergence or divergence among navigational strategies used by macroscopic animals in turbulent odor plumes. Strong effects of advection and turbulence also determine transport of chemical signals at microscopic scales, such as in pheromonal communication between sperm and egg. Here, laminar shears stretch or distort chemical concentration fields and thus substantially modify broadcast distances of signals relative to those produced by molecular diffusion. Independent of organism size or fluid medium, physics tightly constrains the evolution of chemical-signaling processes and dictates the mechanics of orientation.

FISH MIGRATORY PHEROMONES: A CASE STUDY OF A BILE STEROID DERIVED PHEROMOME IN THE SEA LAMPREY

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Many species of fish migrate vast distances over the course of their lives, eventually congregating to reproduce in habitats suitable for their young. Although it is well known that salmon achieve this feat by following unknown odor(s) associated with their natal streams, it is known by relatively few that many other fish instead employ pheromones released by aggregating conspecifics to achieve this task. The latter strategy makes 'sense' for fish which are poor swimmers such as the sea lamprey, *Petromyzon marinus*. This ancient species reproduces in freshwater streams but spends its juvenile (parasitic) phase being carried throughout the Atlantic Ocean/ Great Lakes before migrating back into streams. Fisheries research long ago established that migratory lamprey have a strong tendency to enter streams which contain conspecific larvae, suggesting a pheromone. Recent work by my laboratory and colleagues has now shown that the odor of larval lamprey is highly attractive to adults and that a single larva can activate 100,000 liters of water. Pursuing the hypothesis that bile steroids might make suitable migratory cues because of their unique structures and association with feeding, we have discovered that larval lamprey produce and release large quantities of 2 unique bile steroids, petromyzonol sulfate (PS) and allocholic acid (ACA). Further, we have found both of these steroids in stream waters and by using EOG recording have established that they both function as potent olfactory stimuli with picomolar detection thresholds. Finally, behavioral tests have found that migratory lamprey are attracted to low (realistic) concentrations of PS and ACA, although less so than to larval odor itself suggesting the pheromone has other components.

HORMONALLY-DERIVED SEX PHEROMONES IN FISH: A CASE STUDY OF STEROID AND PROSTAGLANDIN PHEROMONES IN THE GOLDFISH

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Living in water that transports hormonal products released by conspecifics, fish evolved sensitive and specific reproductive responses to these potentially important chemical cues (*hormonal pheromones*). Indeed, steroids, prostaglandins and/or their metabolites are reported to be hormonal pheromones in major groups of fishes including carps (goldfish), catfishes, gobies, cichlids, and salmon. Hormonal pheromones are best understood in goldfish, where females sequentially release a preovulatory steroid pheromone and a postovulatory prostaglandin pheromone that dramatically affect male behavior and physiology. Major components of the preovulatory pheromone are the oocyte maturation-inducing steroid 17□,20ß-dihydroxy-progesterone (17,20ß-P), a sulfated metabolite (17,20ß-P-20ß-Su), and the androgen, androstenedione (AD). These steroids are released by different routes (17,20ß-P and AD across the gills; 17,20ß-P-Su in urine), and act through separate olfactory receptor mechanisms. Although not completely characterized, interactions of these three steroid odorants induce both behavioral responses that enhance male spawning success, and endocrine responses that increase releasable sperm. At ovulation, pheromonal steroid release is greatly reduced, and egg-oviduct interaction stimulates synthesis of hormonal prostaglandin F2□ (PGF2□) that induces female spawning behaviors. At this time, released PGF2□ and a metabolite, 15-keto-PGF2□, act through separate olfactory receptor mechanisms to trigger male courtship behaviors that further increase releasable sperm. Hormonal pheromone compounds in other carps are similar to those of goldfish, but differ markedly in more distantly related fishes.

CHEMICAL MEDIATION OF COMPLEX INTERACTIONS AMONG SEAWEEDS, INVERTEBRATES, AND THEIR PREDATORS: CONSEQUENCES FOR MARINE COMMUNITIES

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Chemical ecologists have long recognized the ecological and evolutionary importance of conflict between plants and herbivorous animals because of the critical role that plant chemistry plays in mediating these interactions. However, we have generally been slower to recognize the importance of cooperation among plants and animals, and the ways in which these mutualistic or commensal interactions are mediated (sometimes indirectly) by chemistry. For example, the persistence of corals on temperate rocky reefs in North Carolina is linked to their ability to harbor a herbivorous crabs that are resistant to chemical defenses (mostly small, lipophilic metabolites) produced by seaweeds that compete with corals. Herbivores exploit plant chemical defenses for shelter as well, as occurs when the decorator crab *Libinia* places the seaweed *Dictyota*, on its back as a form of chemical camouflage from predators. The preference of crabs for this alga is cued by dictyol E, a diterpene alcohol specific to *Dictyota* that deters feeding by both crab and algal predators. Animals often benefit their chemically defended hosts, as occurs when a polychaete worm festoons its tube with noxious seaweeds that float by, "rescuing" these plants from being washed ashore. Chemical complexity can thus enhance local diversity in the same way that the provision of physical shelter by structurally complex habitats does. We are currently addressing whether the effectiveness of structurally vs. chemically based refuges might differ by comparing epifaunal density and diversity among cnidarians that use chemical vs. morphological defenses against predators.

THE PHYLOGEOGRAPHY OF HERBIVORE TOLERANCE FOR CHEMICALLY-RICH SEAWEEDS

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Herbivores that feed on chemically-rich plants must combat the metabolites by behavioral or physiological means. Spatial variation in such herbivore tolerance is well documented for many insects but largely unexplored for other groups, including marine herbivores. The amphipod *Ampithoe longimana* is a polyphagous, tube-dwelling herbivore found in estuaries along the eastern United States coastline. The geographic range of the amphipod extends beyond that of one of its preferred foods, tropical seaweeds of the genus *Dictyota*. As a result, populations of the amphipod sympatric with *Dictyota* have higher affinity for, and fitness on *Dictyota* than do populations outside the plants' geographic range. These geographic differences are mediated by differential tolerance for the diterpenoid alcohols produced by *Dictyota*. Integrating behavioral and fitness data with a molecular phylogeography of several populations (based on mtDNA and nuclear ITS sequences) suggests that drift cannot completely explain such evolution; rather, local selection must have played a role. Within the geographic range of *Dictyota*, omnivorous fishes select for herbivores with high preferences for *Dictyota*. Outside of the range of *Dictyota*, negative selection acts to reduce herbivore tolerance for the plants, though the mechanism is unclear. Overall, my research indicates that marine herbivores can respond evolutionarily to locally available seaweeds by maintaining their tolerance for the plants' metabolites. Further, it appears that evolving high tolerance for one seaweed does not correlate with reduced tolerance for alternative seaweeds, a phenomena which may help explain the predominance of a generalist lifestyle among other marine herbivores.

HERBIVORE ATTRACTANTS AND DETERRENTS FROM AQUATIC MACROPHYTES: EXAMPLES FROM WATERCRESS AND WATERMILFOIL

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We studied the chemical ecology of two freshwater plant-herbivore systems: watercress and Eurasian watermilfoil. The watercress glucosinolate-myrosinase system is a deterrent to feeding by generalist herbivores. In North America, where watercress is exotic, many stream invertebrates (limnephilid caddisflies, snails and the amphipod *Gammarus pseudolimnaeus*) will not feed on live, chemically-defended watercress, but readily consume undefended, but lower nitrogen, senescent watercress. In England herbivores have evolved in streams with watercress and may have adapted to watercress defenses. Two snails, *Gammarus pulex* and one caddisfly were deterred from feeding on fresh, but not senescent or heat-deactivated tissue, similar to North American herbivores. In contrast, the limnephilid caddisfly *Limnephilus lunatus* preferred fresh green to senescent tissue and consumed similar amounts of fresh and senescent tissue. Thus, one English herbivore appears adapted to the watercress defense system, but most herbivores are deterred. The North American weevil *Euhrychiopsis lecontei* is a specialist herbivore of watermilfoils that recently expanded its host range from the native northern watermilfoil to the exotic Eurasian watermilfoil. The weevil is attracted to Eurasian by chemicals released from the plant and adult exposure to Eurasian watermilfoil results in an induced preference for Eurasian over northern watermilfoil. Bioassay-driven fractionation of watermilfoil exudates resulted in the identification of one small (MW = 112) water-soluble compound that attracts the weevil; other attractive components may be present. These two systems indicate that chemical mediation of freshwater invertebrate-macrophyte interactions is important, and comparisons with terrestrial and marine systems will advance our overall understanding of plant-herbivore interactions.

CHEMICAL DEFENSES OF FRESHWATER MACROPHYTES: CONTRASTS WITH MARINE PATTERNS

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Herbivory in marine communities is a major force affecting seaweed populations and the structure of marine systems in general. In contrast, herbivory on freshwater macrophytes has been assumed to be slight, suggesting that selection for the evolution of chemical defenses against consumers would be minimal. In contrast to expectations, about 60% of 57 species (from 27 families) of macrophytes we tested from the Southeastern US had extracts that deterred feeding by an omnivorous crayfish. The frequency and magnitude of deterrence from freshwater macrophytes exceeded that of marine seaweeds from the same geographic region. Reciprocal tests of marine and freshwater extracts against marine and freshwater herbivores demonstrated that this difference in deterrence was due to the chemical defenses produced, rather than to differing susceptibilities of the herbivores tested. Deterrence of water-soluble extracts was common for freshwater, but rare for marine, macrophytes; however, deterrence was not correlated with phenolic levels, suggesting that other classes of compounds were involved. In contrast to general statements from text books and older reviews, an overview of available literature on grazing in freshwater systems shows that herbivory rates are much higher than generally assumed. Much of the general impression of low grazing impact is due to a focus on snails and insects; vertebrates and larger generalist invertebrate consumers commonly have dramatic impacts on freshwater macrophytes and may thus select for potent chemical defenses.

Do macronutrients interfer with allelopathic interactions of submersed macrophytes with other primary producers?

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Freshwater angiosperms living submerged in the littoral zone of lakes face adverse interactions with primary producers and herbivores. Their soft tissue lacking lignification or other structural defences renders them palatable to invertebrate and vertebrate herbivory. Shading by other macrophytes, phytoplankton or epiphytes often offsets a positiv net productivity. To counteract herbivory and shading aquatic plants should produce effective allelochemicals. Since all aquatic angiosperms are secondarily aquatic, –i. e. evolved from terrestrial ancestors, we assume a comparable protective role of secondary metabolites in freshwater as known for terrestrial systems. We have isolated allelochemicals preventing algal and cyanobacterial growth from *Myriophyllum spicatum* L., a highly competitive submersed macrophyte. The major inhibitor is tellimagrandin II, a hydrolyzable polyphenol which strongly inhibits exoenzymes of target organisms, e.g., alkaline phosphatase, an exoenzyme vital for algae during summer phosphorus depletion in lakes. Furthermore, we observe a negative correlation between the nitrogen content and the content of polyphenolic allelochemicals in milfoil *(M. spicatum)* tissue during the vegetation period. A higher concentration of polyphenols during spring and summer is adaptive to counter shading by other primary producers. In fall, higher nitrogen allocation in the apical shoots favors vegetative dispersal. Laboratory studies confirm this reciprocal interaction of nitrogen and polyphenolic allelochemicals. The interaction of macronutrients with defensive compounds in submerged macrophytes increases our understanding of allelopathic interactions *in situ*. In addition, larvae of the aquatic herbivorous *Acentria ephemerella* (Lepidoptera) grow significantly slower on the polyphenol-rich milfoil than on low-tannin food, indicating that polyphenols in this macrophyte have multiple protective functions.

THE ROLE OF SYMBIONTS IN THE PRODUCTION OF COMPOUNDS FOUND IN MARINE INVERTEBRATES

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In the marine natural products literature, it is not unusual for authors to suggest that bioactive compounds could be produced by associated or symbiotic microorganisms. This is particularly common when the bioactive compound isolated from a marine invertebrate is structurally similar or even identical to a known microbial product. There are, however, relatively few examples where it has been demonstrated that the compound is of microbial origin. The major difficulty faced by those who wish to study chemical production by symbionts is that the majority of symbionts are difficult or currently impossible to culture outside of the host. Alternatively, the symbionts might be culturable but may not produce their bioactive materials outside the host.

We are making steady progress in studying the production of bioactive metabolites by symbionts by combining marine natural products chemistry with bacterial genomics and microscopy. After reviewing past research, recent studies of the patellamides from the ascidian *Lissoclinum patella* and the halogenated metabolites of the sponge *Dysidea herbacea* will be presented.

CONSEQUENCES OF CONSUMING CHEMICALLY RICH PREY

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The advantages of chemically repelling consumers are obvious and have been studied extensively in both marine and terrestrial communities. Much of the current knowledge about chemical defenses against consumers in the marine environment is based on immediate behavioral responses of consumers to prey chemistry. It is largely unknown, however, whether marine consumers limit ingestion of chemically rich prey due to their negative post-ingestive effects or whether prey are capitalizing on idiosyncrasies of consumer taste and producing compounds consumers find distasteful, but that have little effect on consumer physiology and fitness. Relatively few studies have assessed how consuming ecologically realistic amounts of chemically defended prey over longer periods of time impacts the feeding behavior and fitness of marine consumers and thus the evolution of effective chemical defenses in marine prey. This talk presents the results of long-term feeding assays offering structurally diverse secondary metabolites of marine invertebrates to an omnivorous marine fish. Comparisons between the fish's immediate reaction to the compounds vs. the compounds' longer term effects will be discussed.

LATENT ALARM SIGNALS: A NEW APPROACH TO THE STUDY OF CHEMICAL COMMUNICATION IN AQUATIC ENVIRONMENTS

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Chemical alarm signals have been reported in several animal phyla. Such alarm signals are released by injured specimen to evoke behavioral alarm reactions in conspecifics. Predators may be 'labeled' by alarm signals from prey, and their release of prey specific signals may over time cause morphological changes in conspecific prey. Surprisingly, chemical alarm signals seem absent in some species, but still these animals respond with alarm reactions to chemical cues from predators. The predator cues may also induce morphological changes in the prey, and may even result in life-history changes. However, chemical alarm cues of predator origin (*i.e.* kairomones) seem to contradict a theory of evolutionarily stable strategies, and the origin of the predator signals was therefore questioned. The questioning was supported by the fact that in many papers reporting kairomones, the predators were fed the prey species of study. Accordingly, a hypothesis was developed on predator labeling by chemically 'silent' alarm signals from prey, combined with signal activation in the GI tract of predators. An important issue in this context was 'zero-setting' of predators with regard to prey signals. This was obtained by either using alien predators, or by feeding common predators for prolonged time periods with taxonomically distant prey. In this way, latent alarm signals that induce morphological changes in conspecifics were demonstrated in the water flea *Daphnia galeata*. In the green sea urchin (*Strongylo-centrotus droebachiensis*), strong magnification of behavioral responses was found in response to alarm compounds that had passed through the GI tract of predators.

MACROALGAL DEFENSES IN THE NITROGEN REPLETE, CARBON LIMITED SOUTHERN OCEAN: NO EVIDENCE FOR NITROGENOUS SECONDARY METABOLITES

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Antarctic macroalgae have low C:N ratios, high nitrogen contents, and are usually growth limited by carbon (light). The Carbon Nutrient Balance Hypothesis (CNBH) predicts that macroalgae would produce nitrogenous secondary metabolites for defense rather than high levels of non-nitrogenous defenses under these conditions. We examined palatability of Antarctic Peninsula macroalgae in feeding bioassays with three common, sympatric macroalgal-consuming omnivores. To date, feeding bioassays have been performed on fragments of thallus from 26 macroalgal species and 21 (81%) were rejected by at least one omnivore. Organic extracts from 13 macroalgal species rejected as thallus were used in feeding bioassays. At least one extract from 12 species (92%) was rejected by at least one omnivore, suggesting that chemical defenses against herbivores probably are present in at least some of the macroalgal species. We have identified a number of specific, non-nitrogenous secondary metabolites in these extracts and previous workers have also reported non-nitrogenous secondary metabolites from antarctic macroalgae. Additional extracts targeting nitrogenous metabolites from 25 species were subjected to thin layer chromatography and visualized by stains specific for nitrogenous compounds. No nitrogenous secondary metabolites were identified by this or other methods. Nitrogenous secondary metabolites are also extremely rare in macroalgae from other areas of the world. Consequently, although our bioassays suggest that chemical defenses probably do occur, our data cast doubt on the applicability of the CNBH for predicting the chemical composition of macroalgal defenses under carbon limited conditions. (NSF OPP9814538, OPP9901076)

CHEMICAL DEFENSE OF THE INVASIVE GREEN ALGA *CAULERPA TAXIFOLIA*

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Since its accidental introduction into the Mediterranean Sea in 1984, the tropical green macro-alga *Caulerpa taxifolia* has spread tremendously. This aquarium-derived strain of *C. taxifolia* was also recently found in Moreton Bay, Australia and in the lagoon of San Diego, California. As a bioinvasive species it represents a threat to temperate marine ecosystems. The most important key to its success is its efficient chemical defence towards potential predators such as sea urchins. It is believed that this defence is almost exclusively based on the acetylenic sesquiterpene caulerpenyne (**1**) that can account for up to 1.3 % of the algal fresh weight. We show that *C. taxifolia* reacts with a rapid transformation of (**1**) upon wounding. The three acetate groups of this sesquiterpene are enzymatically cleaved within minutes after mechanical damage. Transformation of the 1,4-*bis*-enol acetate moiety of caulerpenyne (**1**) results in labile 1,4-dialdehydes after enolisation.

Caulerpenyne (**1**) Oxytoxin 2 (**2**)

Normal phase HPLC/APCI-MS measurements of freshly prepared algal extracts allow characterization of these reactive products. Structural elucidation was achieved after trapping of the highly unstable aldehydes with 2,4-dinitrophenylhydrazine. These aldehydes belonging to the oxytoxin family have been detected in marine opisthobranch molluscs, natural predators of algae of the order Caulerpales. The role of wound-induced transformation in activated chemical defence of *C. taxifolia* is suggested.

HYDROID DEFENSES AGAINST PREDATORS: IMPORTANCE OF SECONDARY METABOLITES VERSUS NEMATOCYSTS

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Marine hydroids are commonly thought to be defended by organelles called nematocysts that penetrate predator tissues and inject proteinaceous venoms, but not all hydroids possess penetrating nematocysts. Recently, an increasing number of novel secondary metabolites have been isolated from marine hydroids – their ecological roles are largely unknown. We tested the hypothesis that nematocysts and noxious secondary metabolites represent alternative hydroid defenses against predation by examining: (1) palatability of whole polyps before and after nematocysts had been deactivated, (2) crude extract palatability, and (3) hydroid nutritional value. Our results suggest that chemical defenses may be at least as common as nematocyst-based defenses and that the two may represent largely alternative defensive strategies. Species with deterrent extracts represent four families and both sub-orders of hydroids, suggesting that chemical defenses among hydroids may be widespread and have multiple origins.

CHEMICAL ECOLOGY AND BIODIVERSITY OF *PHESTILLA* NUDIBRANCHS

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We investigated the chemical ecology and biodiversity of coral-eating nudibranchs in the genus *Phestilla* (Gastropoda: Tergepedidae). Most *Phestilla* nudibranchs specialize on corals in the genus *Porites* (with the exception of *Phestilla melanobrachia*) and rely on waterborne chemical cues released by corals to induce settlement and metamorphosis. We searched for nudibranchs on corals from a variety of locations on Guam and noted a diversity of species previously unknown, including one species that associates with the coral *Goniopora fruticosa.* Larvae from different *Phestilla* spp. were hatched, raised to an age competent for metamorphosis, and used to study settlement specificity. The waterborne inducer for settlement and metamorphosis in *Phestilla sibogae*, released from its preferred food *Porites compressa* in Hawaii*,* has been studied extensively. This small molecule (MW 300-500) has defied attempts at isolation and structural characterization for over two decades. It is a minor component of a complex mixture of metabolites released by the coral into seawater; therefore, obtaining enough material for structural elucidation has been difficult. We have developed some different and rapid isolation techniques and obtained NMR spectral data at each step of the isolation process to aid in the characterization of the inducer.

BEHAVIOURAL AND ELECTROPHYSIOLOGICAL RESPONSES OF SEA LICE *LEPEOPHTHEIRUS SALMONIS* (COPEPODA: CALIGIDAE) TO SEMIOCHEMICAL STIMULI

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Sea lice infestation of farmed Atlantic salmon, *Salmo salar* L., costs the Aquaculture industry millions of pounds annually. To investigate the potential for using semiochemicals in sea lice control, their role in host location was confirmed by assessing responses to host (salmon), and non-host (turbot) conditioned water using Y-tube bioassays. Chemical components were extracted from fish conditioned water using solid-phase extraction (SPE) techniques, and extracts were further separated into volatile and non-volatile fractions by vacuum distillation. Although both host and non-host conditioned water activated adult male sea lice, directional responses were only obtained for salmon conditioned water (SCW), the SPE extract of SCW and the volatile fraction of this. No directional responses were obtained for the non-volatile fraction of the SPE extract of SCW, turbot conditioned water (TCW) or TCW SPE extract, confirming that host location is mediated by low molecular weight, host specific semiochemical cues. In addition, electrophysiological recordings from the first antenna show dose-response effects to SCW and SCW extract. Behavioural, electrophysiological and organoleptin assays are beginning to reveal those individual components concerned with host location. These results indicate the potential value of host location semiochemicals as a basis for developing trapping and/or disruption techniques for sea lice control.

SALMON SIBLINGS SCHOOL DURING DOWNSTREAM SMOLT MIGRATION

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The ability to recognize kin is an important mechanism in order for altruistic behavior to evolve. Juvenile salmonid fish have the ability to discriminate between unfamiliar siblings and non-siblings by using chemical cues. Kin recognition in salmon and trout is important for favoring close relatives on basis of lower aggression in territorial defense. This study demonstrates, for the first time, that kin-recognition is also present in a non-aggressive situation in salmonids, i.e. when Atlantic salmon (*Salmo salar* L.) smolt migrate to sea. We followed the downstream migration of individually tagged fish (PIT) when descending a 400 m long enclosure of a river. The release groups were composed of fish from three full sibling groups and siblings were both familiar and unfamiliar with each other (reared in the same tank or in a different tank). The release sites were in the upstream end of the enclosure and tagged fish were registered in real time (hrs, sec) when passing an antenna in the downstream end. For each fish registered during the night (peak migration) the nearest neighbour fish in time (before and after) was estimated and identified. The results showed that Atlantic salmon smolt preferred to migrate downstream with familiar siblings (reared together), but also with unfamiliar siblings. No significant preference was observed suggesting that fish migrated with individuals from the same release group. The results strongly suggest that salmon smolts prefer to migrate together with related individuals.

A NEW DEFENSE STRATEGY AGAINST MARINE PATHOGENS AND SAPROPHYTES

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Seawater can contain 10^6 microbes per mL. Marine microorganisms pose challenges to reef plants and animals due to their fouling, pathogenic, and parasitic activities. Seaweeds, which must remain unfouled in order to protect their photosynthetic ability, deter fouling as well as colonization by pathogenic and saprophytic organisms by unknown mechanisms. In the course of exploring the frontier of macro/micro interactions, we asked whether macroalgae utilize chemical defenses against microbial attack. Extracts of 48 macroalgal species were tested at natural concentration in agar matrices for deterrence of growth by 1 pathogenic fungus, 2 saprophytic fungi, and 1 pathogenic bacterium, all collected in similar marine environments. Over half the algal extracts surveyed significantly deterred growth of against at least one microbe, relative to controls. Bioassay-guided fractionation of extracts of one brown alga and one green alga led to the identification of potent antifungal compounds. The antifungal constituent from the decumbent form of Carribean *Lobophora variegata* is a polycyclic macrolide of unprecedented molecular structure active against one marine pathogen, one saprophyte, and some fungi of biomedical importance.

THIOGLYCOSIDES: VALUABLE MOLECULAR PROBES FOR THE ANALYSIS OF SEQUESTRATION PROCESSESS IN LEAF BEETLE LARVAE

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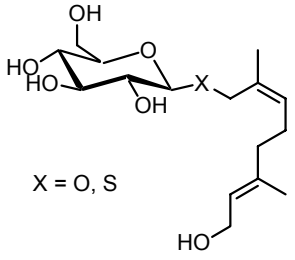
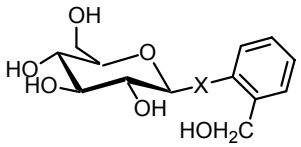
Thioglycosides combine a unique structural similarity to natural substrates with an exceptional chemical and biological stability against hydrolytic enzymes and can be, therefore, used to study transport phenomena of glycosides. Species which specialized on Salicaceae, i.e. *Phratora vitellinae* and *Chrysomela* spp., derive salicylaldehyde from plant phenolglycosides, salicin and salicortin. Larvae of the chrysomelid beetles *Phaedon cochleariae* and *Gastrophysa viridula* use iridoid monoterpenes for defense. The latter are synthesized *de novo* in the glandular tissue and the reservoir of the defense system. However, larvae feeding on leaves impregnated with thioglycosides of early precursors of iridoid biosynthesis such as the thioglycosides of 8-hydroxygeraniol rapidly accumulate these probes in their defensive secretion.





Salicin: X = O
Thiosalicin: X = S

The successful import suggests that the larvae possess, in addition to the *de novo* biosynthesis, the capability to sequester appropriate glycoside precursors that are transformed to iridoid monoterpenes in the reservoir. Thiosalicin is effectively accumulated by larvae of *Phratora* and *Chrysomela* spp., demonstrating that the plant derived salicin is ingested and transported **intact** to the defensive gland prior to further modification. The successful demonstration of such a transport mechanism in salicylaldehyde and iridoid producing leaf beetle larvae using thioglycosides as stable glycomimics of the unstable natural *O*-glycosides not only sheds new light on the origin and the complexity of iridoid defense in Chrysomeline larvae, but also raises new hypotheses on the evolution of salicylaldehyde secretion in Salicaceae-feeders [1].

B. Feld, J. Pasteels, W. Boland. (**2001**) Chemoecology, submitted.

MOLECULAR APPROACHES TOWARDS UNDERSTANDING INSECT PHEROMONE BIOSYNTHESIS AND REGULATION

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For several decades, questions about the endocrine regulation, tissue localization, and biochemical pathways of pheromone biosynthesis have long been addressed through biochemical, physiological, behavioral, and genetic analyses. Recently, molecular biology techniques have been brought to bear on these questions as well. The combination of molecular and biochemical techniques can have a synergistic effect on our understanding of various processes, and molecular techniques often provide keys to questions that are otherwise difficult to solve. Relatively new genomics technology also has great potential in revealing the components of pheromone biosynthetic pathways, endocrine regulatory elements, and their interactions. The use of biochemistry, "classical" molecular biology, and genomics (EST database construction and microarray expression analysis) is explored here in the context of juvenile hormone-regulated aggregation pheromone production in pine bark beetles and cotton boll weevils.

FROM FLY TO FLEA BEETLE: IDENTIFYING NON-LEPIDOPTERAN SEMIOCHEMICALS WITH THE AID OF GC-EAD

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Over the years coupled gas chromatography-electroantennographic detection (GC-EAD) has seen numerous successful applications, including characterization of antennal responses to pheromones and host odor. Originally developed for lepidopteran antennal responses, this technique is used more and more for the detection of non-lepidopteran semiochemicals. The equipment and setup for lepidopteran GC-EAD is relatively standardized due to the more uniform sizes and shapes of lepidopteran antennae. However, the diversity in antennal morphology of non-lepidopteran antennae dictates some minor but often unreported adaptations to the method of GC-EAD analysis. Examples of such adaptations are discussed with newly identified pheromones of sawflies (*Janus integer*, *Cephus cinctus*), flea beetles (*Aphthona* spp., *Phyllotreta* spp.), a nitidulid (*Colopterus truncates*), and the cereal leaf beetle (*Oulema melanopa*).

PRACTICAL USE OF SPME FOR QUALITATIVE AND QUANTITATIVE ANALYSIS

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Solid phase microextraction (SPME) is a commercially available technique for extracting organic chemicals from the air or aqueous media. The SPME device looks like a syringe, and its needle houses a polymer-coated silica fiber, into which the compounds are absorbed. For subsequent GC analysis, the samples are thermally desorbed from the fiber within the hot inlet of the gas chromatograph. The technique is solventless, sensitive, simple to conduct, rapid, and has become a valuable tool in chemical ecology. It has been used for both qualitative and quantitative analysis of insect host volatiles, pheromones, and other behavioral chemicals. Various fiber coatings are available, such as polydimethylsiloxane (PDMS) and carboxen/PDMS, and qualitative analyses can be optimized by choosing the appropriate fiber type. Quantitative analysis is somewhat complicated because the SPME fiber does not extract the sample matrix exhaustively, but instead reaches an equilibrium with it. Still, the kinetic behavior of the PDMS fiber is predictable and has been successfully modeled in terms of readily available information about the analytes and system being sampled. This model allows SPME to monitor the absolute flux of compounds from a natural source such as a fungal culture very quickly and with virtually no prior standardization. Thus SPME, along with suitable behavioral bioassays and methods for formulating synthetic compounds, can allow the effects of individual components in complex natural mixtures to be understood on a quantitative basis.

VIDEO METHODS FOR BEHAVIORAL BIOASSAYS AND ANALYSES

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Behavioral bioassays are used in the identification of semiochemicals and the delineation of the reactions they mediate. One approach is to design an uncomplicated assay that generates a (+) or (-) response. In assaying attractants, such all-or-nothing responses typically involve arrival at an upwind source of odorant, an event which can be monitored either by direct observation or by capture in a trapping device. A technically sophisticated approach might be to record the organism's movements and interpret these to establish attraction. Video records of flight tracks in a wind tunnel can provide a rich source of information, but does an analysis of such maneuvers provide insight into whether the full identity of semiochemicals is known or how odorants modulate orientation maneuvers? In the case of female mosquitoes flying to a bouquet of human skin odors and CO_2, the outcome of an attraction (or capture) bioassay can be sensitive to the duration of the test, and flight behavior near odor sources can be crucial to the assay's outcome. Flight tracks provide unique information about how odorant quality and plume structure modulate attraction. Such insight can be lost when the only measure of attractiveness is arrival at the odorant source.

COEVOLVED AND NON-COEVOLVED *Telenomus* PARASITOIDS (HYMENOPTERA: SCELIONIDAE) OF *Euschistus* spp. STINK BUGS (HETEROPTERA: PENTATOMIDAE): COMBINING THE BEST OF NORTH AND SOUTH AMERICAN STRAINS

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We are investigating the semiochemical cues used by geographically isolated strains of *Telenomus podisi* to find eggs of stink bugs in the genus *Euschistus*. Two strains of *T. podisi* are being maintained at Beltsville on eggs of the South American species, *Euschistus heros*; one parasitoid strain originated from specimens collected near Bras×lia, Brazil (SA strain), and a second strain originated from specimens collected at Beltsville, Maryland (NA strain). Preliminary evidence indicates that both strains are attracted to the vicinity of *Euschistus* spp. by host allomone and/or pheromone compounds. Furthermore, experiments using *E. heros* showed that SA *T. podisi* females specifically recognize traces left on the substrate by walking *E. heros* females and then intensively search the area of the "footprints", apparently looking for an egg mass to parasitize. On the contrary, NA *T. podisi* females are incapable or recognizing the footprints of *E. heros* females, despite the fact that these parasitoids were reared from eggs of *E. heros*. Separate experiments testing the cold tolerance of the NA and SA *T. podisi* strains demonstrated that NA *T. podisi* females are extremely cold tolerant (≈80% survival after 5 days at –4°C), a trait which facilitates handling of this potential biological control agent. Efforts are underway to identify the footprint contact pheromone and to artificially select a *T. podisi* strain combining cold tolerance and complete semiochemical recognition of *E. heros*, with the hope of utilizing this improved parasitoid strain for biocontrol of *E. heros* in Brazil and *Euschistus* spp. in the U.S.

CUES MEDIATING HOST LOCATION IN *Trissolcus basalis*: THE ROLE OF VOLATILES INDUCED BY HOST EGGS-PLANT INTERACTION

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Abstract: Parasitoids of herbivorous, sessile and non active hosts face the problem of using reliable and detectable cues during host location more than parasitoids of mobile and active hosts. While interactions between host eggs and plant are known when oviposition causes a wound, none have been shown for exposed eggs simply glued on the plant. In the first case, egg parasitoids were demonstrated to be attracted by host-induced volatile synomones, originated from the oviposition activity. In the second case, parasitoids are known to use cues from other host stages, like pheromones, which help in restricting the searching areas, thus enhancing the chance to encounter host eggs. Here we report laboratory observations, conducted in Y-tube olfactometer with the aid of an automated image system, on the influences of volatiles induced by host egg-plant interactions on *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae). *T. basalis* females were attracted to the odor of broad bean (*Vicia faba*) leaves on which eggs of the southern green stink bug, *Nezara viridula* (L) (Heteroptera: Pentatomidae) had been laid, whereas females were significantly less attracted by the other combinations tested, such as eggs alone or laid on filter paper, host-contaminated or uncontaminated leaves and others. Open arena bioassays with host egg clusters or glass models of the clusters are in progress in order to evaluate combined effects of visual cues from the eggs and/or chemical cues from the eggs, the substrate and their interaction.

NOVEL TECHNIQUES FOR UNRAVELING THE COMPLEX PHEROMONE MEDIATED BIOLOGY OF *DERMESTES MACULATUS* DEGEER

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An insect tracking system has proved to be a useful tool for distinguishing between responses to multiple pheromone signals in *Dermestes maculatus*. *D. maculatus* attacks animal products such as skins and hides but is most damaging as a pest to dried fish causing significant losses in many developing countries. Faecal pheromones and pheromones secreted by males and females have been shown to play an important role in the behaviour of this insect.
A computer tracking system, called Ethovision (Noldus Information Technology 1998), was used in this study. The equipment enabled insect movements to be detected and recorded as co-ordinates which could then be used for later analysis. The package allowed many parameters of behaviour to be analysed giving a broader behavioural description beyond simple preference testing, and enabled the specific behaviour of conspecifics in the presence of different pheromone sources to be characterised. This approach was used to differentiate between the pheromones secreted from the female beetle.

ANALYSIS OF HONEYBEE MANDIBULAR GLAND PHEROMONES USING SILICONE TUBING

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Martin Luther Universität Halle-Wittenberg, Halle, Germany

The development of an improved technique for the trapping and analysis of honeybee mandibular gland pheromone components using thin silicone tubing will be discussed and an application of the technique explained. The mandibular gland pheromones of honeybees consist of a series of C10 acids that can be analyzed gas chromatographically after derivatisation. The silicone tubing is pre-loaded with the derivatising agent and then used to trap the pheromones from living honeybees in a manner that is analogous to the use of SPME fibres. The silicone tubes with the trapped pheromones are then extracted in dichloromethane and the solution analysed gas chromatographically. The method allows for sequential analysis of pheromone signals from individual bees as they age. This technique has allowed us to follow changes in pheromone signals that are associated with changes in reproductive competence and made possible an analysis of pheromonal contests between individual worker bees in social groups. The technique could be used to explore pheromonal changes associated with dominance relationships in a variety of groups of social animals.

LAKE TAHOE: MOVING BEYOND THE CONFLICTS THROUGH FOUR DECADES OF CHANGE

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Aquatic ecosystems worldwide are under increasing anthropogenic stress and Tahoe is no exception. To solve this increasingly important problem necessitates rapid conversion of basic environmental studies into far-reaching management decisions. The construction of hydroelectric reservoirs in Africa and Central and South America, the proposed Three Gorges project in China, the continuing loss of clarity in Lake Tahoe, the demise of the Aral Sea in Russia, and new developments along Lake Baikal's shores in Russian Siberia all provide clear demonstrations of the global problems we face in the 21st Century. The conservation of lakes and streams as well as the protection of drinking water sources is of particularly urgent concern. Lake Tahoe is losing its remarkable transparency at a rate of 0.3 meters annually as algal growth rates increase about 5% per year. Atmospheric loading of nitrogen has converted this classic nitrogen limited lake to a lake now highly sensitive to phosphorus pollution. A multidisciplinary approach has been essential to developing effective water management strategies for solving increasingly complex environmental problems. Long-term data collection, including paleolimnological studies of sedimentation and pollutants, has been key to better understanding and managing the lake, its surrounding watershed, and basin air quality. In the past, many policy decisions by regulatory agencies have been based on scanty short-term data that are sometimes lacking methodologically or subject to superficial interpretation. Such a case occurred during a short-term drought-related improvement in transparency at Tahoe. Modern ecologists and limnologists have a responsibility to help meet the growing global challenge for restoration and preservation of increasingly threatened water supplies. Strong environmental science based on long-term studies must be at the forefront in developing improved adaptive management practices for aquatic ecosystems worldwide.

PHEROMONE BIOSYNTHESIS: DESATURASE GENES

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Research on biosynthetic pathways of sex pheromones in moths has revealed that the majority of known pheromone components are produced by various combinations of two unique enzyme systems, desaturases and chain-shortening enzymes, that produce an array of unsaturated long-chain fatty acetates, aldehydes and alcohols. The unusual desaturases discovered in moth pheromone glands are of interest because they function to produce unsaturated products that differ from the normal metabolic Z9-desaturases found in the rest of the insect body. Not only do they produce various Z11 fatty acids, but some generate mixtures of Z and E geometrical isomers, which are rare in nature. The evolution of these desaturases has played a key role in the generation of the diversity of chemical blends used as species-specific mating signals by many moth species, and, thus, is significantly involved in the speciation process.

Full-length cDNA that encodes a Z11 desaturase from the sex pheromone gland of the cabbage looper moth was initially characterized, and a functional assay utilizing a mutant yeast expression system confirmed that it produces Z11-16 and Z11-18 acids. cDNA clones for pheromone desaturases of the redbanded leafroller moth and European corn borer, which produce mixtures of E/Z11-14 acids, were characterized, but different expression systems had to be developed for the functional assays. Desaturase genes were then characterized for the lightbrown apple moth, which uses E11-14 and E9,E11-14 pheromone components, and for a primitive New Zealand leafroller that uses a Z10-16 desaturase to produce its pheromone.

PBAN REGULATION OF LEPIDOPTERAN PHEROMONE PRODUCTION

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Pheromone Biosynthesis Activating Neuropeptide (PBAN) has been implicated in the stimulation of pheromonotropic activity of several lepidopteran species. The neuropeptide shares a common C-terminal ($FXPRL\text{-}NH_2$) with a functional diverse peptide family (PBAN/pyrokinin/myotropin). It has been isolated and its sequence determined from six different species of Lepidoptera although its regulatory role in pheromone biosynthesis has been demonstrated in over 20 different lepidopteran species. Much evidence has accumulated indicating its direct role on the pheromone producing cells associated with the intersegmental membrane situated between the 8^{th} and 9^{th} abdominal segments of the ovipositor tips of female moths. Normal activation of pheromone production may involve several interdependent mechanisms including both neural and humoral modulators. These modulators may be differentially activated but closely coupled depending on both physiological and environmental factors. The present review focuses on PBAN's mode of action and its modulation at the tissue and cellular levels with special emphasis on signal transduction and receptor activation.

PHEROMONE BIOSYNTHESIS IN MOTHS

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Sex pheromone biosynthesis in female moths typically involves fatty acid synthesis followed by desaturation, chain shortening or elongation, and functional oxygen group synthesis. Most moth sex pheromones, thus utilize acetate esters, alcohols, aldehydes, and epoxides. Some moths utilize hydrocarbons lacking an oxygen functional group. The biosynthesis of these molecules is determined by the type and specificity of the enzymes involved in the biosynthetic pathway. This talk will focus on the biosynthesis of sex pheromone in moths that utilize oxygenated molecules and in moths that use hydrocarbons. Pheromone biosynthesis in female moths will also be discussed with regard to the regulation of biosynthetic pathways. The production of oxygenated pheromones is usually regulated by the peptide hormone PBAN (Pheromone Biosynthesis Regulating Neuropeptide). Background information will be presented on the biosynthesis of oxygenated pheromones and the regulation of their biosynthesis by PBAN. The regulation of the production of hydrocarbon pheromones is not clearly understood but may not require the presence of PBAN. The biosynthesis and regulation of hydrocarbon based pheromones will also be discussed.

REGULATION OF PHEROMONE PRODUCTION BY ECDYSTEROIDS AND JH

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Ovarian produced 20-hydroxyecdysone induces the biosynthesis of the female housefly sex pheromone components, including (Z)-9-tricosene, cis-9,10-epoxytricosane, (Z)-14-tricosen-10-one and a series of methylalkanes. About two days post-emergence, the female becomes vitellogenic and begins producing ecdysteroids which induce pheromone production. The pheromone components accumulate first in the hemolymph associated with lipophorin and, followed by about a 24 hr delay, on the surface of the insect.. The hydrocarbon components are produced by the chain elongation of fatty acyl-CoAs, which are then reduced to the aldehyde and decarboxylated to the hydrocarbon. Ecdysteroids regulate the chain length specificity of the fatty acyl-CoA elongases, and not the reductive conversion of very-long chain fatty acyl-CoAs to hydrocarbons. Putative cytochrome P450s, which function in the decarboxylation of the aldehyde, have been cloned, sequenced and modeled. JH induces monoterpenoid (C10) pheromone production in male *Ips pini* and *Dendroctonus jeffreyi*. In *I. pini*, feeding induces JH production which upregulates 3-hyrdroxy-3-methylglutaryl-CoA reductase (HMG-R), and to a lesser extent, HMG-CoA synthase. *In situ* hybridization localized pheromone production to midgut tissue. The first geranyl diphosphate synthase to be isolated from animal tissue was cloned and sequenced from a midgut library from *I. pini*. Molecular modeling of this putative geranyl diphosphate synthase showed interesting similarities and differences to farnesyl diphosphate synthase.

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PRODUCTION OF CUTICULAR PHEROMONES OF DROSOPHILA MELANOGASTER AND RELATED SPECIES

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The cuticle of *Drosophila melanogaster* is covered with a layer of lipids including long-chain hydrocarbons. Two of them are mainly produced by mature males males (7-tricosene,7T and 7-pentacosene,7P) and can act on conspecifics either as attractants for females and repellents for other males. The main mature female hydrocarbons are longer and act as contact pheromones after males tap female cuticle (7,11 heptacosadiene, 7,11 HD and 7,11nonacosadiene, 7,11 ND). Contrary to those of the rest of the world, females from sub-saharan African strains show low levels of 7,11 HD and high levels of its position isomer, 5,9 HD. Other species as the sympatric sibling species *D. simulans* are sexually monomorphic, with a large production of 7T and 7P together with 2methyl-branched HCs as in *D. melanogaster* flies of either sex; in 5 such species of the melanogaster subgroup,7T acts as a sex pheromone.

The biosynthesis of these compounds shares a number of steps (synthesis of unsaturated fatty acids, desaturation, elongation and decarboxylation) while some steps are sex-specific, especially for female diene production (female-specific desaturation of monoenic fatty acids and their elongation and decarboxylation.) The common steps involve among others a Δ9 desaturase and one or more elongase(s). We have characterized molecularly and functionally a Δ9 desaturase in *D. melanogaster* and in *D. simulans*, Desat1, which transforms palmitic acid into palmitoleic acid and seem to lead to ω7-HCs. Indeed in position 7 *desat1* mutants show low levels of such unsaturated hydrocarbons. Another desaturase gene has been characterized in *D. melanogaster*, which is translated only in females of the 5,9 HD rich morph. *desat2*, located near *desat1*,encodes another Δ9 desaturase which uses myristic acid as substrate leading to the expected ω5-fatty acids necessary for the diene biosynthesis. We are searching for the enzymes involved in the female-specific steps and a number of preliminary data will be presented and discussed.

SYNTHESIS AND HEMOLYMPH TRANSPORT OF HYDROCARBONS AND SEX PHEROMONES

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The outer surface of insects is covered with a lipid layer that provides water-proofing and protection against environmental stresses. Hydrocarbons are major constituents of this epicuticular wax and in some insects they are also exploited as biosynthetic precursors for contact pheromones. In the German cockroach hydrocarbons and pheromones are synthesized by oenocytes which are situated in the integument. Synthesis of hydrocarbons is dependent to a large extent on food availability, but its sex-specific conversion to pheromone in the adult female is regulated by juvenile hormone. An important site for deposition of hydrocarbons in females is the ovary. Shuttling of hydrocarbons from the abdominal integument to the epicuticle, fat body, and gonads uses a versatile lipoprotein, lipophorin, which carries both hydrocarbons and the contact sex pheromone, and also serves as a juvenile hormone-binding protein. In several tiger moth species and in the housefly, short- and medium-chain hydrocarbons sex pheromones are also transported by lipophorin from integumental biosynthetic sites to sex pheromone glands or the epicuticle. In *Holomelina* tiger moths pheromone is synthesized by tissues associated with the abdominal integument. Lipophorin transports the pheromone to an abdominal gland that stores and releases the pheromone only during active calling behavior. We suggest that such transport pathways are common not only among insects that emit hydrocarbon pheromones, but also among insects that sequester hydrophobic plant-derived metabolites.

PHEROMONE BIOSYNTHESIS IN THE YELLOW MEALWORM BEETLE

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The biosynthetic pathways used by beetles to produce their pheromones, and the mechanisms by which these pathways are regulated, have been the targets of much interest. However, most studies have focused on the biochemistry of *aggregation* pheromone production. We have examined the biochemistry of *sex* pheromone production in a "model" beetle, the yellow mealworm, *Tenebrio molitor*. Early studies by other workers established that mature females elicit a greater response from males than immature females, and that production of the female-produced sex pheromone is regulated by Juvenile Hormone (JH). The long-term objective of our study is to determine the mechanisms by which pheromone production is regulated in female yellow mealworms. We have elucidated the biosynthetic route to 4-methylnonanol, and used this information to develop both *in vivo* and *in vitro* radio-assays for 4-methylnonanol biosynthesis. We have also developed bioassays to study the pheromone-mediated mating behavior. Using both biological and biochemical approaches, we have examined the effects of aging (post-eclosion), mating, and JH analogue treatment on pheromone biosynthesis. We have confirmed that 4-methylnonanol production is stimulated by JH, and identified one enzyme involved in the biosynthesis of 4-methylnonanol that is regulated by JH. We have also determined that mating induces a temporary decrease in sex pheromone production in the female. We are currently investigating the biochemical mechanisms for these processes. With this information in hand, we can begin to determine the mechanisms by which JH and mating exert their effects on pheromone biosynthesis in female *T. molitor*.

REGULATION OF SEX PHEROMONE BIOSYNTHESIS IN SCARAB BEETLES

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Previously, we have demonstrated that biosynthesis of buibuilactone [(*R,Z*)-(—)-(oct-1-enyl)oxacyclopentan-2-one], japonilure [(*R,Z*)-(—)-(dec-1-enyl)oxacyclopentan-2-one], and other lactones, utilized in the chemical communication of scarab beetles (subfamily Rutelinae), starts from saturated fatty acids, stearic and palmitic acid, involves their desaturation, followed by enantiospecific 8-hydroxylation, chain shortening, and cyclization. Biosynthesis of these scarab beetle pheromones is regulated by a brain factor. The pheromone titer in the glands of decapitated *A. cuprea* females dramatically decreased 24 h after surgery, but it was resumed after the injection of extracts from the brains of virgin females. These extracts lost activity after treatment with proteinase K. On the other hand, synthetic *Bombyx mori* PBAN mimicked the effect of brain extracts in *A. cuprea*. These findings suggest that in scarab beetles, a PBAN-like factor is involved in regulation of biosynthesis. Attempts to characterize the gene encoding the peptides by library screening and PCR were unrewarding. On the other hand, a bioassay-oriented strategy led to the isolation of active fractions separated by HPLC and ion-exchange chromatography. Due to the scanty amounts of material, characterization of the PBAN-like factor in scarab beetles is yet to be acoomplished.

I appreciate the direct or indirect participation in this project of various members of my past and present research groups, in particular, Jong-yoon Kim, Hubert Wojtasek, Paulo H. G. Zarbin, Makoto Hasegawa, Jean-Francois Picimbon, Zhang Deyu, Larisa Nikonova, Yasuo Murata, Göde Schüler, and Yuko Ishida.

TWO ROUTES FOR PHEROMONE BIOSYNTHESIS IN THE MOTH, *Epiphyas postvittana* (Walker): POSSIBLE CONSEQUENCES FOR REGULATION.

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The sex pheromone components of many species of moths are structurally similar. This is largely a consequence of common biosynthetic and regulatory mechanisms used by these species; i.e, these, compounds are biosynthesized de novo, through synthesis and limited metabolism of saturated fatty acids, and their biosynthesis is regulated by the neuropeptide PBAN (itself a fairly, well-conserved structure among species). Given these common features, it is somewhat surprising, in the limited number of studies that have been attempted, that PBAN has been found to influence many (but only one for a given species) of the different steps involved in pheromone biosynthesis. Thus, PBAN has been found to influence fatty acid synthesis, □–oxidation, the reduction of fatty acyl precursors, the hydrolysis of triglycerides, and an acetyl transferase. Taken together, these studies suggest that either the role of PBAN in regulating pheromone biosynthesis in moths is more complex than thought, or that other regulatory factors confound the role of PBAN in some of the studies. Our recent work on pheromone biosynthesis in the moth, *Epiphyas postvittana*, has identified two routes that pheromone biosynthesis may proceed; a direct route proceeding through the various fatty acyl CoA esters or a less direct route involving hydrolysis of fatty acyl pheromone analog-containing triglycerides. The consequences of this result in terms of PBAN regulation and its study will be discussed.

PBAN REGULATION OF SEX PHEROMONE BIOSYNTHESIS IN TWO MOTH SPECIES, *Dendrolimus punctatus* and *Operophtera brumata*

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Both *in vivo* and gland *in vitro* experiments showed that the sex pheromone production in the female pine moth, *D. punctatus* (Lepidoptera: Lasiocampidae) is controlled by PBAN or a PBAN-like factor found in the head of female moths. *In vitro* experiments showed that the presence of calcium in the incubation medium was necessary for stimulation of pheromone production. The calcium ionophore, A 23187, alone could stimulate pheromone production. The pheromone component (*Z,E*)-5,7-dodecadienol and its acetate and propionate esters were produced in these experiments but in addition the aldehyde, (*Z,E*)-5,7-dodecadienal, was also found. This indicates that females are capable of producing four oxygenated functional groups. Labeling experiments suggest that PBAN controls pheromone biosynthesis by regulating an early step in or prior to fatty acid biosynthesis.

The titre of the pheromone, (*Z,Z,Z*)-1,3,6,9-nonadecatetraene in the female winter moth, *O. brumata* (Lepidoptera: Geometridae) was significantly decreased after decapitation. However, when a decapitated female was injected with as little as 0.1 pmol Bom-PBAN or 0.2 head equivalent of female head extract, the pheromone titre was restored to a level that was found in normal females. These data suggest that the hydrocarbon pheromone production in *O. brumata* is regulated by PBAN or a PBAN-like substance.

DE NOVO ISOPRENOID PHEROMONE PRODUCTION OCCURS IN MIDGUT TISSUE IN BARK BEETLES

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3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-R) has been shown to be a key regulated enzyme in the pathway leading to the isoprenoid pheromone ipsdienol in the pine engraver beetle, *Ips pini* (Say), and frontalin in the Jeffrey pine beetle, *Dendroctonus jeffreyi* (Coleoptera:Scolytidae). Reasoning that *HMG-R* expression would be greatly elevated in tissue(s) producing the pheromone, a 3201 bp *HMG-R* cDNA from *I. pini* was isolated and sequenced, revealing a 2601 bp open reading frame encoding 867 amino acids. Upon alignment, the catalytic domain was 41% identical to four other HMG-R proteins (three insects and human) with less than 9% identity to the prokaryotic HMG-R from *Pseudononas mevalonii.* For JH III-treated males of both species, Northern blotting indicated the highest transcript level for *HMG-R* at the abdominal-thoracic interface. *In situ* hybridization with a riboprobe prepared from the 3' end of *HMG-R* localized these high transcript levels to the anterior midgut. For *I. pini*, *HMG-R* expression in the anterior midgut was sex, juvenile hormone, and feeding dependent, as indicated by *in situ* hybridization. Further, isolated anterior midgut tissue from fed or juvenile hormone III-treated males incorporated radiolabeled acetate into ipsdienol in *I. pini* and frontalin in *D. jeffreyi*, providing strong evidence that this is the site of pheromone production. The up-regulation of a metazoan *HMG-R* does not support bacterial synthesis of pheromone. To our knowledge, this is the first time that alimentary canal tissue has unambiguously been shown to produce pheromone in insects.

A NOVEL SEX-SPECIFIC AND INDUCIBLE MONOTERPENE SYNTHASE ACTIVITY ASSOCIATED WITH THE BARK BEETLE, *IPS PINI* (SAY)

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Myrcene is an acyclic monoterpene that has long been associated with pheromone synthesis in pine bark beetles [*e.g.* the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae)]. The logical source of myrcene for bark beetle pheromone biosynthesis has been the oleoresin of the host pines. While monoterpene synthases, which convert geranyl diphosphate to monoterpenoid natural products, have been frequently characterized from conifers and other plants, they have not yet been found in animals. We report the discovery of a monoterpene synthase activity in two populations of an insect, *I. pini*. Cell-free assays of *I. pini* revealed that ^{3}H-geranyl diphosphate is converted to ^{3}H-myrcene in whole-body extracts from male, but not female, *I. pini*. Furthermore, the enzyme activity in males can be induced by prior treatment with juvenile hormone III (JH III) or by feeding on phloem from the host trees, Jeffrey pine, *Pinus jeffreyi* Grev. & Balf, or red pine, *Pinus resinosa* Ait. The sex-specificity and endocrine induction of this activity argue for its involvement in the biosynthesis of monoterpenoid pheromones mediated by enzymes from *I. pini* rather than from microbial symbionts. We believe that the discovery of a myrcene synthase activity in *I. pini* provides the strongest evidence to-date for synthesis of a monoterpene in the Metazoa.

The terpene synthase gene family in *Zea mays* and its role in defense against herbivory

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Secondary plant metabolites provide protection against pathogens and herbivores not only by acting as phytoalexins, toxins and feeding deterrents (direct defense), but also by attracting natural enemies of the herbivores (indirect defense). Several volatile metabolites are thought to have a role in indirect defense by serving as a signal in tritrophic interactions between plants, herbivores and parasitoids.
Upon attack by lepidopteran larvae, maize (*Zea mays*, L.) emits a mixture of volatiles that is dominated by mono- and sesquiterpenes. In order to understand the function of volatile emission and its regulation by herbivore damage, we are investigating the molecular genetics and biochemistry of terpene biosynthesis. A key step in terpene biosynthesis is catalysed by the enzyme class of terpene synthases. We have isolated several maize terpene synthase genes which form a heterogeneous family. To identify the function of these genes, the catalytic properties of five genes have been determined after functional expression in *E. coli*. Each terpene synthase forms a more or less complex mixture of mono- and sesquiterpene products, which are constituents of the terpene blend of maize plants. We are currently studying the regulation of terpene biosynthesis in maize with the aim to identify the functional role of specific terpenes in the direct and indirect defense of the plant.

ROLE OF CUTICULAR HYDROCARBONS IN MATE RECOGNITION OF LONG-HORNED BEETLES

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The rustic borer, *Xylotrechus colonus*, is a crepuscular long-horned beetle whose larvae feed under the bark of many eastern American hardwood trees, especially hickory. Adult rustic borers are brought together by their mutual attraction to the larval host; once on the host, males locate and recognize females by touching them with their antennae. Male *X. colonus* did not respond to hexane-washed females, but attractiveness was restored when the hexane extract was applied to these females, demonstrating that the active compounds were isolated in the extract. We have identified cuticular hydrocarbons present in females but absent (or in small quantities) in males, and bioassays confirm these are the contact pheromones. These compounds include pentacosane, 9-methylpentacosane, and 3-methylpentacosane.

Chemically Mediated Interactions Between Native Ants and the Invasive Argentine Ant: The Role of Cuticular Hydrocarbons in Species Recognition

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Ants, like all terrestrial arthropods, possess a coating of lipids on their cuticle that functions, in a primary sense, to prevent desiccation. Qualitative and quantitative variation in these lipids, particularly in cuticular hydrocarbons, can act to communicate information between conspecific and heterospecific ants. In this study, I used a behavioral bioassay to measure the response of Argentine ants (*Linepithema humile*), an invasive pest of Mediterranean-type climates worldwide, to 1) whole lipid extracts of native ants versus a blank control and 2) cuticular hydrocarbon versus polar lipid fractions of the native ant whole lipid extracts. The Argentine ant causes dramatic reductions in native ant abundance as it invades new areas by out-competing natives for food resources and also by fighting. The current study was conducted using three species native to Northern California (*Messor andrei*, *Formica Moki* and *Prenolepis imparis*) that are known to interact with Argentine ant. The whole lipid extracts of all three native species elicited more aggression from Argentine ants than the blank control. Further, the cuticular hydrocarbon fraction of the whole lipid extract elicited higher levels of aggression from Argentine ants than the polar lipid fraction for all three native species.

DISCRIMINATION OF BLENDS OF HONEY BEE RECOGNITION PHEROMONES

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Honey bees, *Apis mellifera*, use colony-specific blends of fatty acids to discriminate nestmates from non-nestmates. Palmitic, palmitoleic, oleic, linoleic, linolenic, and tetracosanoic acids all occur in bees' wax. These compounds are transferred to the surface of the bees, giving them a recognitive signature. In an artificial five-compound blend of acids, each acid is treated as an important component of the colony's signature. Guard bees learn and use artificial blends in making discriminations between nestmates and non-nestmates. Foragers treated with the same blend as the guard are accepted as nestmates by the guard. The absence of any one acid from the blend causes guard bees to treat the forager as a non-nestmate. Experiments using pairs of the pheromones suggest that bees do not make discriminations by using simple ratios of compound concentrations. Instead they rely on the concentrations of each compound, but may use compounds as referrents for interpreting concentrations of other compounds. This system of acquisition of recognition pheromone blends from the comb wax gives guard bees a simple and efficient method of detecting non-nestmate intruders in the colony.

IDENTIFICATION AND ASSAY OF CHEMICAL RECOGNITION SIGNALS FOR SCORPIONS (Scorpionida: *Hadrurus arizonensis*)

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Scorpions have never been shown to use chemical signals for intraspecific communication. We used "tail-rubbing" behavior of *Hadrurus arizonensis*, the largest of North American scorpions, as a model for isolation and assay of chemical signals used by this species. Previous studies show non-polar extracts of whole animal cuticle elicit sex and species-specific behaviors. The whole extract contains a mixture of long chain hydrocarbons (C20 to C41), *n*-alcohols (C18 to 26), aldehydes (C22-C31), and not common acid esters with long chain alcohols (C26 to C30) esterified to short chain (C2 to C6) acids. Principal component analysis of >80 cuticular compounds of individual adult *Hadrurus* (n=10 each sex) showed males expressed elevated levels of the esters. These substances were particularly abundant in tail gland extracts of reproductively active males suggesting they may be some forms of aphrodisiac. The main esters were synthetized and a first trial with the triacontyl-acetate (the major male compound) shown to be active in eliciting tail-rubbing behavior. This C30 alcohol ester thus becomes the first semiochemical identified for any member of the Order Scorpionides. Behavioral and electrophysiological tests of other C4 and C6 esters, also sexually dimorphic and characteristic of seasonal males, are underway. The apolar nature of these molecules suggests they are appropriate for deposition on the natural substrate (sand) where they can be detected by surface contacting chemoreceptive organs.

HYDROCARBONS AND NESTMATE RECOGNITION IN ARGENTINE ANT, *LINEPITHEMA HUMILE*

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Intercolonial aggression in the Argentine ant, *Linepithema humile*, is observed between colonies reared on different insects. This aggression behavior is mediated by cuticular hydrocarbons the ants acquire from the insect prey. The prey hydrocarbons that elicit the strongest nestmate aggression are similar to but different from previously unknown groups of long-chain hydrocarbons in Argentine ants. Variations in these groups of hydrocarbons are also responsible for intercolonial aggressions observed in field Argentine ant populations.

PROBING THE PLANT SURFACE: THE COMPOSITION OF LEAF EPICUTICULAR WAXES AND THEIR POTENTIAL EFFECTS ON INSECTS AND FUNGI

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Plant leaves are covered by a cuticle consisting of a polymer matrix and soluble cuticular waxes. A specific portion of these waxes is located in a thin film on the very surface of the organ. As these 'epicuticular waxes' play an important role in host recognition by fungi and insects, detailed information on their composition is needed.

We have developed methods for the first time enabling the selective preparation and quantitative analysis of epicuticular wax films. On *Prunus laurocerasus* leaves large amounts of pentacyclic triterpenoids were located in the inner parts of the cuticle, while long-chain aliphatics were concentrated in the epicuticular film. Thus, the leaf surface consisted of ubiquitous plant wax constituents, while species-characteristic lipids were hidden underneath. It is especially noteworthy that the triterpenoids, known to possess insecticidal and fungicidal properties, were not located at the surface where they could serve as a first line of defense. A detailed analysis of the seasonal development of *P. laurocerasus* leaves revealed distinct stages of epicuticle formation. Acetates, alcohols and alkanes consecutively accumulated in a film with steadily increasing thickness (≈ 70 nm after 60 d). The accumulation rates are indicative for diffusional transport and spontaneous segregation of intra- and epicuticular fractions.

For other plant species we also found characteristic layers of cuticular components: on leaves of *Cerinthe minor* fungicidal δ-lactones are concentrated near the surface; similarly, the furanocoumarins synthesized by *Apium graveolens* are located at the leaf surface shortly after fungal infection and hence can serve as contact phytoalexins.

INSECT EGG DEPOSITION INDUCES *PINUS* TO "CALL" FOR EGG PARASITOIDS

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Egg depositions of the herbivorous pine sawfly *Diprion pini* were shown to induce the pine (*Pinus sylvestris*) to emit volatiles (synomones) that attract the egg parasitoid *Chrysonotomyia ruforum*, an eulophid wasp. Studies on the spatial and time scale of this induction process showed (a) that oviposition of *D. pini* may induce the emission of synomones locally and systemicly, and (b) that synomones are released already 24 h after egg deposition and are still emitted 72 h later. Experiments on the mechanisms of this induction revealed that an elicitor is located in the sawfly´s oviduct secretion which is attached to the eggs. Pine twigs supplied systemicly with jasmonic acid also released volatiles that attract the egg parasitoid. Chemical analyses of volatiles released by oviposition-induced pine twigs are currently conducted. The results of this study parallel those obtained by our earlier studies of oviposition-induced emission of synomones in elm. Thus, up to now, both a gymnosperm and an angiosperm tree have been shown to be able to respond to herbivore attack prior to feeding damage by the larvae and to „call" for natural enemies as soon as eggs are laid on them. The emission of synomones in response towards egg depositions may be considered as a preventive strategy which acts just in time prior to (further) damage by feeding of herbivorous larvae.

Compositional changes of monoterpene enantiomers in response to fungal inoculation in Scots Pine *

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Scots Pine trees (*Pinus sylvestris*) were subjected to pretreatment by wounding only or inoculation with either one of the blue-stain fungi *Leptographium wingfieldii* or *Ophiostoma canum*. Four weeks after pretreatment, the trees were mass inoculated with *L. wingfieldii*. Absolute amounts, as well as relative and enantiomeric compositions of monoterpene hydrocarbon were determined in the phloem before and after pretreatment and mass inoculation, using 2D-GC and GC-MS. The 2D-GC system (Borg-Karlson et al 1993) was a successful tool to monitor the dynamics of resin constituents in pine tree phloem during fungal growth. The absolute amounts of most monoterpenes decreased in the phloem sampled >20 cm from the fungal infection, and increased in the phloem sampled within the infection reaction zone. After mass inoculation, the relative amounts of both (–)-□-pinene and (–)-limonene increased in phloem samples taken >20 cm from the fungal inoculation in the pre-inoculated trees compared with phloem sampled from the control trees. The enantiomeric compositions of (–)-□-pinene and (–)-limonene changed after fungal growth at defined distances from the inoculation site, being most pronounced in phloem sampled >20 cm from fungal inoculation. Pathogen virulence did not show any effect on the enantiomeric composition, but growth of the highly virulent *L. wingfieldii* enhanced monoterpene production in the phloem at the site of inoculation to a greater extent compared to growth of *O. canum*. The biosynthesis of certain monoterpene enantiomers evident in Scots Pine is discussed in relation to induced resistance.

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TESTING THE DEFENSIVE FUNCTION OF TERPENOID RESIN IN NORWAY SPRUCE (*PICEA ABIES*)

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The terpenoid oleoresin of conifers has long been thought to function in defense against herbivores and pathogens, but its actual role has been difficult to demonstrate. We have begun to investigate the biochemistry and molecular biology of terpenoid resin formation in Norway spruce (*Picea abies*) in order to develop tools to test resin function under natural conditions. The major constituents of *P. abies* resin are monoterpene olefins and diterpene carboxylic acids, which accumulate in ducts found in stems and foliage. Bark beetle or pathogen attack on *P. abies* stems leads to the formation of additional resin at the wound site. We demonstrated that this effect can be mimicked by application of methyl jasmonate, providing a convenient tool for manipulating resin accumulation. Additional resin formation is preceded by increased activities of two classes of enzymes, prenyltransferases and terpene synthases. The isolation of genes encoding these enzymes is now underway and several cloned genes are being transformed into *P. abies* embryogenic cell cultures in an effort to produce transgenic plants with altered resin composition.

Abstracts for Posters

CHARACTERIZATION OF A MYRCENE SYNTHASE FROM BIOREACTOR CULTURES OF THE MARINE RED ALGA *OCHTODES SECUNDIRAMEA*

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A monoterpene synthase from suspension cultures of the marine red alga *Ochtodes secundiramea* is shown to biosynthesize myrcene from geranyl diphosphate (GPP) using cell free extracts. This is the first in vitro characterization of any monoterpene synthase from a marine organism. Myrcene is the likely progenitor of the unusual halogenated monoterpenes characteristic of this marine alga and, as such, represents a key step in this metabolic pathway.
Mechanistic considerations based on reaction products from the biologically relevant substrate GPP, as well as from neryl diphosphate (the cis isomer of GPP) and linalyl diphosphate suggest that the enzyme is of evolutionarily ancient origin. The ability to assay and quantitatively monitor the expression of this enzyme in suspension cultures of the alga, under strictly defined growth conditions, provides an unparalleled opportunity to delineate, at the molecular level, factors eliciting the biosynthesis of this class of secondary metabolites, to evaluate the metabolic pathway leading to the halogenated monoterpenes and to investigate their role in the chemical ecology of marine algae.

HYDROCARBON SYNTHESIS BY OENOCYTES AND ITS DELIVERY BY LIPOPHORIN TO OOCYTES AND CUTICLE OF THE GERMAN COCKROACH

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Hydrocarbons (HCs) water-proof the insect cuticle and also serve as semiochemicals. In the German cockroach, HCs are synthesized by the abdominal integument, and they are deposited primarily on the epicuticular surface. A mild enzymatic dissociation and differential filtration separated oenocytes from epidermal cells. Only the oenocytes, identified by TEM, synthesized HCs, while the much smaller epidermal cells did not. Our results conclusively show that purified oenocytes produce HCs. The ovaries contain a major fraction of the internal HC pool in adult females. Because oocytes and associated tissues do not synthesize HCs we hypothesized a hemolymph transport axis. *In vivo* studies with a native *Blatella* HC, [^{3}H]3,11-dimethylnonacosane, or with[1-^{14}C]propionate, which becomes incorporated into methyl branched HCs, revealed that newly synthesized HCs were taken up by the ovaries in relation to oocyte maturation. *In vitro* incubations of ovaries with sternites, which synthesize HCs, showed that hemolymph was mandatory for ovarian uptake of HCs, with maximum uptake requiring >10% hemolymph. Similar results were obtained with high density lipophorin isolated from hemolymph and purified by KBr gradient ultracentrifugation; maximum uptake required >1 mg/ml lipophorin. This is the first report showing that large amounts of maternal HCs are provisioned in oocytes, and the existence of a HC transport pathway involving hemolymph lipoproteins. How HCs are delivered by lipophorin to the ooplasm, and the fate and role of embryonic HCs is under investigation.

PARTHENOGENESIS, CALLING BEHAVIOUR AND POTENTIAL SEX PHEROMONE OF THE LEAFMINER MOTH *PHYLLONORYCTER EMBERIZAEPENELLA*

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It was proved that the leafminer moth *Phyllonorycter emberizaepenella* (Lepidoptera: Gracillariidae) reproduced by parthenogenesis of the thelytoky type. Despite a complete lack of males, parthenogenetically reproducing females diurnally demonstrated the calling posture, normally used for releasing signalling compounds. Two compounds, which we collected from a calling female, were identified as potential sex pheromone components: (8*E*,10*E*)-8,10-tetradecadienyl acetate and (8*E*,10*E*)-8,10-tetradecadienol, the latter occurring only in trace amounts. In the field experiments no males were attracted to traps baited with either the potential sex pheromone or with virgin females. Both the pattern of behaviour and the chemical characteristics of the pheromone of *Ph. emberizaepenella* species were similar to those known for Lepidoptera with the usual amphimictic mode of reproduction. Theoretical speculations that in thelytoky, where there is no need to find a sexual partner, the individuals would obtain certain advantages due to reduction in their sexual behaviour, were not confirmed for *Ph. emberizaepenella*.

BEHAVIORAL AND ELECTROPHYSIOLOGICAL RESPONSES OF *SUPELLA LONGIPALPA* TO SEX PHEROMONE STEREOISOMERS

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Females of the brownbanded cockroach, *Supella longipalpa*, produce a sex pheromone (supellapyrone), which has four possible isomers: RR, RS, SR, and SS, the first one being the natural compound. Behavioral and EAG dose-response curves were developed for each one of the four stereoisomers. 50% of the males responded to 0.3 pg of RR and to 30 pg of SR and SS. However, no response was observed at any concentration of RS (0.3 pg to 1 ng). The addition of RS to RR did not decrease the attractant potency of the later. RR and SR produced similar EAG amplitudes at the same concentrations. However, the antenna was not very responsive to SS or RS. Therefore, EAG was not a good predictor of behavior. EAG peak-width at half peak-height was larger for SR than for RR, which suggests that some aspect of the interaction between the pheromone and the sensillum environment or the receptor neuron is different between these two compounds. Alternatively, RR and SR may stimulate different types of antennal sensory neurons. This possibility will be investigated with electrophysiological recordings of individual sensilla.

Coronatine Analoga as Molecular Probes of the Secondary Plant Metabolism

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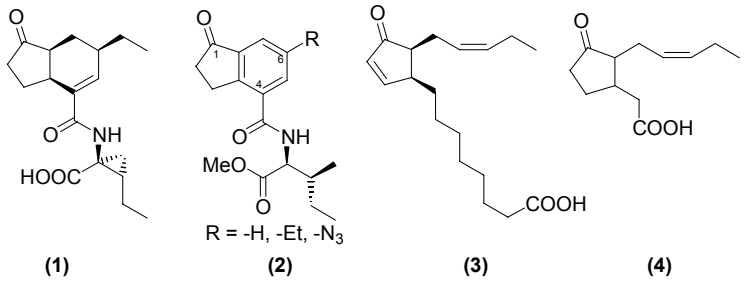
Coronatine **(1)** is known as a highly active phytotoxin, which is produced by several pathovars of *Pseudomonas syringae* (eg. *tomato, glycinea*). Coronatine can activate the stress signaling of plants, which results for example in volatile emission, tendril coiling or highly increased production of Taxol®. Its biological properties are very similar those of the phytohormone jasmonic acid (JA, **4**) and its early intermediate 12-oxo-phytodienoic acid (PDA, **3**). Due to this structural analogy and its wide range of biological activities coronatine is an important tool to study the secondary plant metabolism.



As the synthesis of coronatine is very difficult and as preparation in large scale is almost impossible, we looked for a similar structure that is easy to prepare. Beside the unsubstituted 1-oxo-indanoyl-isoleucine conjugate **(2a)** and its azido derivate **(2c)** we now synthesized the 6-ethyl-1-oxo-indanoyl-isoleucine **(2b)**. This new compound is almost as active as the original coronatine. It showed an enormous effect on the production of benzo[c]phenanthridine alkaloids in *E. californica.* Various other bioassays showed its ability to mimic the JA- and PDA-like effects of coronatine. This coronatine substitute is an easily accessible tool to increase the production of commercially interesting compounds of the secondary plant metabolism.

OCCURRENCE AND SIGNIFICANCE OF PHEROMONE VARIATION AMONG DENNING POPULATIONS OF RED-SIDED GARTER SNAKES (*THAMNOPHIS SIRTALIS PARIETALIS*)

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In species that utilize pheromones to initiate and orchestrate reproductive behaviors, intraspecific variation in pheromone production and expression can have significant reproductive consequences. For example, variation in pheromone expression among geographically isolated populations may result in sexual isolation among the populations if the variation disrupts behavioral interactions. Utilizing behavioral trials and chemical analyses, we examined whether pheromonally mediated sexual isolation exists between isolated populations of red-sided garter snakes (*Thamnophis sirtalis parietalis*) in Manitoba, Canada. In simultaneous choice tests, adult males from a population of red-sided garter snakes in central Manitoba displayed a strong courtship preference for females from their own population over females from a population in western Manitoba, whereas males from the western Manitoba population showed no such preference. Chemical analyses of the female sexual attractiveness pheromone, the cue primarily responsible for eliciting male courtship behavior in garter snakes, revealed significant variation in the composition of the pheromone among the two populations. Specifically, the two populations varied in the relative concentrations of individual unsaturated methyl ketones expressed by females on their dorsal surface. These results suggest that a degree of sexual isolation via variation in pheromone expression does exist among isolated populations of red-sided garter snakes. Furthermore, populations of red-sided garter snakes in central Manitoba, unlike populations in western Manitoba, share hibernacula with the plains garter snake (*T. radix*), suggesting that pressure to recognize conspecific females may drive the pattern of sexual isolation observed in this species.

INTERACTION BETWEEN FRUGIVOROUS BATS AND *Piper gaudichaudianum* FRUITS

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Some frugivorous bats, as *Carollia perspicillata* and *Sturnira lilium,* are the most important consumers of Piperaceae ripe fruits. These bats are important for the regeneration of disturbed areas because they defecate viable seeds during the flight. We are studying the interaction between *C. perspicillata* and *P. gaudichaudianum* fruits in a reserve (Brazil). The essential oil of ripe (RF) and unripe fruits (UF) were extracted by headspace: morning, afternoon and night. The analysis of the oils was made by GC/MS and the identification of the components was made by RI and MS. We observed that the night sample of RF showed a main component: Hexanedioic acid ester derivative. This compound was present in lower concentrations in other RF samples and it was not detected in the UF samples. A field test was conducted using 8 baited mist nets with *Piper*-like fruits impregnated with the essential oil of night RF and 8 unbaited mist nets were used as controls. Sixteen bats were captured. *Carollia perspicillata* (n=2) and *S. lilium* (n=5) were captured exclusively in nets baited, while *Artibeus lituratus* (n=9) were captured at random. The analysis of their feces was made and only in the samples of *C. perspicillata* and *S. lilium* revealed the presence of seeds of *Piper* sp., indicating these bats were feeding on fruits of these genus when captured. More field tests will be made to confirm the role of essential oil of night RF in bat attraction and if the main component of this sample is the responsible for such attraction.

IDENTIFICATION OF THE CITRUS ODOR OF A SEABIRD (CRESTED AUKLET: *Aethia cristatella*)

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The exogenous application of chemical repellents is widespread in birds, but endogenous production is exceedingly rare. We herein report a new class of avian defensive compounds isolated from the feathers and volatile odor of the crested auklet (*Aethia cristatella*). Mass spectra indicated that a series of saturated and unsaturated aldehydes comprise the auklet odorant. The structures of the unsaturated compounds were suggested by the mass spectra of their dimethylhydrazine derivatives and confirmed by comparison with authentic samples. Similar compounds are also secreted in the repugnant metasternal gland emissions of heteropteran insects and are known to be potent invertebrate repellents. We suggest that the auklet odorant functions as an ectoparasite repellent and a signal of mate quality. This would represent a rare and direct link between vigor, quality and parasite resistance, one of several putative bases for mate selection. This is the first report of defensive compounds produced by a seabird or colonial bird and one of the few examples of chemical defense in a polar or subpolar marine vertebrate.

IS *HELICOVERPA ZEAE* SURVIVAL IN CORN EARS AFFECTED BY *FUSARIUM GRAMINEARUM* TOXINS?

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The corn earworm, *Helicoverpa zeae*, can often be found in ears of corn infected with *Fusarium graminearum*. This fungal pathogen is known to produce toxic secondary metabolites such as the tricothecene mycotoxin deoxynivalenol (DON) which is known to be an inhibitor of protein synthesis for plants and an anti-feedant for mammals. Levels of DON were monitored by a GC assay with a new extraction procedure in field grown ears artificially inoculated with a suspension of *Fusarium* macroconidia. Earworm infestation was detected 15 days after inoculation for 2 varieties with no differences between control and inoculated ears. The toxicity of DON to earworms was tested in a corn-based diet. Our results show that DON has a chronic toxicity, but non acute toxicity under 25 ppm, a concentration occurring in the field. Persistence of DON was also measured in diet and adults insects by GC.

MATING DISRUPTION OF MEDITERRANEAN CORN BORER *Sesamia nonagrioides* (Lepidoptera : Noctuidae) USING SPRAYABLE FORMULATIONS OF PHEROMONE IN FRANCE

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Mating disruption of the Mediterranean Corn Borer *Sesamia nonagrioides* (Lepidoptera : Noctuidae) using sprayable formulations of pheromone in France. *S. nonagroides* Lefèbre is present in the Mediterranean area and is the most harmful pest to corn crops. In France two generations are observed, first generation is controlled by seed dressing with neonicotinoid (Imidacloprid), second generation emerging mid-July is very difficult to control.
Using a binary blend (two major components) Z11-16 Ac: Z11-16 OH : 90/10 at 80 g/ha as a liquid sprayable formulation, it is possible to control the second generation.
In first large scale field trials in 1997, 1998 and 1999 promising results were obtained using the following parameters :

- Trap catches in treated fields
- EAG measurement in field at various levels of the crop
- Reduction in second generation damage

Results obtained showed 66 to 84% reduction of attacks, equivalent to chemical standard control.
An IPM programme is now offered to corn seed producers and sweet corn growers in Southern France using seed dressing for G1 control, mating disruption for G2 control and *Trichogramma* for those areas where Mediterranean and European Corn Borer are present.
Results presented are part of EEC programme FAIR CT 96-1302.

PRECISION DELIVERY OF SEMIOCHEMICALS/OLFACTORY STIMULI USING A SPRAYER

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In the field of olfactory communication, studying the response of animals to olfactory stimuli is hindered by the general lack of a release method that allows a precise release rate to be maintained during the course of an experiment. A novel device for quantitative and dynamic application of semiochemicals was developed that allows precise control of the chemical stimuli emitted from a point source. The sprayer consists of a motor-driven or a geared syringe driver that pushes the syringe slowly at a known constant rate to dispense semiochemicals in solvents through a micro tubing to glass a capillary micropipette fixed in a piezoelectric high frequency vibrator. The high-frequency vibration of the capillary tip atomizes the released solution and complete evaporation occurs within a few centimeters of the tip into micro-droplets that evaporate completely within a few centimeters of the tip. The ratio of the components of a chemical stimulus chemical stimulus can be set and calculated straightforwardly from the dilution factor and the dynamically controllable speed of the syringe plunger. The sprayer permits the delivery of chemical stimulus stimuli independent of relative vapor pressures of the components and of environmental factors such as temperature, relative humidity, etc.

The sprayer has been used successfully with many insect species, e.g., Lobesia botrana, Cydia pomonella, Ephestia kuehniella, Choristoneura rosaceana, Pityogenes bidentatus, Contarinia pisi and many more. Several versions of the sprayer are available depending on the type of the behavioral experiments. The basic sprayer version is suitable for the use in laboratory experiments; the portable version is suitable for field experiments and the dynamic version is used for altering the quality of the odor stimulus while the animal in the response mode.

IDENTIFICATION, SYNTHESIS, AND FIELD EVALUATION OF THE SEX PHEROMONE OF THE CITRUS FRUIT BORER *Ecdytolopha aurantiana*

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The sex pheromone of the citrus fruit borer *Ecdytolopha aurantiana* (Lepidoptera: Tortricidae) has been identified by gas chromatography coupled to an electroantennographic detector (GC-EAD). The electron impact mass spectral (EI-MS) fragmentation of the major EAD-active peak gave identifying features for a monounsaturated acetate. Further analyses by chemical ionization mass spectrometry (CI-MS), vapor-phase infrared spectroscopy (GC-IR), along with chemical derivatization (DMDS reaction) led to full characterization of the major component as (*E*)-8-dodecenyl acetate (E8-12Ac). The second constituent was identified as the related alcohol, (*E*)-8-dodecenol (E8-12OH). The two compounds were indistinguishable from the authentic synthetic standards in chemical and EAD analyses. Samples of the two compounds were obtained by a facile synthesis utilizing lithium chemistry. Field tests showed that captures in traps baited with a mixture of E8-12Ac and E8-12OH at the 100:1 and 10:1 ratios were not significantly different from the catches in traps having two virgin females. Dosage tests showed better performance of traps baited with 1 mg than those with 0.1 mg of the pheromone blend, either in 100:1 or 10:1 ratio.

WATER AS AN ATTRACTANT FOR STORED PRODUCT MOTHS REVISITED: A COMPARATIVE STUDY INCLUDING *EPHESTIA CAUTELLA* AND *E. KUEHNIELLA*

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Mass trapping with sex pheromone-baited traps have been used in attempts to control several species of moths that infest stored product food products. This approach suffers from the fact that males only are attracted to the pheromone-baited traps. A few males escaping the trapping might suffice to fertilise most females in the population. As a result, trapping of males may not cause any subsequent reduction in larval numbers and damage. Water has previously been reported as an attractant for pyralid moths. In the present study, we tested the attractive properties of water for both male and female moths in two Swedish food factories, a chocolate factory infested with *Ephestia cautella* and a flour mill, infested with *E. kuehniella*. Buckets lined with sticky paper were baited with water, pheromone or water plus pheromone. Unbaited buckets were used as controls and commercial funnel traps baited with pheromone served as active controls. In the chocolate factory, water-baited buckets attracted large and approximately equal numbers of male and female *E. cautella*. Synthetic pheromone attracted large numbers of males, but no females. Water was actually superior to pheromone for attraction of males. This surprising observation may get support from earlier reports that water is a limited resource for *E. cautella* and that *E. cautella* moths drink water to increase their life span. In the flour mill, we observed no attraction of *E. kuehniella* to water, whereas pheromone attracted large number of males. A dramatic reduction of the number of *E. cautella* in the chocolate factory was observed after six months of trapping, suggesting that water traps may actually be used for efficient control of *E. cautella* infestations.

DEVELOPEMENT OF OLFACTOMETRIC TOOL TO STUDY BEHAVIORAL RESPONSE OF LARGE SIZE INSECTS

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We developed a 4-way olfactometer (55 × 55 × 5,5 cm) adapted to large sized insects (3-6 cm) coupled to an olfactory stimulator able to deliver highly volatile semiochemicals in constant concentration. To examine air turbulence in the olfactometer and its tightness, we materialized air flows by discoloring sensitive papers with acid vapors in various configurations. The comparison of the digitized acidic prints allowed to fix an optimal configuration (air straightening stainless steel grids in inputs and output, 500 ml/min) for bioassays. The olfactory stimulator allow to deliver a broad range of concentrations of a volatile organic compound (VOC) in the air from a diluted aqueous solution, ventilated by a gentle air flow. We calibrated the olfactory stimulator using *Rhynchophorus palmarum* pheromone and solid phase microextraction. We evidenced that hyphenating the olfactory stimulator to the 4-way olfactometer provided a physically functional system. Its biological relevance was proved by establishing the dose-response curves to pheromones in three Coleoptera palm pests, *R. palmarum* (Curculionidae), *Strategus aloeus* and *Oryctes rhinoceros* (Scarabaeidae). The hyphenated system allowed a precise determination of the response thresholds to the pheromones (as low as 30 pg/s for *R. palmarum*) that could not be measured with the devices used previously. The system could also highlight the synergistic response of *R. palmarum* to its pheromone (P) associated to a natural kairomone (K: sugarcane VOCs) when submitted to a multiple choice between P, K and the combination of both. The hyphenated system provides a powerful laboratory tool to study the behavioral responses large insects to controlled low doses of semiochemicals VOCs.

HOW INSECT POLLINATORS ANALYZE FLORAL ODORS

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Floral odors can vary with respect to genotype, pollination status, developmental stage, and local environmental conditions. Yet insect pollinators depend these odor cues to discriminate among flowers. As the chemical components of this odor cue may vary over time and among flowers, what aspects of a particular floral scent do insects learn and remember? We hypothesize that insects utilize the least variant component or submixture of an odor mixture to "perceptually" characterize a floral perfume, particularly when it might be the best predictor of reward. To test this hypothesis, we composed 3-component odorant mixtures comprised of compounds that were either similar or dissimilar in chemical composition. We then systematically varied the concentration of two components (low, intermediate and high by way of electroantennograms) while keeping the remaining component at a constant level. Using these mixtures, we trained individual honeybees in a proboscis extension response conditioning paradigm and then tested them with the individual components of the mixtures at low and/or high levels. We show that concentration and identity of components influence generalization from the mixture to a component. Bees "filter out" low-level "minor" components when a high, relatively invariant component is present in the mixture. Thus the impact of a component on the perceptual properties of a mixture depends on qualitative (similar/dissimilar), quantitative (low/intermediate/high) and possibly variance properties of the components. These data suggest that analysis of floral odors should focus not only on quantitative and qualitative nature of each component, but also attention should also be devoted to variance.

HERITABLE CHARACTERISTICS INFLUENCE OLFACTORY DISCRIMINATION BY HYBRID MOTHS

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Insects are the most speciose terrestrial animal group and have evolved communication strategies to prevent interactions amongst related species. Chemical communication is abundantly evident amongst the Lepidoptera (in particular moths) where females often produce a pheromone that is highly attractive only to conspecific males. Our understanding of the genetic control and evolution of male olfactory and behavioral traits remains somewhat limited. Two closely-related species of moth in the genus Heliothis have proven advantageous for examining genetic regulation of olfaction/ behavior because of their ability to interbreed and produce viable hybrid offspring. Importantly, the pheromonal blends produced by female *Heliothis virescens* (Hv) and *Heliothis subflexa* (Hs) differ in their 2° components. The female emissions of each species are well correlated with conspecific male behavioral preferences and the specificities of olfactory projection interneurons (PNs) in the sexually-dimorphic glomeruli that comprise the macroglomerular complex (MGC) of the male antennal lobe. The number and spatial configuration of MGC glomeruli in the two parental species are essentially identical despite differences in the pheromonal blends of the two species. Behavioral bioassays conducted in a wind tunnel have revealed that Hs-Hv hybrid males possess novel olfactory characteristics. The observed behavioral differences correspond to changes in the specificities of antennal lobe PNs with no obvious modification of MGC organization. These results suggest that heritable physiological and glomerular-association characteristics of MGC PNs play a pivotal role in governing the behavioral ability of hybrid males to discriminate between specific odor blends.
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MALE PEA MIDGE, *CONTARINIA PISI*, RESPONSE TO STEREOISOMERS NOT PRODUCED BY THE FEMALES: BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDIES

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The sex pheromone of the pea midge consists of 2-acetoxytridecane, 2*S*,11*S*-diacetoxytridecane and 2*S*,12*S*-diacetoxytridecane. The response of male pea midges to the corresponding stereoisomers of 2*S*,11*S*-diacetoxytridecane and 2*S*,12*S*-diacetoxytridecane was tested, both in field trapping experiments and by electroantennographic (EAG) recordings. When added at 20% to the sex pheromone blend, two of the stereoisomers, 2*S*11*R*- and 2*R*,11*S*-diacetoxytridecane, were shown to have a strong inhibitory effect on male attraction in the field. At the same concentration, the other stereoisomers, 2*R*,11*R*-diacetoxytridecane, 2*R*,12*R*-diacetoxytridecane, and *meso*-2,12-diacetoxytridecane, did not have a significant effect on male behavior. It was also shown that substitution of either 2*S*,11*S*-diacetoxytridecane or 2*S*,12*S*-diacetoxytridecane with the corresponding stereoisomers reduced trap catches to the level of blank traps. The EAG-recordings showed similar dose response curves for the pheromone components and the stereoisomers shown to have an inhibitory effect. It seems likely that male antennae have receptors for both pheromone components and for inhibitory stereoisomers.

COMPUTATIONAL APPROACH FOR PHEROMONE- PHEROMONE BINDING PROTEIN INTERACTION

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Pheromone-pheromone biding protein(PBP) interaction is one of major interests for both protein chemistry and chemical ecology. PBP is first molecular interface to invoke instinct behaviour by transferring information to nerve system. On the other, recent progress of computing power is incredibly higher and, no doubt, is helpful to use this tool for studying molecular-molecular interaction, *i.e.* Pheromone-PBP system. For analyzing macromolecules (biomolecules), empirical molecular dynamics-based calculation has been proposed, but in fact, this method will not be able to solve detailed binding mechanism. Another method named molecular orbital calculation is a good prospect, although requiring enormous or astronomic orders of calculation ability. It has been suppose to be impossible to a pply for macromolecules such as PBP.

A new approach, fragment molecular orbital calculation (FMO) has been developed and applied onto *Bombyx mori* pheromone-PBP complex. The calculation starts with protein data bank coordinates file 1DQE(the dimer complexes of *Bombyx mori* PBP-bombykol). Hydrogens were generated and then minimized. The target molecules are divided into relatively small fragment entirely. Consequently, the molecular orbital through whole of complex(es) is calculated.

Principle, strategy and first stage result of FMO calculations will be presented and discussed.

NEW APPROACH - NEW PRODUCTS: HPLC PURIFICATION AND MICRO-QUANTITY NMR ANALYSIS OF TERMITE DEFENSIVE SECRETIONS

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The soldier caste of termites of the genus *Reticulitermes* (Isoptera, Rhinotermitidae) possesses a large frontal gland, that secretes through a pore on top of the head toxic compounds used for defense of the termite colony. During earlier analyses of the secretions of several French *Reticulitermes* species, and the North American species *R. flavipes,* α-pinene, β-pinene, limonene, geranyl linalool, and γ-cadinene and its corresponding aldehyde have been identified, as have a number of undetermined terpenes.
Here, the frontal gland secretion of seven European species of the genus *Reticulitermes* has been fully analyzed, using GC-MS in a first step. We observed similarities between groups of species corresponding to their geographical distribution. In the next step, we used HPLC equipped with a DAD detector to purify and isolate compounds from the secretions one by one. With subsequent micro-quantity NMR analyses we succeeded in verifiying for the first time the chemical structures of all compounds in the frontal gland secretions of *Reticulitermes* soldiers. Most interesting, the NMR spectra revealed that sesquiterpene analysis by GC-MS can give misleading results due to temperature by a Cope rearrangement (compounds such as elemenes found in GC-MS are finally the rearrangement of germacrenes). Thus, when natural products are analysed, GC-MS analysis might be insufficient to determine chemical structures. Now, the new technology in NMR analysis equipped with microprobe or cryoprobe is available and allowed us to carry out this new approach which requires only few micrograms even for 2-dimensional NMR correlations. These results open new possibilities for structure elucidation of semiochemicals in insects.

WORKERS, SOLDIERS, FRONTAL GLANDS: COMMUNICATION OF ALARM AND DEFENSE IN EUROPEAN TERMITES OF THE GENUS *RETICULITERMES*

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Termite colonies of the genus *Reticulitermes* (Isoptera, Rhinotermitidae) have a soldier caste, which up to now was assumed to be solely responsible for alarm and defense of the colony. The soldiers possess a so-called frontal gland (FG) in the head for chemical defense, its secretion (FGS) being released through a pore on top. In European *Reticulitermes,* it contains as main component the toxic diterpene alcohol geranyl-linalool. A function as alarm pheromone for its minor, volatile components has been assumed, but never verified.
Here, the communication of alarm and defense has been investigated in detail in four European *Reticulitermes* species (*R. santonensis, R. grassei, R. lucifugus, R. banyulensis*). It could be demonstrated that workers play a crucial role. When exposed to an odour source containing soldier FGS, workers display an alarm reaction, are attracted to FGS, and recruit further workers as well as soldiers. In the absence of workers, soldiers respond significantly less to FGS, an alarm response can not be triggered. Histological and chemical (GC-MS) investigations of soldiers and workers showed that only soldiers have a well developed FG. Its secretion contains, apart from geranyl-linalool, monoterpenes and sesquiterpenes in species-specific compositions (see also abstract by Quintana et al.). None of the soldier components is present in worker extracts. Instead we found in workers of all species investigated a similar pattern of comparatively volatile compounds (alcohols, aromatics, fatty acids), the source of which is still unclear. The possible role of both worker and soldier components in alarm communication is currently investigated.

BEHAVIOUR AND POSSIBLE PRODUCTION SITES OF MALE EMITTED SEX PHEROMONES IN *TRIBOLIUM CONFUSUM*

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Behavioural studies on attractiveness of both sexes of the confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) was tested in a laminar flow walking bioassay. Results show that females were highly attracted to live male beetles ($p<0.001$). Males were, however, not attracted to conspecific males or females. The definition of the substance would therefore be a sex pheromone and not an aggregation pheromone.
Different tests in the walking bioassay conclude that the sex pheromone is partly emitted from the legs of the male beetle. Leg extracts were significantly ($p<0.01$) attractive to females. Scanning electron microscopic pictures and transmission electron microscopic pictures show that *T. confusum* males differ from *T. castaneum* in that *T. castaneum* males have production sites and gland structures only on the prothoracic femurs, whereas they are present on all three pairs of femurs in *T. confusum* males. Behaviour tests with extracts of male bodies without legs still evoked a significant attraction by the females, indicating that production of sex pheromone is not limited to the production sites present on the femurs, but are also emitted somewhere on the body.
The results show that aggregation pheromones may not exist in *T. confusum*, but instead a system based on sexual pheromones emitted by the male attracting the female.

ELECTROPHYSIOLOGICAL STUDIES AND IDENTIFICATION OF POSSIBLE SEX PHEROMONE COMPONENTS OF THREE DIFFERENT BRAZILIAN POPULATIONS OF THE SUGAR-CANE BORER *Diatraea saccharalis*

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The sugar-cane borer, *Diatraea saccharalis* (Fabr. 1794) (Lepidoptera: Pyralidae), is an important pest of maize and sorghum crops and the key pest of sugar-cane in the Americas. Damage occurs by mechanical action as the borer builds internal galleries causing apical bud death, weight loss, atrophy and contamination by yeasts that cause red rottenness in the plants attacked.

The sex pheromone of the sugar-cane borer has been reported by Carney and Liu as *Z*9,*E*11-16:Ald. Various field tests have been carried out to verify the attractiveness of pheromone traps using *Z*9,*E*11-16:Ald and in all tests the traps with virgin females have shown significantly higher collecting efficiency than those using synthetic pheromones.

The knowledge of the total composition of the female sex pheromone may increase the capture of *D. saccharalis* males in pheromone traps since there might be one or a few minor compounds in the pheromone blend which play important roles in male attraction. Therefore, we investigated the composition of sex pheromone glands of *D. saccharalis* females. By gas chromatographic analysis we showed the presence of *E*9,*Z*11-16:Ald, *Z*9,*Z*11-16:Ald, *Z*11-16:Ald and 16:Ald as minor compounds in virgin female gland extracts of *D. saccharalis* of three different locations in Brazil, beyond *Z*9,*E*11-16:Ald already reported. Although there were variations in the gland extract compositions of the locations investigated, only two compounds, *Z*9,*E*11-16:Ald and *Z*11-16:Ald, elicited antennal responses in electroantennography (EAG) and electroantennographic detection after GC separation (GC-EAD).

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EVIDENCE FOR A SEX PHEROMONE IN THE BARK BEETLE PARASITOID *ROPTROCERUS XYLOPHAGORUM* (HYMENOPTERA: PTEROMALIDAE)

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Male *Roptrocerus xylophagorum* exhibited courtship/mating behaviors including wing fanning, antennation, mounting, and copulation attempts when exposed to glass bulb decoys (~1.5 mm diam.) coated with a whole-body solvent extract of females. Males responded much less strongly to freeze-killed female cadavers extracted with solvent than to unextracted cadavers; treatment of extracted cadavers with female extract restored male responsiveness. The source of the pheromone could not be readily localized to any single body region. Pheromone activity was minimally affected by either female age or mating status. The pheromone exhibited no long-range or anemotactic activity, and casual observation suggested it had an active space of less than a few centimeters. However, the activity of extract-treated decoys persisted longer than 1 week, and males exhibited arrestment on a glass surface on which females had walked, suggesting that the pheromone might be substrate-born. Recent exposure to females reduced male responsiveness, but responsiveness was fully restored after only a few hours of male isolation from females. When whole-body female extracts were fractionated on silica gel, the pheromone's activity was largely recovered with the first, most non-polar fraction. These data suggest that the pheromone is a large, stable hydrocarbon present across the insect's entire body surface, hence it likely consists of one or more female-specific components of *R. xylophagorum's* cuticular wax.

HERITABLE VARIATION OF SEX PHEROMONE PRODUCTION AND THE POTENTIAL FOR RESISTANCE EVOLUTION TO PHEROMONE-BASED MATING DISRUPTION OF THE INDIAN MEAL MOTH, *PLODIA INTERPUNCTELLA* (LEPIDOPTERA: PYRALIDAE)

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The heritable variation of the sex pheromone blend and the potential for evolution of resistance towards pheromone-based mating disruption due to a shift of this blend of the Indian meal moth, *Plodia interpunctella*, was investigated. The female-produced sex pheromone of the species consists of (*Z*,*E*)-9,12-tetradecadienyl acetate, (*Z*,*E*)-9,12-tetradecadienal, (*Z*,*E*)-9,12-tetradecadienol and (*Z*)-9-tetradecadienyl acetate. The heritability of the pheromone blend, i.e. the ratio of the two major components, (*Z*,*E*)-9,12-tetradecadienyl acetate and (*Z*,*E*)-9,12-tetradecadienol, was estimated to 0.65±0.14. To study if selection could increase mating in pheromone-permeated air, three independent selection lines were established and the mating ability of selected and unselected moths in plastic tents treated with high doses of a four-component pheromone disruptant was compared for two consecutive generations. No change in blend composition could be detected in selection lines compared to control lines and no increase in mating ability of females in pheromone-permeated air was observed in the selection lines compared to control lines, indicating weak selection on the pheromone blend or other traits influencing mating ability under mating disruption conditions.

ABSOLUTE CONFIGURATION OF TWO ACTIVE COMPONENTS FROM *SCOLYTUS INTRICATUS* ABDOMINAL EXTRACT

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To identify the chemical basis of aggregation behavior of the European oak bark beetle, *Scolytus intricatus*, behavioral tests, gas chromatographic (GC-MS) and GC-EAD analyses were performed on male and female abdominal extracts.
Behavioral observations indicated that female abdominal extract attracts beetles of both sexes. GC-EAD and GC-MS experiments revealed that extracts of both sexes contain two antennally active compounds: 4-methyl-3-heptanol and 4-methyl-3-heptanone. Different stereoisomers of 4-methyl-3-heptanol were proved to mediate aggregation behavior in closely related species *S. scolytus* [1], *S. multistriatus* [2] and *S. amygdali* [3]. Chiral column chromatography showed that extracts comprise of (3*R*,4*S*)-4-methyl-3-heptanol and (*S*)-4-methyl-3-heptanone in both sexes.
Since two antenally active compounds are present in male as well as in female abdominal extracts, we hypothesize that female abdominal extract contain also some other component than (3*R*,4*S*)-4-methyl-3-heptanol and (*S*)-4-methyl-3-heptanone.

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GUT CONDITIONS OF TANNIN-TOLERANT LARVAE OF *ACENTRIA EPHEMERELLA* (LEPIDOPTERA: PYRALIDAE)

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Myriophyllum spicatum, a freshwater macrophyte, is known to contain high concentrations of tannins (polyphenols). Growth of the oligophagic larvae of *Acentria ephemerella* is supressed when feeding on *M. spicatum* compared to other host plants, but the larvae can fully develop. To understand the mechanisms of adaptation, it is necessary to know about the fate of the tannins in the caterpillar gut. Caterpillar guts are in general alkaline, thus tannins are usually readily oxidized and turn brownish. However, the gut content of *Acentria* larvae retained its green color after feeding on *M. spicatum* and we could not find any free tannins and no products of hydrolyzation in the the different parts of the gut after freeze-drying. Microelectrode measurements revealed that the gut of *Acentria* larvae is anoxic, whereas the redox conditions are oxidizing. Since anaerobic gut conditions were independent of the host plant, oxygen may be consumed by mechanisms other than chemical oxidation of phenolics. The mechanism(s) generating oxidizing but anoxic conditions remain to be established. An alternative route of tannin removal may be covalent binding to proteins, e.g. digestion enzymes, which may cause the above mentioned growth reduction.

SPECIFICITY IN HOST ODOUR ATTRACTION OF CO-EXISTING BEETLES IN THE GENERA *PHYLLOTRETA* AND *MELIGETHES*

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The chemical diversity and host odours within the cruciferous plants are investigated for beetles species within the genera *Phyllotreta* and *Meligethes*. Cruciferous plants are known to contain glucosinolates which during wounding hydrolyse to form the corresponding isothiocyanates (IT) and thiocyanates (TC). A number of these degradation products have regarded as cues in insect-host-plant recognition. In this study, the attraction to alkyl-TC, alkyl-IT, aryl-TC and aryl-IT were compared using the two beetle genera *Phyllotreta* and *Meligethes*.
The field tests were performed at certain distance from crucifer-fields and on the edge of an oilseed rape field at Juuru, Northern Estonia. The tests were made at the end of May on a meadow with flowering dandelions (*Taraxacum* spp.) and in July-August while new generation of *Phyllothreta* spp were feeding on leaves of *Sinapis arvensis* L. and other cruciferous wild plants.
In our tests *Phyllotreta spp* were most attracted to butenyl TC and 3-butenyl IT, whereas Meligethes spp were most attracted to 2-phenethyl IT. Inhibitory effect of cis-3-hexenol and the lower odours were found, which might be of importance in controlling the attraction of the pollen beetles.

OVIPOSITION STIMULANTS FOR THE SULFUR BUTTERFLY, *COLIAS ERATE*: D-(+)-PINITOL AS A HOST-RECOGNITION CHEMICAL

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The sulfur butterfly, *Colias erate polyographus*, feeds on various legumes. We have previously identified two cyanoglucosides, i. e., linamarin and lotaustralin, as oviposition stimulants involved in host preference from one of its major host plants, white clover (*Trifolium repens*). These cyanoglucosides were inactive by themselves, however, they synergistically and significantly increased female's acceptability of an aqueous fraction, which strongly evoked oviposition response. Further chemical investigation of the active fraction revealed that it contained pinitol, methyl ß-glucoside and glycerin as characteristic components together with ubiquitous sugars. Of these, only pinitol, which was finally identified as D-(+)-pinitol, induced egg-laying. Although the other compounds did not elicit oviposition behavior, methyl ß-glucoside and glycerin slightly but significantly enhanced the ovipositional activity of females when tested in combination with D-(+)-pinitol. We then examined the distribution of pinitol in other potential host plants, and found that all of the plants that were accepted by ovipositing females contained varying quantities of pinitol, suggesting that pinitol is a crucial oviposition stimulant involved in host recognition. In addition, we observed that the females occasionally lay eggs on a non-host plant, *Aristolochia debilis* (Aristolochiaceae). On examining the constituents of the plant, it was found to contain pinitol in an amount enough to stimulate oviposition by the butterfly.

INDEPENDENT EVOLUTION OF PYRROLIZIDINE-ALKALOID-MEDIATED EXOCRINE DEFENSE IN PALAEARCTIC AND NEOTROPICAL LEAF BEETLES

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Leaf beetles of the palaearctic genus *Oreina* and the taxonomically related neotropical genus *Platyphora* comprise species (e.g. *O. cacaliae* and *P. boucardi,* respectively) which sequester pyrrolizidine-alkaloids (PAs) from their respective host-plants. However, they developed completely different biochemical strategies to maintain and exhibit these alkaloids for their own defense. As shown previously *O. cacaliae* sequesters PAs of the senecionine type as non-toxic *N*-oxides from its PA-rich host plants (Asteraceae) and stores them in the body (mainly hemolymph). From the hemolymph the PA *N*-oxides are transferred into the glands and concentrated in the defensive secretions. Pro-toxic tertiary PAs are detoxified by glycosylation. *P. boucardi* sequesters PAs of the lycopsamine type from *Prestonia portobellensis* (Apocynaceae) a PA-poor host plant. PAs are absorbed as pro-toxic free base and efficiently channeled into the glands. There is no noticeable accumulation in the beetle's body outside the glands. Exogenously fed PA *N*-oxide is reduced and rapidly transferred into the defensive glands with the same efficiency as directly fed free base. Thus, chemical defense mediated by PAs in the taxonomically related genera *Oreina* and *Platyphora* must have been evolved independently. Intriguingly, *Platyphora* metabolizes plant acquired PAs (epimerization, necine esterification) in a specific manner, unknown from *Oreina* but well known from taxonomically unrelated lepidopterans.

CHEMICAL BASIS FOR HOST SELECTION IN THE BANDED SUNFLOWER MOTH, *Cochylis hospes* WALLSINGHAM AND THE SUNFLOWER MOTH, *Homoeosoma electellum* HULST

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The banded sunflower moth (BSFM), *Cochylis hospes*, and the sunflower moth (SFM), *Homoeosoma electellum*, are major pests of sunflowers in North America. Previous work, by other authors, had established that plant chemicals are involved in host selection by females of these two species. In the case of the BSFM, Barker (1997) found that hexane extracts, of homogenized pre-bloom sunflower heads, stimulate oviposition by females, whereas pollen (or its aqueous extract) appeared to inhibit oviposition by females (Barker & Grugel, 1996). Work by Delisle et al (1989) on the SFM, established that pollen (or its ethanolic extract) stimulates oviposition by females. We are investigating the comparative host selection by females of these two moths, with the aim of defining the chemical mechanisms by which these pests temporally partition their sunflower host. To date, we have established that host selection in BSFM females is mediated by a number of sunflower chemicals, ranging in polarity and volatility. However, we have been unable to demonstrate that sunflower pollen inhibits host selection in the BSFM towards the stimulatory sunflower head extracts. In the SFM, we have established that chemicals, from the bracts of the sunflower head, are involved in host selection and have also confirmed the role of pollen in stimulating oviposition. A hypothetical model, for chemical partitioning of sunflowers by these two species, is presented.

REMOVAL AND RESTORING OF SEXUAL ACTIVITY IN THE *MALADERA MATRIDA* BEETLE

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The *Maladera matrida* beetle was first discovered in Israel in 1983 and declared as a new species to science. It is a noxious polyphagous pest: both larva and adults destroy crops, the former feeding on the underground organs of crops, such as peanut, sweet potato, potato, carrot, and the latter on flowers and foliage of many plants. At sunset the beetles emerge from the ground, where they stay all day and most of the night, for a brief (45-90 min) feeding and mating episode. During that period the beetles form aggregations on the plants. In laboratory studies it was demonstrated that the females attract the males for mating and that the chemicals involved are probably located on the cuticula of the mature females. Behavioral studies of males in the presence of either live or frozen females showed that chemicals washed from the cuticula of mature females with organic solvents (hexane, dichloromethane and methanol) have a sex pheromone-like activity. Preliminary studies showed that females washed with organic solvents lost their attractiveness for males. Applying the washings back to the washed females restored their attractiveness. Females examined in the morning were not as attractive as females at dusk. In keeping with these results, characterization of the extracted compounds by GC/MS revealed differences between those extracted in the morning and those extracted in the evening.

IDENTIFICATION OF THE MALE-SPECIFIC AGGREGATION PHEROMONE OF THE THE CEREAL LEAF BEETLE (*OULEMA MELANOPA*, COLEOPTERA: CHRYSOMELIDAE)

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The cereal leaf beetle, *Oulema melanopus,* is a serious pest of wheat, oats, and barley. Originally from Europe and Asia, the beetle was first found in the United States in southwestern Michigan in 1962 and has slowly continued to expand its range to include most States east of the Mississippi River. Recently, damaging populations have been reported in the Southeast and some Middle Atlantic States. Current control efforts are focused on the introduction of imported natural enemies. The chemical communication of this leaf beetle was unstudied and no pheromone has been reported. Obtaining a pheromone may help current control efforts by monitoring the expanding populations of cereal leaf beetles. We report here the isolation and chemical identification of a male-specific compound that is electrophysiologically active on both male and female antennae.

MALE-SPECIFIC SESQUITERPENES FROM *APHTHONA* AND *PHYLLOTRETA* FLEA BEETLES

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Volatiles were collected and analyzed from males and females of four flea beetle species (Coleoptera: Chrysomelidae), feeding on host plant material. Six male-specific compounds were isolated from *Phyllotreta cruciferae*, a pest of canola and other crucifers. The same six compounds plus two additional ones were isolated from males of *Aphthona flava*, *A. czwalinae*, and *A. cyparissiae*, which are biocontrol agents of leafy spurge, a serious weed pest. Structures were studied by mass spectrometry, NMR spectroscopy, UV spectroscopy, polarimetry, chiral and achiral gas chromatography, molecular modeling, and microchemical tests. Three of the compounds were identified as the bicyclic sesquiterpenes, (+)-*ar*-himachalene, (+)-*trans*-*alpha*-himachalene, and (+)-*gamma*-cadinene. Two others were himachalene hydrocarbons which were known in general structure from certain fir tree species but with ambiguous stereochemistry. Finally, there were two himachalene alcohols and one norsesquiterpene ketone that is a himachalene analog. Except for (+)-*ar*-himachalene and (+)-*gamma*-cadinene, the compounds are either new natural products or new enantiomers of previously described natural products. Electrophysiological activity was demonstrated for five of the compounds. All of the active compounds (two hydrocarbons, two alcohols, and one ketone) have one specific portion of the ring system in common, including identical configurations at two asymmetric centers. The compounds likely have a pheromonal function.

IDENTIFICATION AND SYNTHESIS OF THE SEX PHEROMONE OF THE VINE MEALYBUG, *Planococcus ficus*

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The production of wine, table grapes, and raisins constitutes a billion dollar industry in the western United States. Recently, economic losses from mealybug infestations in vineyards have increased dramatically. The vine mealybug *Planococcus ficus* Signoret (Homoptera: Pseudococcidae) is one of the two most economically important species. Vine mealybug is distributed throughout southern Europe and the Middle East, and parts of Africa, South America, and North America. It was introduced into California in the early 1990's, and it is spreading rapidly throughout central and southern California. Hosts include grapes, figs, apples, and citrus, and more tropical crops such as dates, bananas, avocados, and mangos. The growth of sooty mold and fungi on honeydew produced by the insects as they feed, and the accumulation of insects and insect detritus renders fruit unmarketable. Vine mealybugs also are proven vectors of viral diseases of grapevines. We will describe the identification of the sex pheromone of the vine mealybug from volatiles collected from virgin female insects, and the synthesis of the major component. The results of lab and field bioassays demonstrating the potential for using the pheromone in mealybug sampling plans also will be described.

IDENTIFICATION OF A SPECIES-SPECIFIC MALE-PRODUCED PHEROMONE BLEND FOR NEOTROPICAL BROWN STINK BUG, *Euschistus heros*

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The Neotropical brown stink bug, *Euschistus heros* (F.) (Heteroptera: Pentatomidae), is one of a complex of stink bugs that are serious pests of soybean, *Glycine max* (L.) Merryl, in Central and South America, especially in Brazil. A blend of three methyl esters from the male is proposed as the species-specific male-produced pheromone based on gas chromatographic-electroantennographic detection (GC-EAD) techniques. Identities of these esters were confirmed by comparison of GC retention times on polar and non-polar capillary columns and of GC-Mass spectra with authentic samples. The three GC-EAD active components reproducibly found in male volatiles were methyl (2E,4Z)-decadienoate (53%), methyl 2,6,10-trimethyldodecanoate (3%), and methyl 2,6,10-trimethyltridecanoate (44%). Laboratory olfactometer behavior bioassays showed that 5 μg of a synthetic blend on a filter paper strip was an attractive source and that responses of females to this blend were equivalent to responses to ten 20-day-old live males.

SEX PHEROMONE STUDIES FOR THE BRAZILIAN POPULATION OF THE FALL ARMYWORM *Spodoptera frugiperda*

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The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a major pest of many crops in the Americas. In Brazil this specie provokes substantial damage in various cultures and is the most important pest in maize. Several studies have shown different sex pheromone compositions due to geographically variation among populations of the same specie.[1,2] A variation in the sex pheromone blend of Brazilian population of *S. frugiperda* would explain lower capture rates of adults using lures from North American or European sources. Therefore, we reinvestigated the sex pheromone composition of *S. frugiperda* in regard to the applicability in integrated pest management in Brazilian plantations.

We observed in virgin female gland extracts, by mass spectrum analysis, Kováts retention index and co-injection with synthetic compounds, the presence of eight peaks with characteristics of long-chain acetates. The ratio of the peak areas found was 0.8 : 1.2 : 0.6 : traces : 82.8 : 0.3 : 1.5 : 12.9 for *Z*7-12:Ac, *E*7-12:Ac, 12:Ac, *Z*9-12:Ac, *Z*9-14:Ac, *Z*10-14:Ac, 14:Ac/*Z*11-14:Ac and *Z*11-16:Ac, respectively. Different to all studies published up to now we found *E*7-12:Ac in the gland extract. However, only two compounds, *Z*9-14:Ac and *Z*7-12:Ac, elicited antennal responses in electroantennographic detection after GC separation (GC-EAD).

In preliminary field experiments, lures containing the binary mixture of *Z*7-12:Ac and *Z*9-14:Ac showed no significant differences related to the attraction when compared to the mixture of *Z*7-12:Ac, *Z*9-14:Ac and *Z*11-16:Ac, although the absolute capture values were higher with the binary mixture.

ANTI-PREDATOR DEFENSE OF THE BIOLOGICAL CONTROL AGENT *OXYOPS VITIOSA* IS MEDIATED BY PLANT VOLATILES SEQUESTERED FROM THEIR HOST PLANT *MELALEUCA QUINQUENERVIA*

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The weevil *Oxyops vitiosa* is an Australian species imported to Florida, USA for the biological control of the invasive species *Melaleuca quinquenervia.* The larvae of this species feed on the leaves of their host and produce a shiny orange secretion that covers their integument. When this secretion is applied at physiological concentrations to a dog food bait, fire ant consumption and visitation are significantly reduced. Gas chromatographic analysis indicates that the larval secretion resembles qualitatively and quantitatively the terpenoid composition of their host foliage. When the combination of the ten major terpenoids from the *O. vitiosa* secretion were similarly applied to dog food bait, fire ant consumption and visitation were reduced. When these ten terpenoids were similarly tested individually, the sesquiterpene viridiflorol was the most active component decreasing fire ant consumption. Fire ant visitation was initially (15 min after initiation of the study) decreased for dog food bait treated with viridiflorol, and the monoterpenes 1-8 cineole, and □-terpineol. Fire ants continued to avoid the bait treated with viridiflorol at 18 µg/mg dog food for up to 6 hrs after the initiation of the experiment. Moreover, ants avoided bait treated with 1.8 µg/mg for up to 3 hrs. The concentrations of viridiflorol, 1-8 cineole, and □-terpineol in larval washes were about twice that of the host foliage suggesting that the larvae sequester these plant-derived compounds and obtain protection against generalist predators.

CONFIGURATION OF TRIOXILIN A, THE PRESUMED EGG-HATCHING FACTOR OF THE BARNACLE, *ELIMINIUS MODESTUS*

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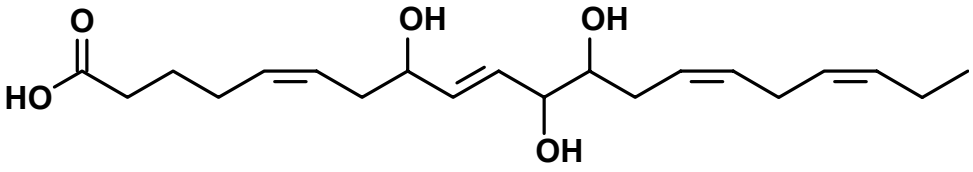
The egg-hatching factor of the barnacle *Semibalanus balanoides* has been intensively studied by the group of Holland [1]. They postulated the active compounds to be trihydroxy eicosanoids like trioxilin A, because fractions of an eicosanoid extract containing these compounds were active in their test organism, *Eliminius modestus*. No attempt was made to clarify the absolute configuration of the trioxilines, which occur also in other organisms and even humans. We could identify trioxilines in *Eliminius modestus* as well. Currently methods for the determination of the absolute configuration of the trioxilines basing on chemical modifications and chromatographic separations are developed. We will report here on the results obtained so far and the identification of further eicosanoids from *Eliminius modestus*.

[1] E. M. Hill, D. L. Holland, J. East, *Biochim. Biophys. Acta* **1157**, 297 (1993).

trioxilin A_4



CHEMICAL DETERRENTS OF LOBSTER FEEDING BEHAVIOR

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Lobsters are important models for understanding neural substrates of chemosensory-mediated behaviors, particularly those associated with detection and recognition of food. We wish to use the lobster as a model for examining the neural substrates of feeding deterrence by allelochemicals. As a first step, we examined sponges as potential sources of feeding deterrents because of their known effects on a number of other species. In addition we tested tannins and related chemicals, which may be associated with reported feeding deterrence of some brown algal species. Food pellets containing fish flakes and a given amount of a putative deterrent were presented to two species of lobsters: *Panulirus argus* and *Homarus americanus*, and amount of time spent manipulating the pellet in the mouthparts as well as rejection or acceptance of food were scored. Aqueous and butanol extracts of six species of Bermudian sponges (*Dysidea etheria, Pseudaxinella explicata, Adocia amphioxa, Xytopsues osburnensis, Aplysina fistularis, Niphates erecta*), some of which are ichthyodeterrent, were not found to deter *P. argus* feeding. Hexane, ethyl acetate, butanol and aqueous extracts of three Caribbean sponges (*Spheciospongia vesparia, Axinella polycapella, Halichondria bowerbanki*) also did not deter feeding in *P. argus*. In contrast, tannins deterred feeding. In *P. argus,* tannic acid was the most deterrent followed by gallic acid and phloroglucinol. *H. americanus* was deterred to a lesser degree by tannic acid and not at all by the other two compounds. We are currently testing the feeding deterrent properties of extracts of brown algae on both species of lobsters.

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INFLUENCE OF FRESHWATER MACROPHYTES ON EPIPHYTIC BACTERIAL COMMUNITIES

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Freshwater macrophytes provide large surface area for colonization by bacteria and algae. It is suggested that bacteria are influenced through physicochemical factors of the plant surface and allelochemicals produced by the macrophytes. Epiphytic bacteria have so far been investigated mainly by classical methods (direct counting, cultivation), thus information of the community structure of attached bacteria is sparse. We investigated bacterial communities by DGGE to address the question, whether biofilms on macrophytes are host specific and if allelopathic substances influence the biofilm structure. Our results indicate that the community structure of selected macrophytes (*Myriophyllum spicatum, Potamogeton perfoliatus, Chara* spec.*, Ceratophyllum demersum, Elodea* spec.) differ between macrophyte species and that the epiphytic communities are dominated by fewer phylogenetic groups than the respective planktonic community. The influence of secondary metabolites produced by macrophyte hosts on the community structure of bacteria are currently under investigation.

JUVENILE HAWAIIAN GOBIID FISH EMPLOY ODOR CUES TO LOCATE FRESHWATER STREAMS FROM THE OCEAN AND TO GUIDE THEM UP THEIR TERMINAL WATERFALLS

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The Hawaiian islands have only 4 species of riverine freshwater fish, all of which are members of the goby family and possess an unusual amphidromous life history. These fish spend their adult lives in the headwaters of streams where they lay eggs in nests. Upon hatching, larvae are swept to sea where they develop for a few months, after which they re-enter streams. Juvenile fish swim great distances inland coming out of the water (using fused fins as a suction cup) to scale the large (100m) waterfalls which characterize these systems. This study asked whether odor cues serve to guide these tiny (1 cm) fish during this journey. Behavioral responses of recently captured juvenile gobies were assayed in 2-choice maze with waterfalls located at the head of each arm. Gobies demonstrated a strong preference to enter and scale flowing stream water when it was tested against seawater. This preference persisted when the former was diluted 100-fold. Stream waters were also strongly preferred over the spring waters from which they originated suggesting that organic compounds released into streams serve as the attractant(s). This was confirmed by tests of conspecific odor (pheromones) which stimulated climbing activity. Tests of different stream waters also found the attractant to be innately recognized and widely distributed. My study is the first to demonstrate that an amphidromous fish uses odor to locate spawning streams, a strategy that appears to make ecological sense given the scale of the aquatic environment and its unpredictability.
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THE CATALYTIC ACTIVITY OF THE BOTTOM SEDIMENTS REGARDING H_2O_2 REACTIONS

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Bottom sediments play an important role in the formation of water quality in natural reservoirs. Organic substances buried in the bottom sediments serve as a source of nutrition for heterotrophic organisms. As a result of their activity, such biogenic elements as nitrogen, phosphorus, and other soluble organic substances return to the water column. Bottom sediments are also a focus for heavy metal ions, due to the sorption of metal ions to surface-bound complexes. The mineral and organic suspended particles act as a kind of a "conveyor belt" to transport metal ions from the bulk water to bottom sediments. In the case of metal ions of variable valency, their oxidation state depends on the redox characteristics of the bottom sediments. Transitions from oxidazing to reducing states may be accompanied by the reduction of metal ions. In this case,Fe (II) and Mn (II) ions may diffuse from the bottom sediments to the water medium where they may be again oxidized into Fe (II) and Mn (III, IV).

Since bottom sediments are, as a rule, the source of reducing agents, they may considerably affect the dynamics of redox processes involving reaction of H_2O_2 and reducing agents in surface waters. In this connection, the redox condition of bottom sediments, the content of substances effectively reacting with H_2O_2 and the rate of their formation, as well as the catalytic activity of the bottom sediments regarding H_2O_2 reactions, seem to be important in assessing ecological conditions of reservoirs. Systematic studies of these parameters are absent in the literature, though redox processes are acknowledged to be very important in the mechanism of chemical transformations of pollutants.

THE WATER QUALITY OF LAKE SEVAN /ARMENIA/

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The role of Lake Sevan was, and still is, very important in the national economy of Armenia. The use of the lake's water has highly developed the hydroelectric power generation and agricultural land irrigation. The lowering of the lake's water level started in 1933, but intense water discharges and a drastic drop in the lake's water level has occurred since 1949 when the annual discharges reached 1.000 to 1.700 bin m^3.

There were a consumer approach towards the resources of the lake and poor ecological management from an oligotrophic level, the lake collapsed into a eutrophic bio-level. At the oligotroph bio-level, no organic matter was accumulated and qualitatively, the water of the lake was regarded as potable. At the eutroph bio-level equilibrium was shattered and organic matter began to accumulate, entailing a positive balance of biogeochemical circulation. All this brought enhanced autotroph subsystem activity and a subsequent sharp decline of water quality.

The main cause of the anthropogenic eutrophication of the lake is the concurrent influence of two effects, e.g. inner-lake occurs as a result of a water level drop of 19 meters, and secondly, the vast quantities of biogen and toxic substances inflow to the lake. Also, heavy metal concentrations have been determined for Lake Sevan trubataries (Fe, Mn, Zn, Cu, Ni, Co).

The estimation of the water quality judging from average values does not reflect the alterations in the ecosystem of Sevan during the process of eutrophication. The growth of the trophic level in many lakes of the world is dependent on the growth of the load of organic elements, particularly, nitrogen and phosphorus, from the catchment basin. The eutrophication causes of Lake Sevan differ from those of other lakes of the world. Lake Sevan eutrophication is attended with profound structural changes in phytoplankton.

OPTIMIZING RESOURCES IN MARINE SPONGES: EVIDENCE FOR MULTIPLE DEFENSE ROLES OF TRITERPENE GLYCOSIDES

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Many marine sponges remain uneaten, unfouled, and uncrowded despite their great abundance on Caribbean reefs and their high nutritional value. Ecological assays using crude extracts of sponges have suggested a chemical explanation; however, very few defensive compounds have been identified. One class of potentially defensive molecules is the triterpene glycosides, which are being increasingly isolated from sponges. We tested triterpene glycosides from *Erylus formosus* and *Ectyoplasia ferox* in field and laboratory assays for deterrence against attachment by biofilm-forming bacteria, fouling by invertebrate larvae and algae, predation by fishes, and overcrowding by neighboring sponges. Formoside and other triterpene glycosides from *Erylus formosus* deterred predation by fishes, microbial attachment, and invertebrate fouling. Similar compounds from *Ectyoplasia ferox* were allelopathic and deterred predation by fishes, all at realistic concentrations. Triterpene glycosides from these sponges were not universally active in all assays, and there was a strong concentration dependence in many of the tests. Small differences in molecular structure apparently affect ecological activity in significant ways. Nevertheless, these compounds appear to fulfill more than one ecological function, thereby maximizing energy conservation.

DETOXIFICATION OF BREVETOXINS BY MOLLUSCS VIA SULFIDE-LINKED DERIVATIVES

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Brevetoxins are neurotoxic polyether metabolites produced by the Florida red tide dinoflagellate *Karenia brevei* (ex *Gymnodinium breve*). These sodium channel activators have caused the deaths of massive numbers of fish, invertebrates, and marine mammals including dolphins and manatees. At usual bloom concentrations, molluscs such as oysters and clams bioconcentrate brevetoxins without being killed. However, at blooms of approximately > 10^8 cells/L, shellfish mortality is significant. A series of cysteine, taurine, and thioglycerol adducts of brevetoxins were previously identified by others from toxic shellfish and urine of humans suffering from neurotoxic shellfish poisoning. In controlled aquarium experiments, we exposed oysters to bloom levels of toxic *K. brevis* followed by several weeks of exposure to non-toxic microalgae to investigate the bioconcentration and detoxification of brevetoxic shellfish. All of the brevetoxin-2 (the major toxic metabolite of *K. brevis*) was immediately converted by oysters to a mixture of sulfide-linked derivatives, which were then slowly lost from shellfish over a period of 2-3 months. Brevetoxin-3 (the second most concentrated toxin of *K. brevis*) was 90 % lost within 2 weeks and without apparent biotransformation. Biotransformation of brevetoxin-2 was also effectively accomplished *in vitro* using water-soluble extracts of oysters and clams. The sulfide-linked derivatives of brevetoxin-2, which bind to sodium channels with lower affinity than the parent brevetoxins and are less toxic to mice, may represent metabolic effort by shellfish to reduce the toxic hazard to themselves.

BIOSYNTHETIC STUDIES ON MARKING PHEROMONES OF THE BUMBLEBEE MALES

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Marking pheromone of the bumblebee males is produced by the cephalic part of the male's labial gland. During the premating behavior, patrolling males scent-mark their territories to attract conspecific females for mating. Each bumblebee species produces a specific blend of compounds. Both straight chain saturated and unsaturated hydrocarbons, alcohols, aldehydes, or esters, and terpenoids are present in the gland secretions.
For our biosynthetic experiments we have used two bumblebee species, *Bombus lucorum* and *B. lapidarius*, producing predominantly aliphatic straight-chain compounds in the glands. Solutions of deuterium-labeled palmitic acid ([$^2H_{31}$]-hexadecanoic acid) were injected either into the head capsules or in the abdomens of males of both species and incubated for 24 or 48 hours. In both cases, the deuterium-labeled metabolites were detected and identified in the labial glands using GC-MS and derivatization experiments. In *B. lucorum*, ethyl [$^2H_{29}$]-(*Z*)-hexadec-9-enoate and ethyl [$^2H_{31}$]-hexadecanoate were identified. In *B. lapidarius*, [$^2H_{31}$]-hexadecan-1-ol and [$^2H_{29}$]-(*Z*)-hexadec-9-en-1-ol were found in the labial gland. Furthermore, the deuterated precursor was built into triglycerides of the fat bodies of males. These results indicate that common lipids found in the body are most probably used as a pool material for semiochemicals and they are transformed to marking pheromone components in the male's labial glands. Thus, the primary and secondary metabolism seems to be connected.

GENOMICS OF PHEROMONE BIOSYNTHESIS IN *IPS PINI*

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The bark beetle, *Ips pini,* attacks host trees *en masse* by utilization of aggregation pheromones released from the male beetle. These monoterpenoid pheromones are produced via the mevalonate pathway. Pheromone production is induced by feeding and/or treatment with juvenile hormone III (JH III). Two enzymes involved in the early steps of pheromone production are HMG-CoA reductase and HMG-CoA synthase. Transcription of both enzymes is upregulated in response to JH III. In order to fully understand pheromone biosynthesis, the rest of the enzymes involved in the pheromone production pathway, as well as elements of the enodocrine regulatory apparatus, need to be identified. A cDNA library, from JH III treated (pheromone producing) male gut tracts, has already been arrayed. In collaboration with the Nevada Genomics Center, high throughput sequencing is being used to develop an EST database of male *Ips pini* gut tract. Expression profiling with microarrays will be used to identify genes that respond to JH and therefore are likely to be involved in pheromone production.

DETERMINATION OF THE *DENDROCTONUS JEFFERYI* AND *IPS PINI HMG-R* PROMOTER REGIONS

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The aggregation pheromones of bark beetles are essential for successful reproduction. The Jeffrey pine beetle, *Dendroctonus jeffreyi,* is a major pest in this area and our lab has studied their pathway for pheromone production extensively. Male *D. jeffreyi* produce the bicyclic acetal frontalin, which likely is as an aggregation pheromone component. JH III stimulates *de novo* frontalin production, and this correlates with an increase in the expression of the 3-hydroxy-3-methylglutaryl-CoA reductase gene (*HMG-R*), a key regulator of the mevalonate pathway. The exact mechanism of JH regulation is still unknown, but it is thought to act directly on the genome by binding (as a hormone/ receptor complex) to specific DNA binding sequences to alter transcription of selected genes. Since *HMG-R* transcripts are induced up to 30-fold by JH III treatment in *D. jeffreyi* and *Ips pini*, these insects provide a useful model for studying the mechanism of JH regulation. Inverse-PCR (IPCR) and Thermal Asymmetric Interlaced PCR (TAIL-PCR) techniques have been used to isolate the 5' flanking region of *HMG-R* in *D. jeffreyi* as a first step to identify potential controlling elements. A putative promoter region has recently been cloned and sequencing is underway. A genomic cosmid library is also being prepared from *I. pini* in an attempt to clone its promoter region as well. Once the promoter regions are isolated, the regulation of the production of pheromones in these pests can be more completely understood.

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ISOLATION AND CHARACTERIZATION OF ISOPRENYL DIPHOSPHATE SYTHASE FROM COTTON BOLL WEEVIL

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The male cotton boll weevil, Anthonomus grandis, produces a four-component pheromone through the isoprenoid biosynthetic pathway. Geranyl diphosphate synthase (GPPS) catalyzes the condensation of isopentenyl diphosphate synthase (IPP) with dimethylallyl diphosphate (DMAPP) to form geranyl diphosphate (GPP), which is the precursor for the male-produced pheromones. GPPS has only been studied in plants, and A.grandis is among the very few animals that contain GPPS activity. Using degenerate primers designed from the conserved domains of isoprenyl diphosphate synthases from other organisms, a full-length cDNA of a putative isoprenyl diphosphate synthase was isolated from a male gut cDNA library of A.grandis through PCR techniques. Analysis of nucleotide sequence revealed that cDNA encodes a 438 residue protein which contains highly conserved regions found in isoprenyl diphosphate synthases. The deduced amino acid sequence showed highest similarity to two insect farnesyl diphosphate synthases (FPPS) from Agrotis ipsilon and Drosophila melanogaster. In order to characterize isoprenyl diphophate synthase from A.grandis, prenyltransferase activity was measured in E.coli cell extracts overexpressing the A.grandis enzyme. Product analysis suggested that the gene isolated from A.grandis likely encodes FPPS. Purification of recombinant protein is in progress and further work is being done to isolate GPPS through PCR techniques and cDNA library screening.

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BIOCHEMICAL AND MOLECULAR ASPECTS OF THE REGULATION OF PHEROMONE BIOSYNTHESIS IN PINE BARK BEETLES (*Ips* spp.)

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Recent research on aggregation pheromone biosynthesis in pine bark beetles (Coleoptera: Scolytidae) by our group has emphasized *de novo* synthesis of ipsenol and/or ipsdienol by two comparative model species, the California fivespined ips, *Ips paraconfusus* Lanier and the pine engraver, *Ips pini* (Say). *De novo* synthesis of these isoprenoids occurs in males of both species, and in *I. pini* the synthesis is stimulated by juvenile hormone III (JH III), which is itself biosynthesized and released from the corpora allata in response to feeding on host pine phloem. However, while feeding stimulates *de novo* ipsenol and ipsdienol biosynthesis in male *I. paraconfusus* (and ipsenol synthesis in the eastern fivespined ips, *I. grandicollis* (Eichhoff), a close relative of *I. paraconfusus*), topical treatment with JH III does not. Thus, two *Ips* species in the *grandicollis* subgeneric group of *Ips* appear to have a different mode of regulation related to JH III than does *I. pini*.

Comparative molecular and biochemical studies of the regulation of the putative key isoprenoid pathway enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-R) in both *I. paraconfusus* and *I. pini* also support different modes of JH III-based regulation of pheromone production. Northern blot and *in vitro* enzymatic assays suggest that feeding on host phloem results in increased transcript abundance and increased activity for both species. However, the identical assays show that topical JH III treatment results in increased HMG-R transcript levels in both species, but the activity of HMG-R is not stimulated in male *I. paraconfusus* as it is in male *I. pini*. A model involving a possible secondary hormone and translational or post-translational regulatory differences will be presented.

MONOTERPENOID PHEROMONE BIOSYNTHESIS: FISHING FOR THE ELUSIVE GERANYL DIPHOSPHATE SYNTHASE IN BARK BEETLES

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The male pine engraver beetle, Ips pini, synthesizes the monoterpene pheromone component ipsdienol de novo through the mevalonate pathway. A key enzyme in the production of these C10 molecules is geranyl diphosphate synthase (GPPS). It is part of a larger family of isopentenyl diphosphate synthases (IPPS) that catalyze the sequential condensation of isopentenyl diphosphate (IPP) with other allylic diphosphate substrates. GPPS activity is the first step in the condensation cascade leading to the formation of a diverse group of isoprenoid products with characteristic chain lengths. GPPS synthesizes geranyl diphosphate (GPP, C10) from the C5 isoprene units IPP and dimethylallyl diphosphate (DMAPP). This C10 product is a putative precursor to the aggregation pheromone ipsdienol in I. pini. GPPS is characterized from a few plant species, but it has not been isolated in any animal system. Coleopterans are unique among animals in that they produce monoterpenoids. Reverse-phase TLC shows that GPP is a major product in homogenized tissue from male I. pini, indicative of GPPS activity. Using PCR amplification, two putative isopentenyl diphosphate synthase genes were isolated from a male I. pini cDNA library. One of the sequences has high sequence similarity with other known insect farnesyl (C15) diphosphate synthases. The other cloned sequence has significant residue differences in key catalytic regions. Northern blot analysis using a probe corresponding to the 5, region of this putative GPPS cDNA hybridized to a 1.5 transcript. Expression levels occur in a JHIII dose- and time-dependent manner in male thoraces which corresponds to pheromone biosynthesis in these insects. Presently, the putative GPPS is being expressed and assayed to confirm its identity.

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CHEMICAL CHARACTERIZATION OF THE GENUS *BURSERA* (BURSERACEAE)

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Burseras are New World tropical trees related to the Old World species which are the sources of frankincense and myrrh. The genus comprises about 100 species distributed from the southwestern United States to Peru and reaches its maximum diversity in the tropical dry forests of Mexico. We performed a chemical characterization of *Bursera* volatile resins using gas chromatography-mass spectrometry. A total of 55 species, each one consisting of 1 to 9 populations and 4 to 20 individuals per population were sampled from a number of field sites in Mexico. The volatile chemistry of the genus is dominated by monoterpenoids. We found much variation among species in both the number and identity of the different volatile compounds and found that closely related species are often very dissimilar in terms of their chemistry. We also found that, in general, species tend to be chemically cohesive, even when there is variation among different populations. We discuss these results in relation to plant-insect coevolution.

AROMA CONSTITUENTS OF THE MEDICINAL PLANT *LIGUSTICUM PORTERI*

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The root of Ligusticum Porteri (Apiaceae) is the most widely used traditional herbal medicine among Hispanics of the Rocky Mountains of southwestern United States and northern Mexico. Commonly called oshá or chuchupate, L. Porteri grows in damp soil at elevations of 2000 and 3500 meters. The roots are used as antiviral, antibacterial, expectorant, and cough suppressant medicines. L. Porteri has not been successfully cultivated, requiring collection of the roots in late summer.

Other members of the Apiaceae, such as the hemlock parsley, Conioselinum scopulorum and the toxic water hemlock, Cicuta Douglasii, grow in close proximity and are similar in appearance to L. porteri. Collectors and users usually identify L. Porteri by the distinctive odor of the roots. Using solid phase microextraction coupled with gas chromatography-mass spectrometry we identified the major volatile chemicals of L. Porteri roots. The roots were extracted, and using a combination of thin layer chromatography-olfactometry and gas chromatography-olfactometry we identified the phthalide ligustilide as the single component responsible for the distinctive odor. Gas chromatography and thin layer chromatography with colorometric visualization distinguishes genuine oshá roots from other potentially toxic species.

STRESS INDUCED CHANGES IN MONOTERPENE COMPOSITION IN *PINUS SYLVESTRIS*

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Results are presented from terpene analyses of *Pinus sylvestris* in order to describe stress-induced changes in the monoterpene composition. This work is part of an EC project where the main objectives are to define indicators for pest and disease in forest stands. Sixteen *Pinus sylvestris* trees in Southwest of Finland have been chosen in a natural stand to undergo four different treatments during four years. The trees have been stress-treated by drought, drought and fertilisation, fertilisation and the fourth group is a non-stressed control group. After four years the trees were inoculated by a blue-stain fungus strain *Leptographium wingfieldii* (associated with *Tomicus piniperda*). Another group of four trees were chosen as a control group without any treatment. Stem phloem samples were taken just before and one day after the inoculation as well as after 3 months in connection to the felling of the trees in September 1999. Chemical analyses of the hexane extracts were made on GC-MS and chiral separation on a 2D-GC. Interesting results about the change of (–)-□-pinene content will be shown. Multivariate data analyses have been done on the relative amounts, absolute amounts and enantiomeric composition. Results will be presented about the profile change between the samplings and the difference between the treatments.

A NEW OVIPOSITION STIMULANT FOR *PAPILIO POLYTES* IDENTIFIED FROM ITS MAJOR HOST PLANT, *TODDALIA ASIATICA* (RUTACEAE).

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Papilio polytes is a Rutaceae-feeding papilionid butterfly utilizing *Toddalia asiatica* (TA) as a major host plant and inhabiting Southeast Asia and Southwestern Islands of Japan. Earlier work on host selection in Papilionidae clearly demonstrates that chemotactile stimuli evoked by certain plant metabolites serve in the final step of oviposition sequence as the cues permitting females to discriminate and to recognize their hosts. We thus attempted to identify active compound(s) present in TA involved in stimulation of egg-laying. A MeOH extract (TA-1) from young leaves of TA that showed potent stimulatory activity was separated by solvent partition into three fractions: $CHCl_3$(TA-2), *i*-BuOH(TA-3) and water(TA-4). Strong activity was found to reside in TA-4, from which one of active compounds was isolated by flash chromatography, preparative TLC and HPLC. The compound, identified as *trans*-4-hydroxy-*N*-methylproline by its FAB-MS and NMR spectra, showed noticeable oviposition-stimulatory activity in itself.

INDUCTION OF TERPENOID VOLATILES RELEASED IN RESPONSE TO INSECT HERBIVORY IN COTTON PLANTS

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Several plant species release volatile compounds after being attacked by herbivorous insects. In cotton (*Gossypium hirsutum*), these herbivore inducible compounds include acyclic terpenoids, (Z)-3-hexenyl acetate, indole, and others. We found that many of these compounds are not only released from the damaged parts of the plants, but also systemically from undamaged parts of caterpillar damaged plants, including (*E*)-ß-ocimene, (*E*)-ß-farnesene, (*E,E*)-α-farnesene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, and (*Z*)-3-hexenyl acetate. We found that cotton plants that release these inducible volatiles systemically were highly attractive to a specialist and a generalist parasitoid in flight tunnel experiments. The compounds systemically released in response to herbivory are known to be synthesized *de novo*. To gain a better understanding of the regulation of inducible terpenes in cotton, we are presently investigating the biochemistry and molecular basis of terpene volatile formation. Plants that were fed upon by caterpillars or sprayed with 0.025% methyl jasmonate showed elevated levels of monoterpene- and sesquiterpene synthase activities compared to untreated control plants. Therefore, the production of herbivore-inducible volatiles, which are known to be synthesized *de novo*, appears to be regulated by changing levels of terpene synthase activity, with a jasmonate signalling cascade involved.

ANTIMICROBIALS FROM TROPICAL RAIN FOREST PLANTS IN GABON

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Species from 44 genera representing 24 plant families were collected as part of a rainforest canopy expedition in Gabon, West Africa (La Makande Station, La Forêt des Abeilles) in Feb./Mar., 1999. Freshly collected leaf and stem tissue was extracted with 95% EtOH and the extracts returned to North America for phytochemical analysis (*i.e.*, HPLC and CI-MS) and bioassay. Antimicrobial activity (+/- UV-A) against *Staphylococcus aureus* (methicillin sensitive and resistant), *Bacillus subtilis*, *Entercoccus faecalis*, *Pseudomonas aeruginosa* and *Mycobacterium phlei* was evaluated using disk diffusion bioassays. A total of 36 extracts had antimicrobial activity against at least one of the bacterial species tested. None of the extracts were phototoxic (*i.e.,* had enhanced inhibitory activity in UV-A than in the dark). Four species were studied in more detail because of their quantitative prevalence in the forest - *Aucoumea klaineana* Pierre (Burseraceae), *Marquesia excelsa* (Pierre) Fries (Dipterocarpaceae), *Paraberlinia bifoliolata* Pellegr. (Fabaceae), and *Xylopia hypolampra* Mildbr. (Annonaceae). Extracts of canopy foliage from all four taxa contained antimicrobial activity, especially *A. klaineana* and *P. bifoliolata*. The crowns of these rain forest trees produced higher levels of secondary compounds and greater structural diversity than understorey saplings suggesting that they may also contain higher levels of biologically activity although this was not specifically quantified. Surveys of plants for biologically active phytochemicals (*e.g.,* viricides, bactericides, fungicides, insecticides, etc.) should emphasize tissues growing in the canopy where greater structural diversity and higher endogenous concentrations appear typical.

INSECTICIDAL COMPONENT IN *SPIRAEA THUNBERGII* (ROSACEAE)

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During our studies on the host selection of *Thrips palmi* (Thysanoptera), we found that *Spiraea thunbergii* (Rosaceae) contains a potent insecticidal component against these insects.
When the insects were given a methanol extract (50 mg weight of fresh leaf equivalent) of the eggplant or *S. thunbergii* leaves containing a 3% sucrose solution, only those on the methanol extract of *S. thunbergii* leaves died within 24 h in spite of the extract containing the 3% sucrose solution which should have provided sufficient nutrition for them to survive for 7 days. Even when the methanol extract was given to the insects without direct contact with it, 100% mortality was observed within 72 h. This result clearly showd that the insecticidal activity in *S. thunbergii* was caused by a volatile substance(s). The volatile component(s) from the methanol extract was absorbed in a Sep-pak C18 cartridge and eluted with methanol. The results of a GC analysis indicate that this active eluate mainly consisted of two compounds, **1** (*Rt* = 9.91 min) and **2** (*Rt* = 18.97 min). Compound **1** (□-methylene-□-butyrolactone, that is, tulipalin A) showed intense insecticidal activity against *T. palmi*. An amount of 25 □g/disc (50 mg weight of fresh leaf equivalent) of this compound caused almost 100% mortality within 72 h without direct contact with the insects and other insect species.
This is the first reported case of finding tulipalin A in other plant family except in the Liliaceae plants. Since tulipalin A was released only when the plant was damaged, this compound plays the role of a protective substance against insect attack besides that of post-inhibitin against fungal invasion.

THE ROLE OF CUTICULAR HYDROCARBONS IN NESTMATE RECOGNITION OF THE SUBTERRANEAN TERMITE, *Reticulitermes hesperus*.

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Using nine colonies of the subterranean termite *Reticulitermes hesperus* from a population in Northern California, we examined the role of cuticular hydrocarbons in nestmate recognition. We showed that *R. hesperus* workers can discriminate between nestmates and non-nestmates using an aggression bioassay. The level of aggression was often variable depending on which colonies were interacting. What chemical cues did the workers use for recognition? We correlated overall similarity of cuticular hydrocarbons among interacting colonies with aggression scores to test the hypothesis that colonies with dissimilar cuticular hydrocarbon profiles are more aggressive towards one another than colonies with similar profiles.

REPRODUCTIVE ISOLATION BETWEEN SYMPATRIC CUTICULAR HYDROCARBON PHENOTYPES OF *Reticulitermes* IN NORTHERN CALIFORINA

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Biogeographical information concerning the common subterranean termites, *Reticulitermes*, in northern California implies that there is one predominant species, *R. hesperus* Banks. The diurnal flights of *R. hesperus* are reported to occur in the spring and early fall. The fall flights have been reported to be very conspicuous, with thousands of alates emerging from many colonies simultaneously. Spring flights are relatively diffuse and usually follow periods of rainfall on clear, sunny afternoons. Recent chemosystematic studies of *Reticulitermes* indicate at least 4 cuticular hydrocarbon phenotypes occurring in northern California. The three most common phenotypes, CA-A, CA-A' and CA-D, are sympatric in the San Francisco Bay Area and can be further distinguished by the defense secretions of their soldiers. Since the spring of 1994, we have been able to collect alates from our monitoring stations, from laboratory cultures, and during flights in neighborhoods in the San Francisco Bay Area. As predicted, there were two flight seasons: one from late March to early May and one from early October through December. The flights in the spring were made only by phenotypes CA-A and CA-A'; those in the fall exclusively include phenotype CA-D. These disparate flight times provide a reproductive isolating mechanism and further evidence that phenotypes CA-A and CA-A' are distinct species from CA-D.

NOVEL TRIMETHYL-BRANCHED HYDROCARBONS IN *HELICOVERPA ZEA* PUPAE

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The hydrocarbon fraction from the internal lipids of developing *Helicoverpa zea* pupae contained methyl-branched alkanes ranging in size from methylhexacosane (C27) to dimethylnonatetracontane (C51). A second component was observed eluting with the monomethylakanes beginning with methylpentatriacontane (C36). The second component was identified as a trimethyl-branched alkane in which the first methyl group was located on carbon 2, and the second and third methyl groups wereseparated by a single methylene. Components identified by gas chromatography-mass spectrometry were 2,18,20-trimethyltetratriacontane, 2,18,20-trimethylhexatriacontane, and 2,24,26-trimethyldotetracontane. These novel compounds were not detected in the hydrocarbon fraction from the internal lipids of developing *Heliothis virescens* pupae. These compounds have not been found in the cuticular surface hydrocarbons of larvae, pupae or adults of either species.

CUTICULAR HYDROCARBONS AND SOLDIER DEFENSE SECRETIONS OF *Reticulitermes* FROM SOUTHERN CALIFORNIA

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Cuticular hydrocarbons and soldier defense secretions were characterized for collections of *Reticulitermes* from Los Angeles, Orange, Santa Barbara, San Bernardino, and San Diego counties in southern California. Collection sites included the type locality of *R. hesperus*. We discovered two distinct taxa, SCA-A and SCA-B, that resemble previously described taxa of *Reticulitermes* from northern California. There are, however, some chemical differences that distinguish samples from the two regions. SCA-A is similar to CA-A and CA-A', but can be separated by variation in the amounts of several cuticular hydrocarbons, and the presence of substantial amounts of germacrene A in the soldier defense secretion. SCA-B is similar to CA-B, but exhibits geographic variability in cuticular hydrocarbons composition; its soldier defense secretion is comprised mainly of germacrene A, which was not detected in CA-B samples.

CUTICULAR HYDROCARBONS OF *Reticulitermes* FROM NORTH AMERICA: ANALYSIS OF CHARACTER EVOLUTION IN PHYLOGENETIC STUDIES SUPPORT THREE MONOPYLETIC LINEAGES

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Cuticular hydrocarbon mixtures can be used to discriminate taxa in *Reticulitermes*. We have used cuticular hydrocarbons as chemotaxonomic characters to construct a phylogeny of this genus from collections from North America, because they are independent characters with discrete states and represent a hierarchical distribution of shared, derived states. Early phylogenetic analyses sorted our collections into 26 chemical phenotypes. Using the wood roach, *Cryptocercus* sp., two species of the dampwood termites, *Zootermopsis*, the Formosan subterranean termite, *Coptotermes formosanus*, and the desert subterranean termite, *Heterotermes aureus*, as outgroups, our analyses separated *Reticulitermes* into 3 distinct monophyletic clades or lineages. *Reticulitermes* in Lineage I make a preponderance of 11,15-dimethylalkanes. Those in Lineage II are defined by a preponderance of 5-methyl and 5,17-dimethylalkanes. Taxa in Lineage III are characterized by olefins and a relative paucity of methyl-branched alkanes. Assessment of character evolution corroborates these lineages because the characters that distinguish the lineages are present in nearly all taxa with few homoplasies or instances of convergent or parallel evolution.

PHEROMONE PRODUCTION IN MOTHS UTILIZING HYDROCARBONS

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The hydrocarbon sex pheromone (13-methyl-Z6-heneicosene) of *Scoliopteryx libatrix* L. (Lepidoptera: Noctuidae) was found to reach its highest levels on pheromone glands of 3-day-old females. Pheromone levels were not different between the time of maximum calling (end of scotophase) and at the middle of photophase. Overwintering females collected in October had sex pheromone present. Decapitation did not lower the amount of pheromone present, indicating that a head factor is not involved in maintaining pheromone titers. Hemolymph also contained the pheromone, indicating that it is made by oenocytes and transported to the sex pheromone gland. Longer chain length hydrocarbons were also identified from the hemolymph and on the cuticular surface. Quantitative differences in hydrocarbon profiles were found with more methyl-branched hydrocarbons found in the hemolymph than on the cuticular surface. Evidence will be presented showing the presence of hydrocarbon pheromone in the hemolymph of several other moth species.

ANTIFEEDANTS FOR PROTECTION OF PINES AND SPRUCES AGAINST THE PINE WEEVIL, *Hylobius abietis*

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The pine weevil, *Hylobius abietis* is the most seriously noxious insect in Swedish forestry. Without treatment of newly planted spruces and pines up to 80% can be killed due to the pine weevil's attack. Today permethrin is used for protection but alternatives has to be found as permethrin is environmentally toxic and used only on exemption until 2003.

We are investigating the use of antifeedants / feeding repellents as an alternative to permethrin for protection of the saplings. The substances has to possess low, preferably none, environmental influence and human toxicity. To ensure longterm effect the antifeedant must also be stable towards UV-light and increased temperatures.

In our search for the optimal antifeedant we are screening commercially available or synthesized substances, possesing the properties mentioned above, in laboratory tests. The promising ones are subjected to further tests, finally in the field.

We will present some results from these activity tests.

TRANS-SEXUAL ANTENNAL TRANSPLANTS ALTER SEX SPECIFIC HOST PLANT-APPROACH BEHAVIOR IN A MOTH

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Using an established surgical transplantation technique, we substituted one antenna, including the associated antennal imaginal disk, in male Manduca sexta (Lepidoptera: Sphingidae) larvae with a corresponding graft from a female donor of the same developmental stage. The transplanted disks developed into apparently normal adult-female antennae. Their sensory receptor cells sent axons to the brain of the host, contributing to the formation of an antennal lobe with female-specific characteristics. The altered adult male moths were then tested for flight responses to a hostplant (tomato) in a laboratory wind

tunnel. Males with one female antenna and one male antenna landed on the

hostplant more than 90% of the time. This response contrasts with the response observed in unaltered males, of which only 40% fly to the hostplant. The behavioral responses of gynandromorphic males resemble more closely the behavioral responses of female moths, which fly to the hostplant in 97% of the cases. The transplanted female antenna in combination with its „feminized‰ antennal lobe dramatically alter the hostplant-approach behavior of gynandromorphic male moths.

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CONIFER MOISTURE RELATIONS, GENETICS, AND THE PRODUCTION OF DEFENSIVE TERPENOIDS IN RESPONSE TO AN INSECT-TRANSMITTED FUNGAL PATHOGEN

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Fifty-eight white fir (Abies concolor) trees representing 17 provenances were inoculated with the pathogenic fungus Trichosporium symbioticum during two years of below-normal precipitation (1990 & 91) and a year with above-average precipitation (1993). The resultant fungal wound lesions were removed from the trees after three weeks and then analyzed by gas chromatography. Results showed that not only did terpenoids increase in response to fungal challenge, but that the size and vigor of response is apparently related to moisture availability. For example, the lowest amounts of total wound-lesion terpenoids were produced in the fall of 1990 (10.0 mg/g). The amounts of total terpenoids double in the early summer of 1991 to about 25.0 mg/g (a year of increased precipitation over 1990 but, still below normal). The early summer 1993 inoculation produced the most dramatic results with total terpenoids again doubling to an average of 45 mg/g. Wound-lesion sizes were similar during the 1990/91 inoculations (150 sq. mm), however, double in size during 1993 (300 sq. mm). Phloem moisture content was also lowest in the 1990 inoculated phloem (41%), 45% in 1991 and 50% in 1993. Pre-dawn moisture stress measured just prior to tree inoculation was highest during 1989/90 at 15.13 bar and only 9.5 bar in 1993. Genetic comparisons indicate that the most susceptible provenances (central California) tend to produce the lowest amounts of terpenoids compared to surrounding provenances. These results suggest that a major factor in the ability of conifers to produce defensive terpenoids in response to a pathogenic fungus may be determined by moisture availability.

ARE THERE COSTS TO DELAYED SEXUAL MATURATION IN RESPONSE TO HABITAT DETERIORIATION?

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A number of migrant lepidopteran delay sexual maturation in response to cues associated with habitat deterioration. It is generally assumed that this delay provides the time window necessary for immature adults to locate another habitat where conditions are conducive for reproduction. We will report on two experiments carried out using the sunflower moth, *Homoesoma electellum*, a species that migrates when the habitat does not contain host plants in the appropriate phenological state for oviposition. The first compared the life time fecundity of females as a function of age at which they became sexually mature, determined by the expression of calling, the behaviour associated with the release of the sex pheromone. The second compared the reproductive output of females that called on the first day following emergence but that were then subjected to a five day delay before being provided either mates or suitable oviposition sites, with those that mated and started ovipositing immediately. The results will be discussed within the context of migration and male nutrient contributions to female reproductive success.

ODOUR-MEDIATED NECTAR FORAGING IN THE SILVERY MOTH, *AUTOGRAPHA GAMMA*

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Floral odours play a significant role in the nectar foraging behaviour in Lepidoptera and other insect orders. Floral odour may elicit searching, alighting and feeding behaviours alone or in concert with visual stimuli. At present there are more than 700 scent compounds from more than 400 species of plants identified. The large number of compounds produced by a flower hampers the elucidation of their behavioural activity. Electrophysiological data may provide useful information, limiting the number of compounds to be tested in behavioural experiments. In a fligth tunnel assay we investigated the attractivity of selected flowers to the silver Y moth *A. gamma* (Lepidoptera: Noctuidae) and identified the constitutents of floral scents responsible for this attraction. We tested the following plants with flowers that are visited by *A. gamma* or other noctuid moths: catnip *Nepeta faasseni* (Labiatae), butterfly-orchid *Platanthera bifolia* (Orchidaceae), red clover *Trifolium pratense* (Fabaceae), creeping thistle *Cirsium arvense* (Asteraceae), greater knapweed *Centaurea scabiosa* (Asteraceae) and soapwort *Saponaria officinalis* (Caryophyllaceae). Volatiles from all plant species were collected, analysed for electrophysiological activity by gas chromatography with electroantennographic detection (GC-EAD) and the active compounds were identified by coupled gas chromatography/mass spectrometry (GC-MS). Lilac aldehydes were found to be the major behaviourally active compounds in the odour emitted by *P. bifolia* flowers. We discuss reasons for differential attraction to flowers, plant-species specific volatiles and overlap in profiles that have been observed between plant species.

SENECIONINE N-OXYGENASE A SPECIFIC DETOXIFYING ENZYME OF LEPIDOPTERANS ADAPTED TO PYRROLIZIDINE ALKALOIDS: CLONING, EXPRESSION AND CHARACTERIZATION

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Pyrrolizidine alkaloids (PAs) are plant secondary compounds, found in certain plant families such as the Asteraceae (e.g., Senecio and Eupatorium). PAs exist in two forms, as pro-toxic tertiary amine (free base) and as non-toxic N-oxide. In the gut of non-adapted herbivores ingested PAs are reduced and absorbed passively as free base which in vertebrates and insects is bioactivated yielding cytotoxic pyrrolic metabolites. Specialized insects like larvae of the European cinnabar moth Tyria jacobaeae (Arctiidae, Lepidoptera) developed adaptations to sequester PAs from their host-plants and utilize them in their chemical defense against predators. In Tyria larvae, the absorbed free base is rapidly N-oxidized in the hemolymph. This reaction is catalyzed by a soluble NADPH and flavin dependent, mixed-function monooxygenase (senecionine N-oxygenase, SNO) which substrate-specifically only N-oxidizes pro-toxic PAs. Thus, SNO must have been recruited from basic metabolism during the adaptation of arctiids to its PA containing host-plants [1]. Here we report on the cloning, sequencing and recombinant expression of SNO from Tyria. High sequence homologies were observed to genomic sequences of putative flavin containing monooxygenases of Drosophila, C. elegans and Arabidopsis. Enzyme activity was expressed in E. coli and Sf9-cells. Mechanistic and evolutionary implications will be discussed.

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SEX ATTRACTANTS OF COCKCHAFERS: QUINONE TITERS IN ADULTS AND GRUBS, RELEASE RATES AND ANTIMICROBIAL PROPERTIES (COLEOPTERA: SCARABAEIDAE)

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The forest cockchafer, *Melolontha hippocastani* Fabr. (Coleoptera: Scarabaeidae, Melolonthinae), is known to produce 1,4-benzoquinone and toluquinone. The first compound was shown to act as a sex attractant that enhances synergistically the attraction of males towards plant volatiles induced by feeding females. Analyses of whole body extracts from adults and grubs (L3) of *M. hippocastani* revealed the presence of 1,4-benzoquinone and toluquinone not only in females but also in males and larvae. Females contained significantly more of both compounds than males. Remarkably, grubs contained significantly higher amounts of 1,4-benzoquinone and toluquinone than females. Dynamic headspace analyses by closed loop stripping (CLS) revealed that females release significantly higher amounts of 1,4-benzoquinone than males. Toluquinone was not detected in the headspace extracts. In bioassays using *Escherichia coli, Saccharomyces cerevisiae,* and the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria brongniartii* both 1,4-benzoquinone and toluquinone showed an inhibitory effect against all tested microorganisms. However, the amounts necessary for inhibition of entomopathogenic fungi and *S. cerevisiae* in the laboratory assay were higher than those found in the extracts from *M. hippocastani.*

IDENTIFICATION AND CLONING OF A PHEROMONE-BINDING PROTEIN FROM THE ORIENTAL BEETLE, *Exomala orientalis*

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We have identified and cloned a pheromone-binding protein (EoriPBP) from the Japanese and American populations of the Oriental beetle, *Exomala orientalis*. The protein showed more than 90% amino acid identity to the previously identified pheromone-binding proteins from *Popillia japonica* (PjapPBP), and *Anomala osakana* (AosaPBP), as well as to one of the odorant-binding proteins from *Phyllopertha diversa* (PdivOBP1). EoriPBP has 116 amino acids, with a calculated molecular mass 12,981 Da, pI of 4.3, and six highly conserved cysteine residues. 5'-RACE amplifications led to the characterization of a signal peptide with 19 amino acids. The signal peptide showed high amino acid identity to the signal peptide for AosaPBP. Comparison of the amino acid sequences of the PBPs involved in the detection of similar ligands, i. e., monounsaturated lactones and ketone, suggests that the most variable residues among the PBPs from *E. orientalis*, *P. japonica*, and *A. osakana*, are probably the most discriminating residues. As with the pheromone-binding protein from *Bombyx mori,* the residues at positions 61, 64, 71, and 82 in EoriPBP, PajpPBP, and AosaPBP, which are either valine, leucine, isoleucine, and methionine, are likely to be specificity determinants.

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CO-EVOLUTION OF PLANT AND INSECT: THE ORIENTAL ORCHID (*Cymbidium floribundum* Lindl.) CONTROLS THE SOCIAL BEHAVIOR OF THE ASIAN HONEY BEES ?

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The Japanese honeybee (*Apis cerana japonica* Rad) and the introduced European honeybee (*Apis mellifera* L.) share the same habitat in Japan. Very little is known about the biology of *A. c. japonica*. The red and white flower varieties of the oriental orchid, "Kinn-ryou henn" (*Cymbidium floribundum* Lindl.: Cf), has been observed to attract workers, drones, queens even the entire swarming colony of *A. c. japonica*. However, the orchid does not attract *A.. mellifera*. This is due to the fact that the flower scent mimics the Nasonov and mandibular glands pheromones of *A. c. japonica*. Both Nasonov gland and mandibular glands extracts induced aggregation behavior in both bee species, but the GC profiles of the extract from *A. mellifera* and *A. c. japonica* were significantly different. It is interesting that a plant like *C. floribundum* can affect the social behavior of A. c. japonica. The experiments using other Asian honeybee such as *Apis cerana cerana* are underway.

We report here for the first time that: (1) The same species of Cf is found in China in 1999. (2) Flower aroma of Chinese Cf were analyzed by GC/MS and these results were compared between Japanese Cf and Chinese one. (3) In search of keys to socialities and diversity in insects, the components of pheromone glands (Nasonov, and mandibular gland) of honey bees in Asia (*A. cerana japonica*, *A. c. cerana*, *A. c., Apis mellifera* etc.) were analyzed by GC/MS. In this paper, we summarize our recent findings on chemical ecology of the flower scent and semiochamicals of the Asian honey bees.