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(July 27, 2023)

Lepidopteran Sex Pheromones: Wonderland for an Agricultural Chemist

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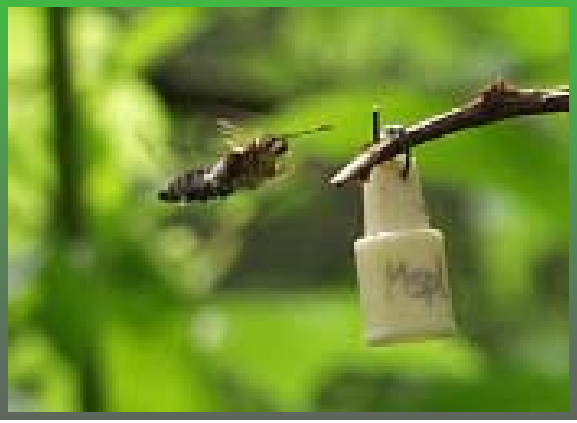
and Technology, Japan



Lepidopteran Sex Pheromones:
Wonderland for an Agricultural Chemist

Sex pheromones have been identified from 722 lepidopteran species.
Male attractants have been reported for other 1323 species.

1) Characteristic chemical structures

Commonality ⇐ speciation from a common ancestor

Diversity ⇐ reproductive isolation (diversity of insect species)

Type I, Type II, and others

2) How to determine the structure?

Bioassay ⇒ EAG (Electroantennography) ⇒ GC-EAD

Instrumental analysis ⇒ GC-MS

3) Application of synthetic pheromones to pest control

Monitoring and mating disruption

4) Type III pheromones (methyl-branched compounds)

5) Database of semiochemicals (pheromones and allomones)

Representative lepidopteran sex pheromones

Sex pheromones have been identified from 722 species.
Male attractants have been reported for other 1323 species.

Lepidoptera (more than 150,000 species)

Type I

silkworm moth

E10,Z12-16:OH
bombykol

smaller tea tortrix

Z9-14:OAc

Z11-14:OAc

rice stem borer

Z11-16:Ald

Z13-18:Ald

Unsaturated fatty alcohols, acetates and aldehydes with a C_{10} – C_{18} chain
Found most commonly (75%)

Type II

Polyunsaturated hydrocarbons and their epoxides with a C_{17} – C_{23} chain
Identified from evolved-insect groups (15%)

Z3,Z6,Z9-21:H

plum cankerworm moth

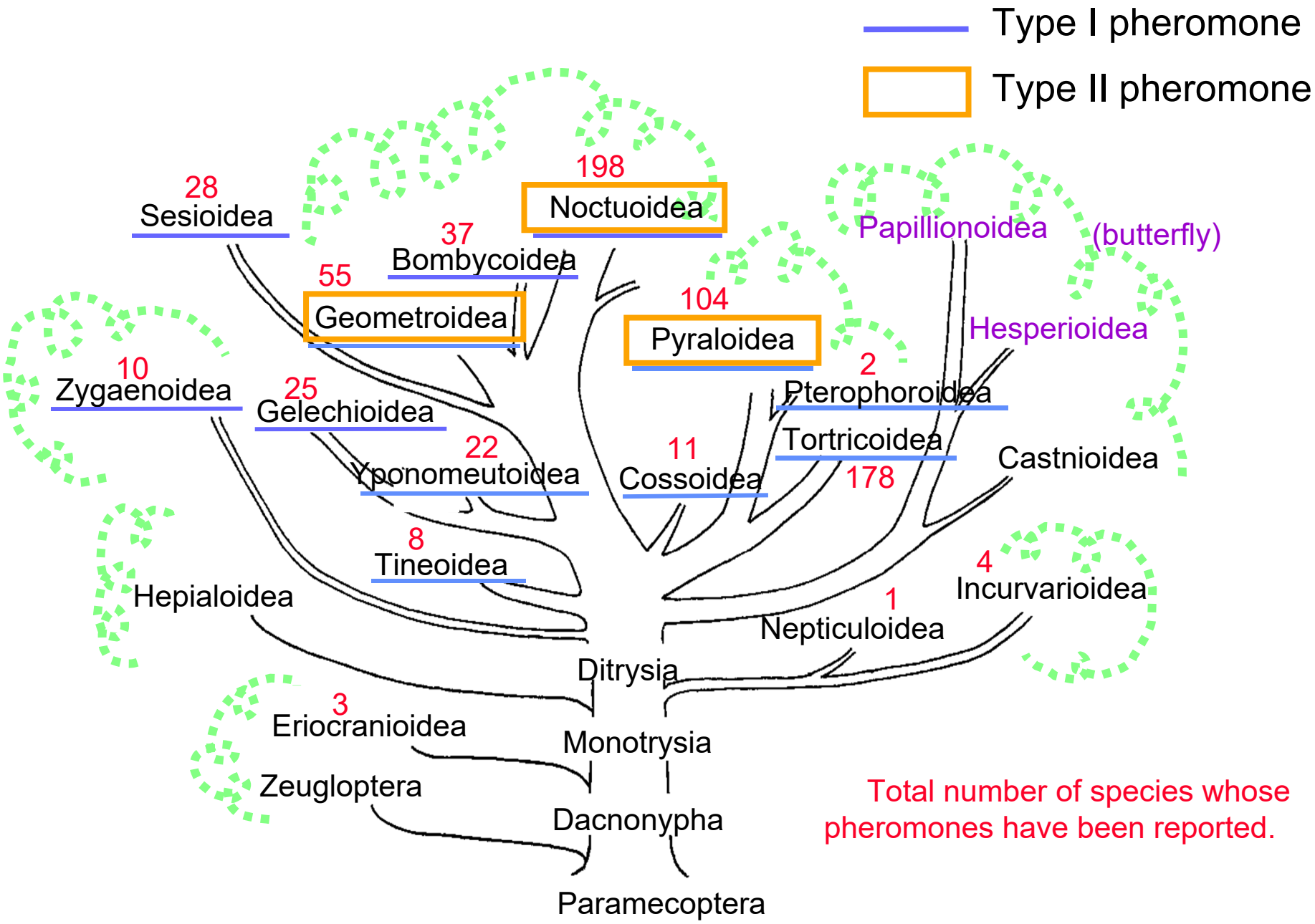
epo3,Z6,Z9-19:H

Milionia basalis

Z3,epo6,Z9-19:H

giant geometrid moth

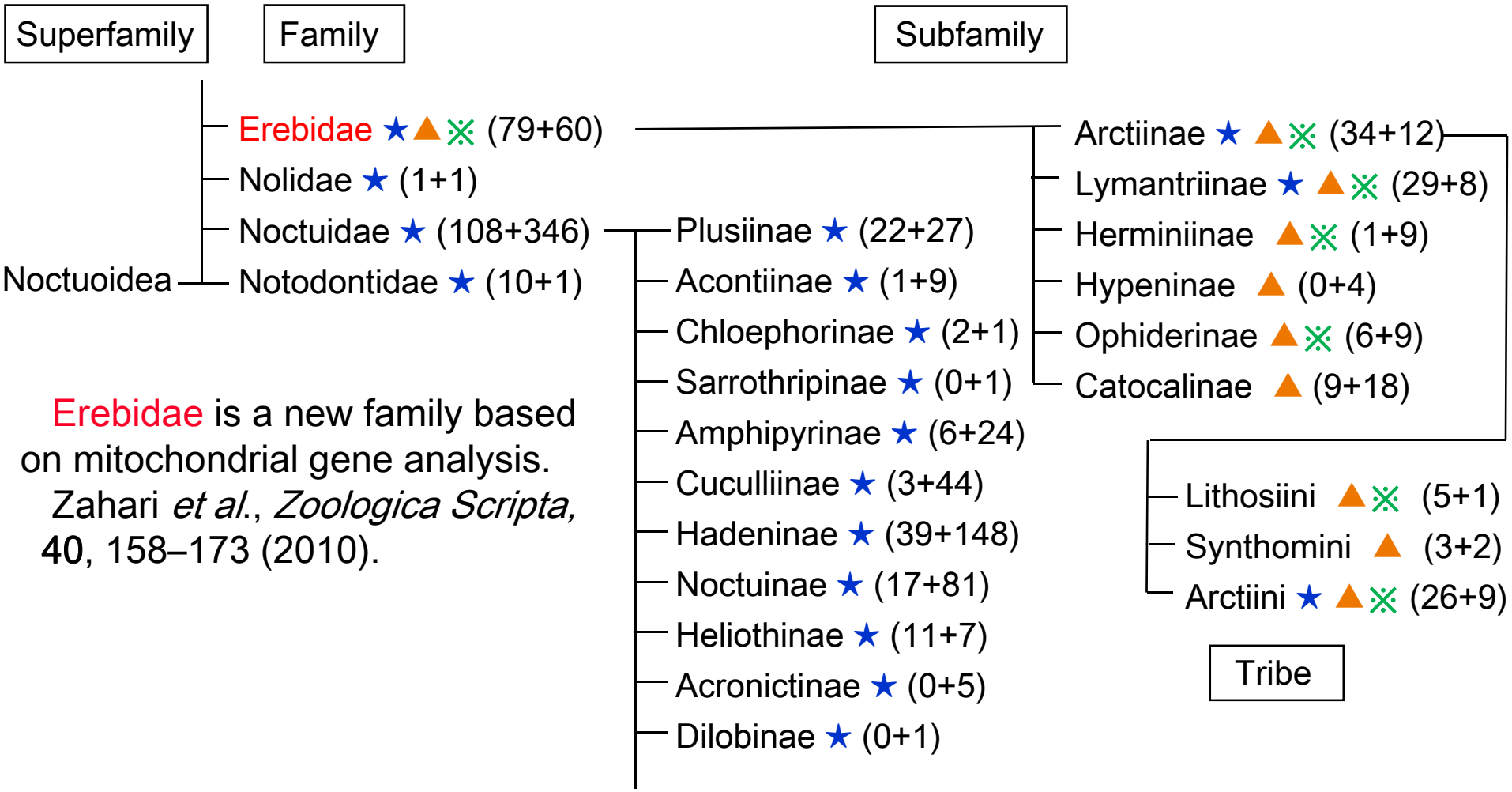
Phylogenetic tree of Lepidoptera



Pheromone studies in the superfamily of Noctuoidea

The numbers in brackets indicate the total number of species whose female pheromones or male attractants have been reported. (pheromone + attractant)

★ Type I compounds, ▲ Type II compounds, ※ others



Erebidae is a new family based on mitochondrial gene analysis.
Zahari *et al.*, *Zoologica Scripta,* **40**, 158–173 (2010).

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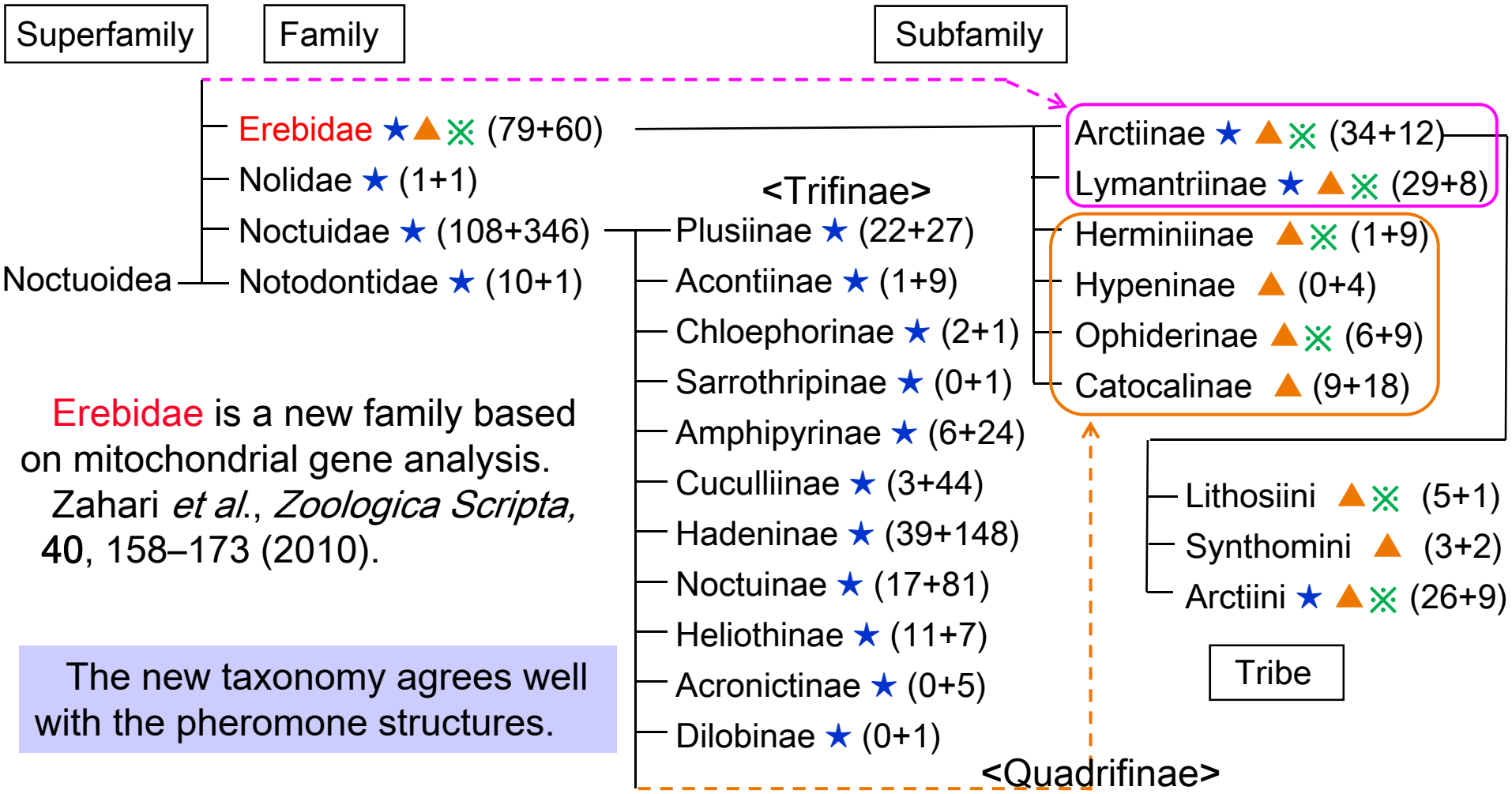

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The new taxonomy agrees well with the pheromone structures.

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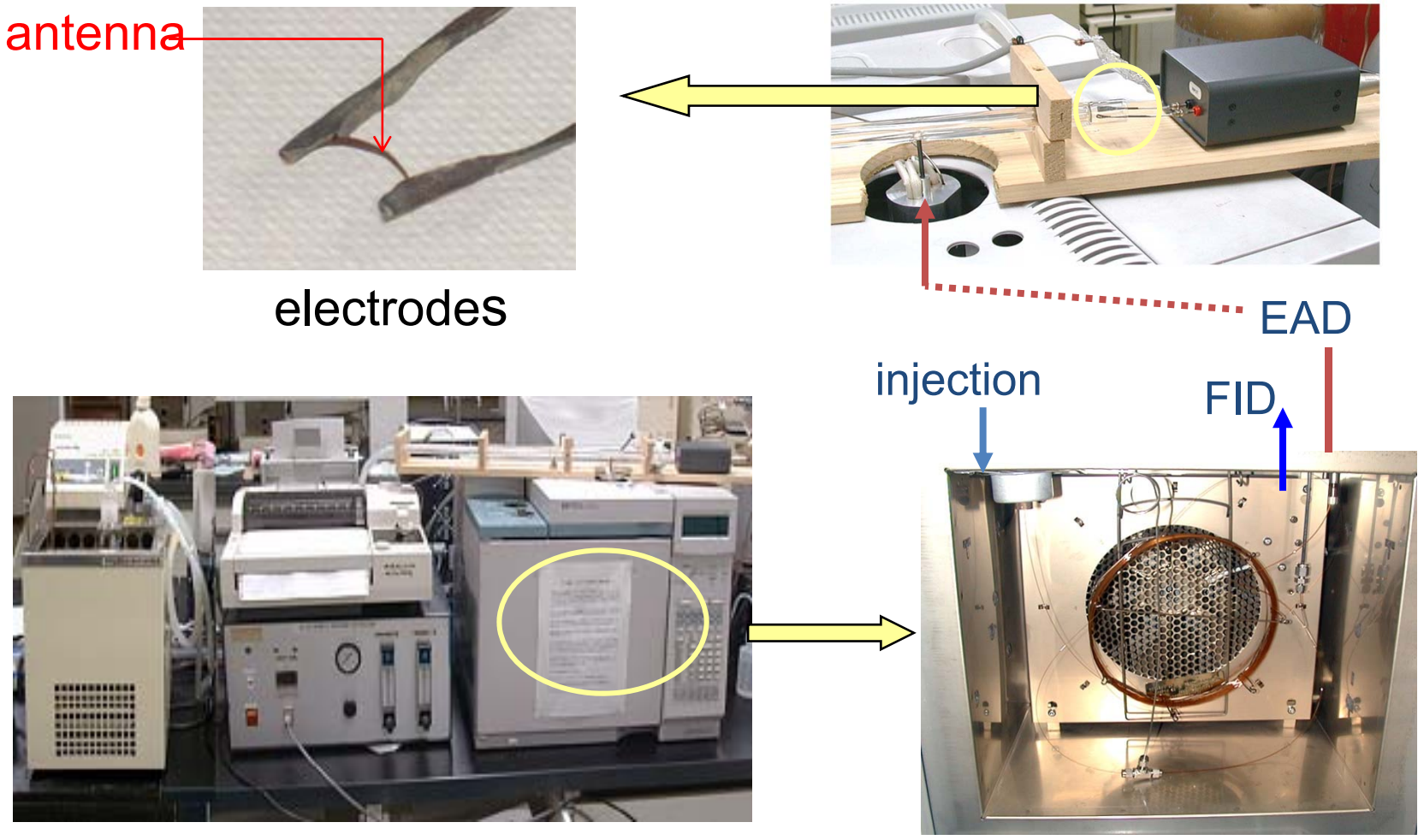
GC-EAD

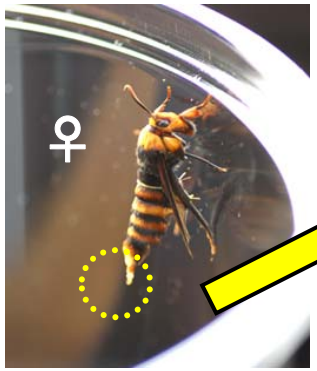
GC equipped with an EAG detector (EAD)

GC-EAD is one of the most important instruments.
Pheromone researches dramatically developed using this instrument.



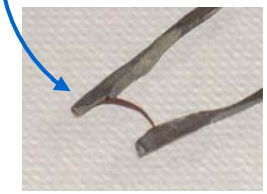
Instrumental analysis of a pheromone extract

Clearwing moth
(*Toleria romanovi*)



Pheromone
gland extract

(A) GC-EAD analysis
Finding of active components
in the female extract



electrodes

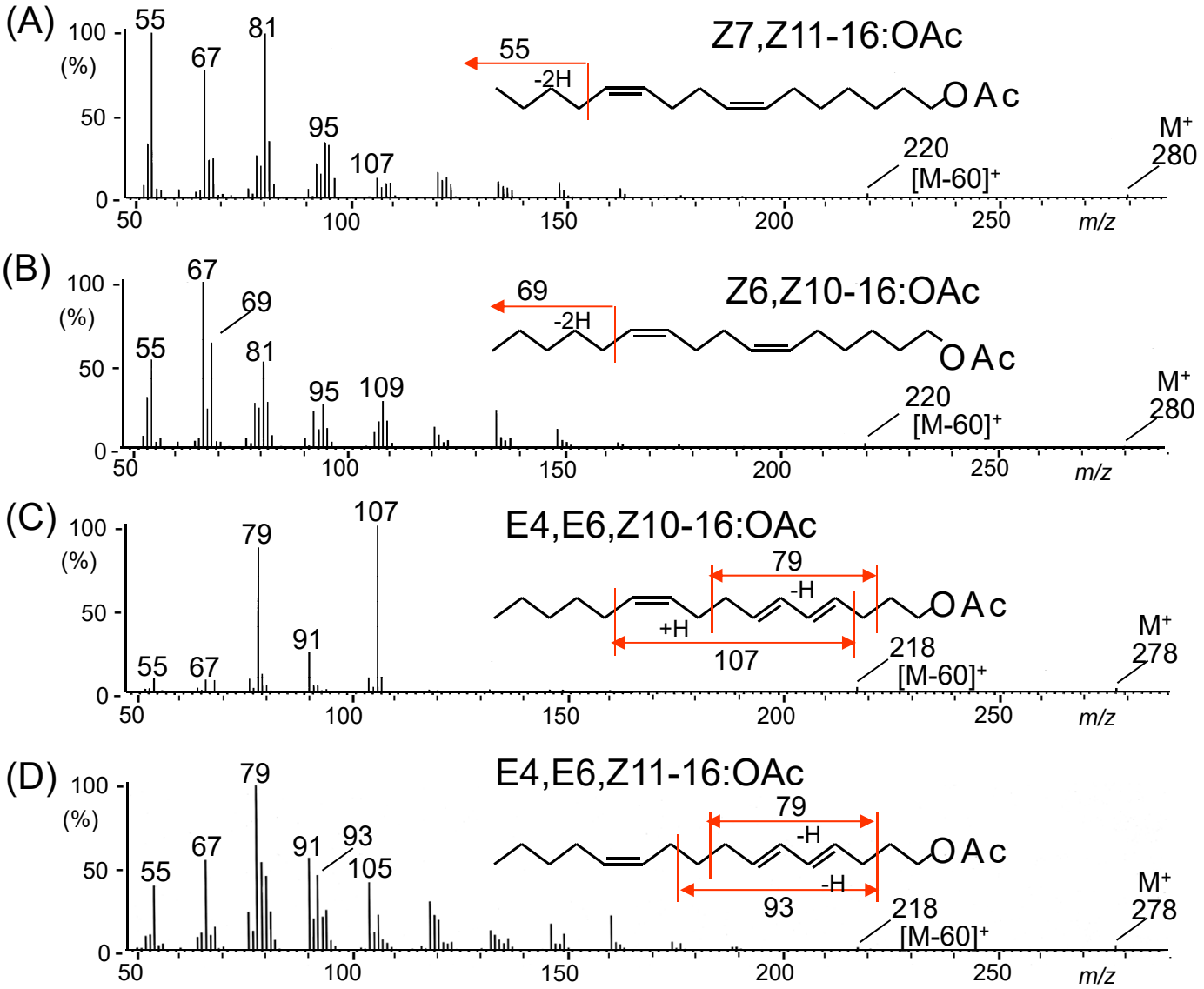
Comp. **III**
Comp. **II**
Comp. **I**
EAD
0.1 mV
FID
23.0
24.0
Rt (min)

(B) GC-MS analysis
Structure determination
by Rt and spectral data

Z3,Z13-18:OH
(Comp. **III**)
Z3,Z13-18:OAc
(Comp. **I**)
TIC (Total ion
chromatogram)
E3,Z13-18:OH
(Comp. **II**)
X 7.5
20.0
21.0
22.0
Rt (min)

Mass spectra of dienyl and trienyl Type I compounds

It is not easy to determine the structure from mass spectral data alone.
However, some compounds exhibit useful diagnostic ions.



[Gelechiidae]
pink bollworm moth

synthetic analogue

[Gracillariidae]
cocoa pod borer moth

[Saturniidae]
eri-silkworm moth

Mass spectra of dienyl and trienyl Type I compounds

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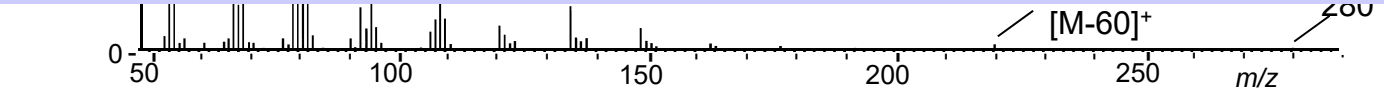
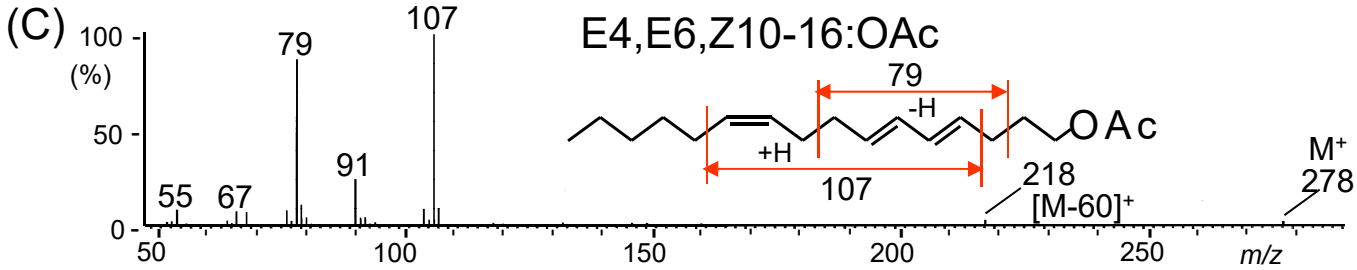
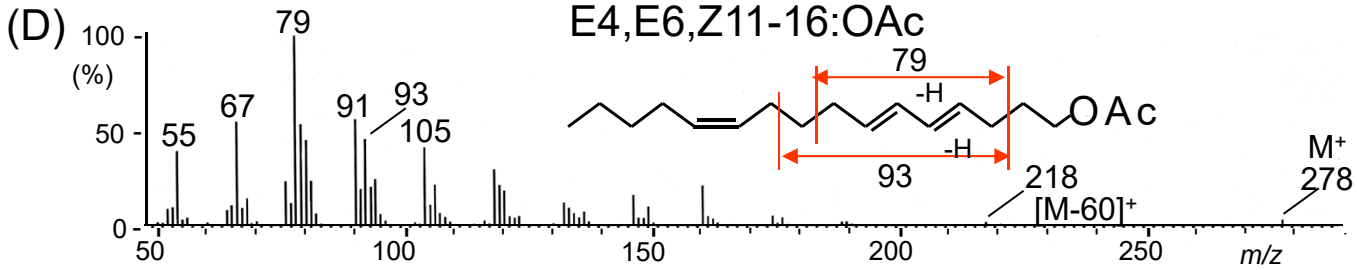
[Gelechiidae]
pink bollworm moth

Published mass spectral data are still limited.
In order to effectively utilize GC-MS for structure determination, further accumulation of spectral data on pheromones is required.





[Gracillariidae]
cocoa pod borer moth

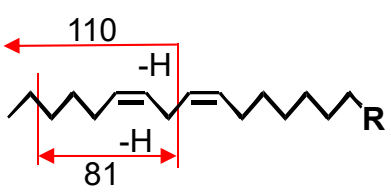


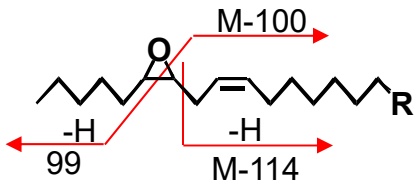
[Saturniidae]
eri-silkworm moth

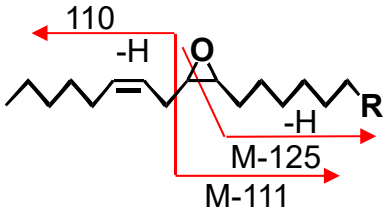
Diagnostic ions of Type II pheromones

Since Type II pheromones are biosynthesized from linolic and linolenic acids, their double bonds are commonly located at the 3-, 6-, and 9-positions. Positions of epoxy-rings and extra double bonds can be deduced by GC-MS analysis.

(A) 6,9-Dienes and the mono-epoxides

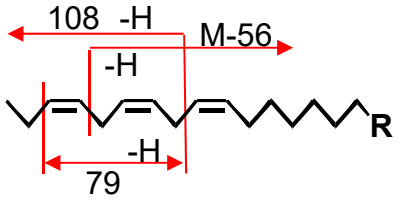


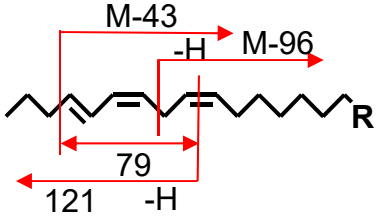


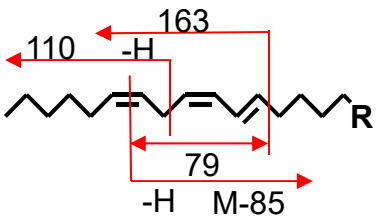


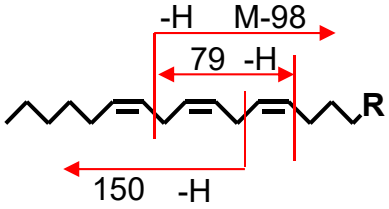
$R = CH_3 - C_7H_{15}$

(B) 3,6,9-Trienes and the positional isomers

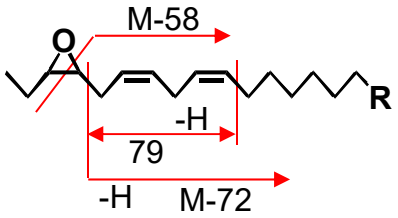


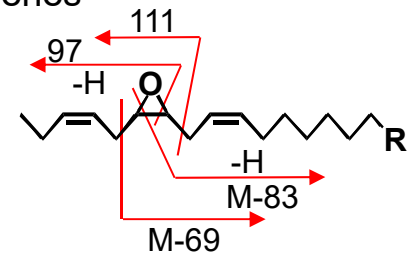


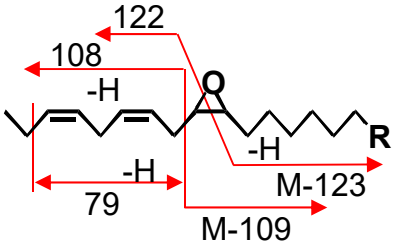


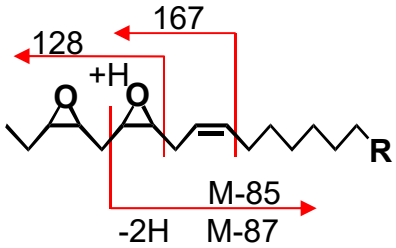


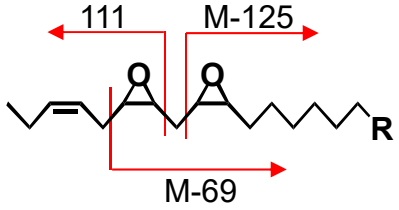
(C) Mono- and di-epoxides of 3,6,9-trienes

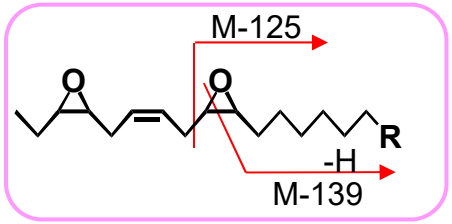












Not yet identified

Joint researches in Japan

Saturniidae

Dr. H. Naka

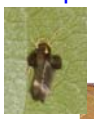
Geometridae

Yponomeutidae

Sesiidae

Stathmopodidae

Drs, H. Naka,
T. Suzuki &
K. Tsuchida



Limacodidae

Noctuidae

Noctuidae

Dr. K. Kishino

Noctuidae

Dr. M. Nomura

Tortricoidea

Drs. M. Ishitani
& F. Komai

Crambidae

Dr. O. Saito

Sphingidae

Drs. H. Honda
& T. Uehara

Noctuidae

Dr. O. Saito

Gracillariidae

Dr. N. Ohbayashi
& S. A. Mafi

Saturniidae

Dr. H. Tatsuta

Geometridae

Dr. H. Yasui

Noctuidae

Dr. M. Nomura

Drs. T. Koshihara
S. Yamada

Crambidae

Geometridae

Dr. K. Ohtani

Gracillariidae

Dr. T. Horikawa

Lasiocampidae

Drs. K. Katagiri, T. Ikeda
& A. Yamane

Geometridae

Dr. M. Kiso

Erebidae

Erebidae

Drs. S Wakamura
N. Arakaki

Tortricoidea

Tortricoidea

Drs. F. Komai & M. Kinjo

Erebidae

Erebidae

Dr. Y. Terashima

Photos from : http://www.jpmoth.org/

Joint researches with CTU

(1) Field screening tests of synthetic pheromone candidates

Hai *et al,,* 2002. *J. Chem. Ecol*., **28**: 1473-1481.

Type I compounds attracted 12 species; Noctuidae (6), Tortricidae (3), and etc.
Type II compounds attracted 7 Erebidae species; Arctiinae (4), and etc.

(2) Identification of sex pheromones

Citrus leafminer moth
Vang *et al.,* 2008. *J. Pestic. Sci.*, **33**: 152-158.
Citrus pock caterpillar
Vang *et al.,* 2011. *J. Chem. Ecol.,* **37**: 134-140. [Mating disruption]
Clear wing moth (*Carmenta mimosa*)
Vang *et al.,* 2012. *Biosci. Biotechnol. Biochem.,* **76**: 2153–2155.
Citrus leafrollers (*Adoxophyes privatana, Archips atrolucens, Homona tabescens*)
Vang *et al.,* 2013. *J. Chem. Ecol.,* **39**: 783-789.
Sweetpotato vine borer moth
Yan *et al.,* 2014. *J. Chem. Ecol*., **40**: 590–598.
Eggplant borer
Vang *et al.,* 2018. *J. Chem. Ecol*., **44**: 631–636.
Cabbage webworm
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I hope that studies on chemical ecology will develop more strongly in Southeast Asian countries.

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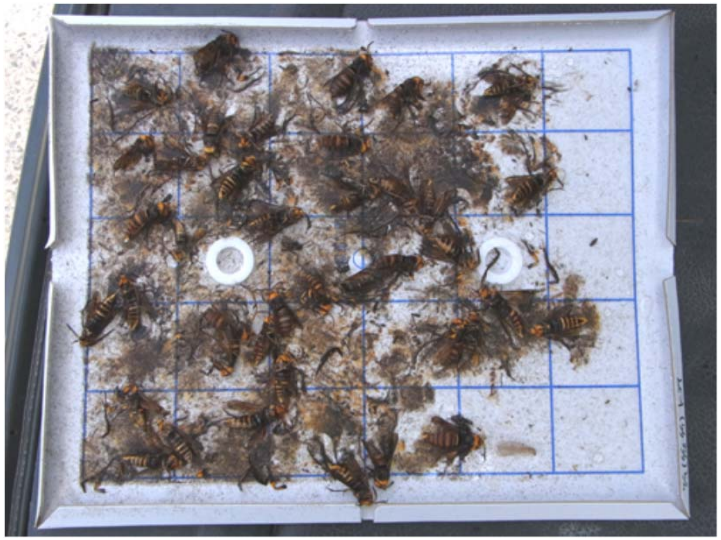
Commercialized lures for monitoring

1) *Toleria romanovi* (pest of vine trees)

Diurnal moth mimicking a wasp
Larvae bore into the trunk,
causing the whole vine tree to death.
Recently, the damage suddenly spread.

(8:1)
Z3,Z13-18:OH
Z3,Z13-18:OAc

Lure: Rubber septum including 1mg of the synthetic pheromone can attract male moths during two months.



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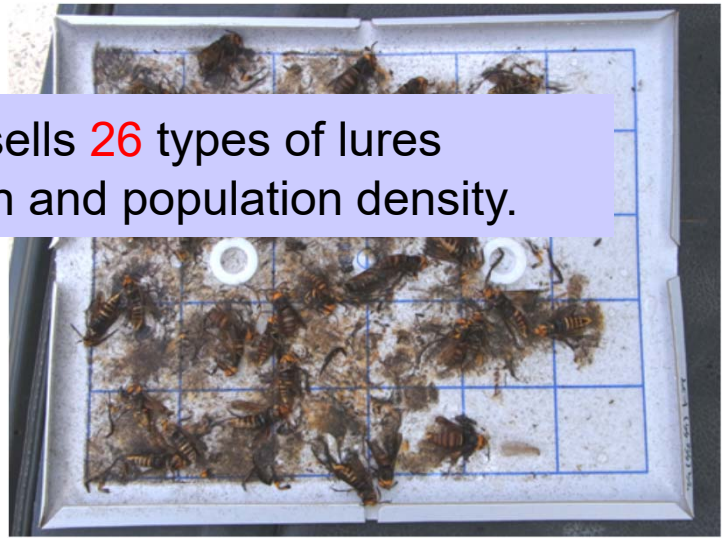
The Japan Plant Protection Association sells 26 types of lures as a monitoring tool to know the flight season and population density.



Mating disruption

High-concentration of a synthetic pheromone blocks the love calls from virgin females.

Dispenser containing 80 mg of compound

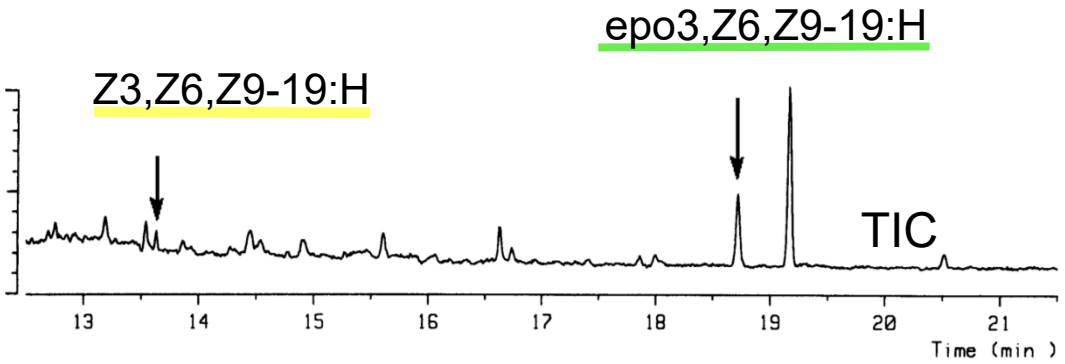
Mating Disruption in the World, 1997 (Type I pheromones)

Crop	Insect	Country	Applied field
Cotton	pink bollworm moth	USA	30,000 ha
		Egypt	328,000 ha
		Israel	8,000 ha
Apple, Pear	coddling moth	USA	13,200 ha
Grape	grapevine moth	Italy	8,800 ha
Tea	small tea tortrix	Japan	400 ha
Vegetable	diamondback moth	Japan	1,000 ha

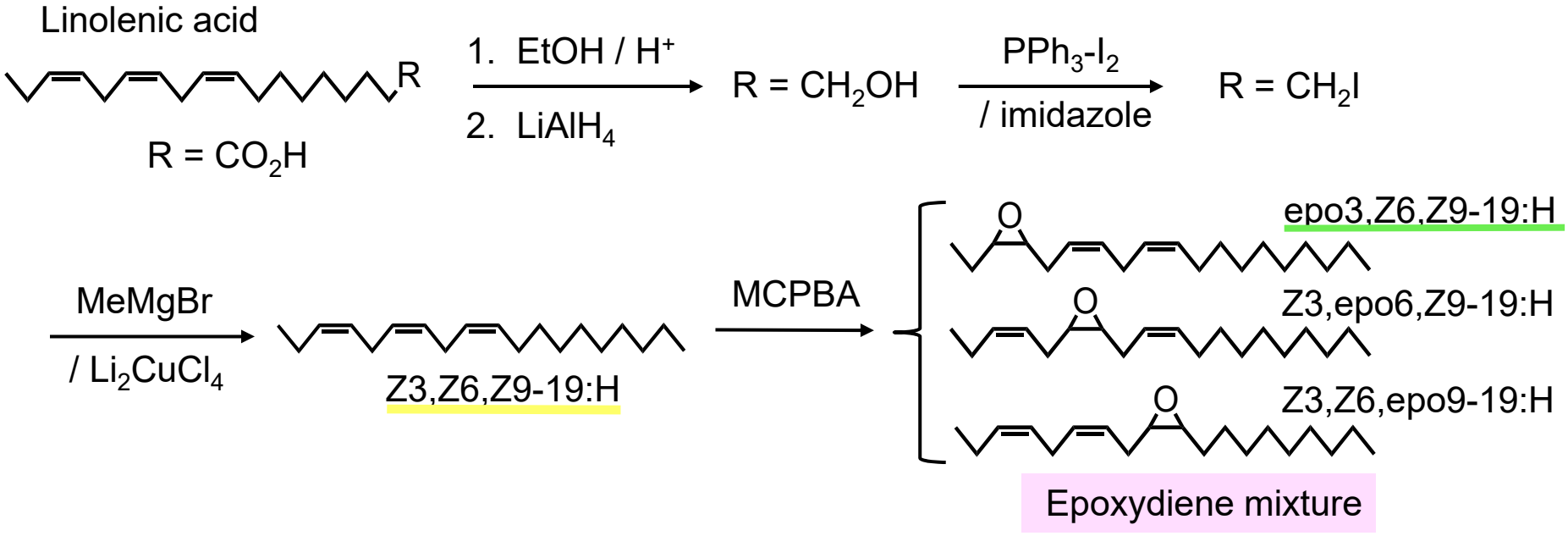
Type II pheromone of the Japanese Giant Looper

Ascotis selenaria
(Geometridae: Ennominae)

(A) GC-MS analysis of a pheromone extract



(B) Synthesis of the pheromone components



Epoxydiene mixture

Mating disruption of the Japanese Giant Looper

Mating ratios of tethered females in the tea gardens which were permeated with triene or epoxydiene mixture released from dispensers（polythylene tubes)

Tubes (N / ha)	
0	In the evening, one tethered virgin female was placed in the center of each test field. Next morning, the female was recovered and examined her mating.
250	
500	
1000	
3000	
5000	

Mating disruption with a Type II pheromone

Mating ratios of tethered females in the tea gardens which were permeated with triene or an epoxydiene mixture released from dispensers（polythylene tubes)

Tubes (N / ha)	(A) Triene [a]			(B) Epoxydiene mixture [b]		
	No. of females		Mating ratio (%)	No. of females		Mating ratio (%)
	Unmated	Mated		Unmated	Mated	
0	0	11	100	0	14	100
250	-	-	-	10	3	23
500	4	6	60	10	4	29
1000	3	6	67	13	1	7
3000	2	8	80	12	0	0
5000	6	4	40	12	0	0

[a] Tested from Sept. 7 to 14, 1999.

[b] Tested from Sept. 7 to 18, 1999.

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Pheromones of lichen moth

(Number of the species whose pheromones were known in 2006)

- Noctuoidea
  - Noctuidae (100)
  - Erebidae (69)
    - Lymantriinae (26)
    - Arctiinae (27)
      - Arctiini (24)
      - Synthomini (3)
      - Lithosiini (0)
  - Nolidae (1)
  - Notodontidae (9)

Because of harmless insects, nobody has been interested in their pheromones.

⇒ Novel pheromones are expected.

Lichen moths

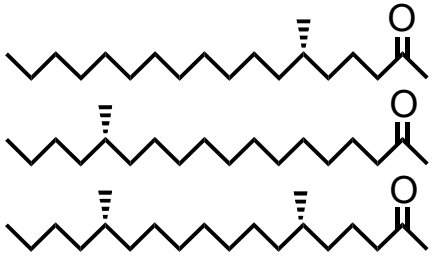
Many species appear throughout the year.

⇒ Moths collected by a light trap were sent to Tokyo, and their pheromones were analyzed.

Iriomote Island

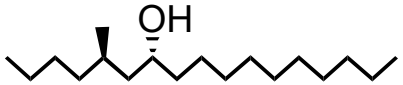
Methyl-branched sex pheromones of Lepidoptera

Lichen moths inhabiting Iriomote Island



Lyclene dharma

Adachi *et al.*, 2010, *JCE,* **36**, 814



Yamakawa *et al.*, 2011, *Tetrahedron Lett.*, **52**, 5808

Miltochrista calamina

Type III pheromones ("Propanogenins")

Leafminer moths (Lyonetiidae)

peach leafminer

Sugiei *et al.*, 1984, *AEZ,* **36**, 814

apple leafminer

Gries *et al.*, 1997, *JCE,* **23**, 1119

mountain-ash bentwing

Francke *et al.*, 1987, *Naturwiss.*, **74**, 143

Hemlock looper (Geometridae)

Gries *et al.*, 1991, *Naturwiss..*, **78**, 315

Gries *et al.*, 1993, *JCE,* **19**, 1501

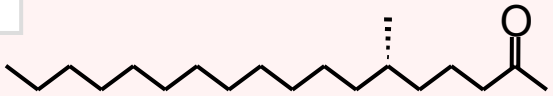
Bagworm moth (Psychidae)

Gries *et al.*, 2006, *JCE,* **32**, 1673

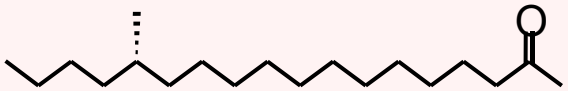
Methyl-branched 2-ketones acting as a pheromone

Lepidoptera

Lyclene dharma



(*S*)-Me6-18: 2-one



(S)-Me14-18: 2-one

Me6,Me14-18: 2-one

Yamamoto *et al.* , *BBB* **71**: 2860 (2007)

Coleoptera

Diabrotica balteata
(banded cucumber beetle)

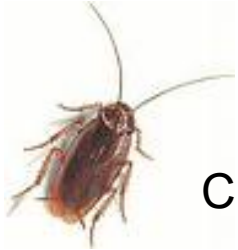
Me6,Me12-15: 2-one

Chuman *et al.* , *JCE* **13**: 1601 (1987)

Diabrotica undecimpunctata
(spotted cucumber beetle)
Me10-13: 2-one

Guss *et al.* , *JCE* **9**: 1363 (1983)

Blattodea



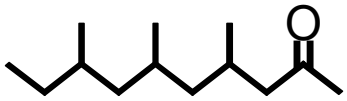
Blattella germanica
(German cockroach)

Me3,Me11-29: 2-one

Nishida *et al.* , *Experientia* **30**: 978 (1974)

Arachnida (Acari)

Chortoglyphus arcuatus
(storage mite)



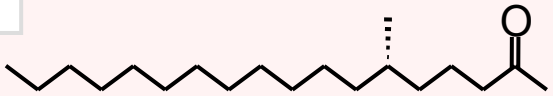
Me4,Me6,Me8-10: 2-one
chortolure

Schulz *et al.* , *Chembiochem* **5**: 1500 (2004)

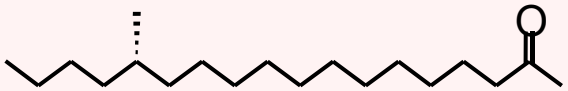
Methyl-branched 2-ketones acting as a pheromone

Lepidoptera

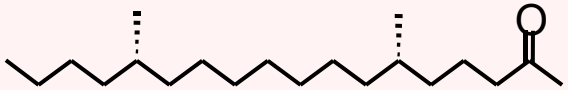
Lyclene dharma



(*S*)-Me6-18: 2-one



(S)-Me14-18: 2-one



Coleoptera

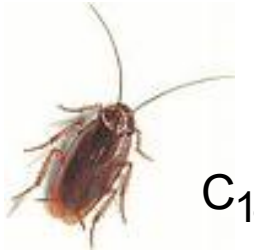
Diabrotica balteata
(banded cucumber beetle)

Me6,Me12-15: 2-one

Chuman *et al.* , *JCE* **13**: 1601 (1987)

These structures have similarities and differences. Therefore, I started making a database to understand the diversity.

Blattodea



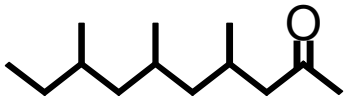
Blattella germanica
(German cockroach)

Me3,Me11-29: 2-one

Nishida *et al.* , *Experientia* **30**: 978 (1974)

Arachnida (Acari)

Chortoglyphus arcuatus
(storage mite)



Me4,Me6,Me8-10: 2-one

chortolure

Schulz *et al.* , *Chembiochem* **5**: 1500 (2004)

Lepidopteran Sex Pheromones:
Wonderland for an Agricultural Chemist

Sex pheromones have been identified from 722 lepidopteran species.
Male attractants have been reported for other 1323 species.

1) Characteristic chemical structures

Diversity ⇐ reproductive isolation (diversity of insect species)
Commonality ⇐ speciation from a common ancestor

Type I, Type II, and others

2) How to determine the structure?

Bioassay ⇒ EAG (Electroantennography) ⇒ GC-EAD
Instrumental analysis ⇒ GC-MS

3) Application of synthetic pheromones to pest control

Monitoring and mating disruption

4) Type III pheromones (methyl-branched compounds)

5) Database of semiochemicals (pheromones and allomones)

Database of semiochemicals ①

https://lepipheromone.sakura.ne.jp/pdb_top_eng.html

Part I. Lepidopteran sex pheromones and attractants

[1630 references (2023, July)]

Chilo suppressalis (rice stem borer)
pheromone: Z11-16:Ald + Z13-18:Ald + other components
1st study: Nesbitt *et al*, 1975. *J. Insect Physiol*., **21**: 1883-1886.

Searching by the database

Ex. 1） *Chilo suppressalis* ⇒ hits of 5 references
studies with the strains in 4 countries
(Philippines, Japan, Korea, and Iran)

Ex. 2） *Chilo* ⇒ hits of 20 references
studies on 10 species of the genus *Chilo*

Ex. 3） Z11-16:Ald ⇒ hits of pheromones for 61 species
and attractants for 121 species
(one of the most common components in the pheromones)

Database of semiochemicals ②

Part II. Arthropod pheromones and related compounds

[2670 references (2023, July)]

Objects recorded in the database

Organisms: Insecta, Arachnida (spider, mite), Diplopoda (millipede), and Chilopoda (centipede)

Semiochemicals:

Pheromones (sex, aggregation, antiaphrodisiac, alarm, trail, marking, primer/queen, death/necromone, and *etc.*)

Allomones (defense)

Gland secretions of unknown role

Grouping of 1592 chemicals

Terpenes (420): acyclic (180), small ring (75), large ring (22), fused-ring (126), heterocyclic (17)

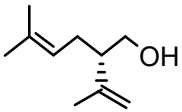
Methyl-branched non-terpenes [Propanogenins] (291): hydrocarbons (74), ring (36), 1º OH & derivatives (39), 2º OH & esters (37), ketones (48), acids & derivatives (57)

Others (881): acyclic (438), ring (96), true alkaloid (208), aromatic (139)

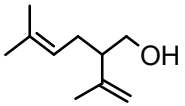
Acyclic terpenes with a C_3 side-chain

C10 (C6 chain)

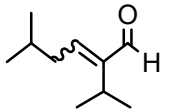
lavandulol



mealybug (F sex phe.)

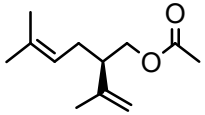


carrion beetle (allomone)

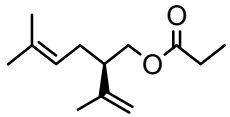


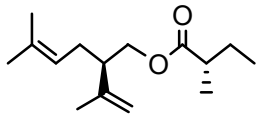
ant (alarm phe.)

lavandulyl acetate



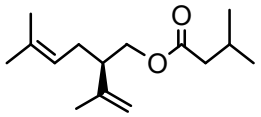
mealybug (F sex phe.)
thrips (aggregation phe.)

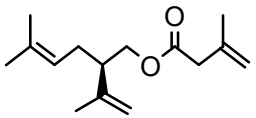




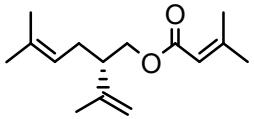
mealybug (F sex phe.)

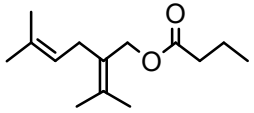
fujikonyl butylate



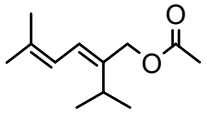


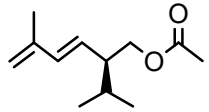
thrips (aggregation phe.)





mealybug (F sex phe.)

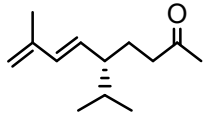




mealybug (F sex phe.)

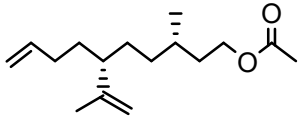
C13 (C9 chain)

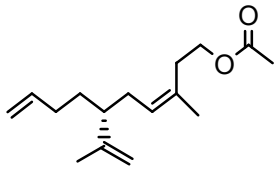
solanone



scale (F sex phe.)

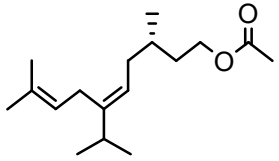
C14 (C10 chain)



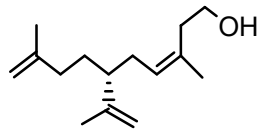


scale (F sex phe.)

C15 (C10 chain)

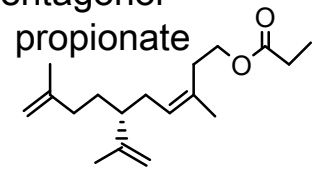


pentagonol



scale (F sex phe.)

pentagonol propionate

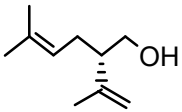


scale (F sex phe.)

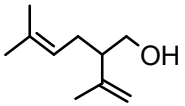
Acyclic terpenes with a C_3 side-chain

C10 (C6 chain)

lavandulol



mealybug (F sex phe.)



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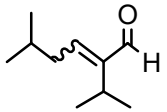
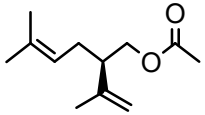
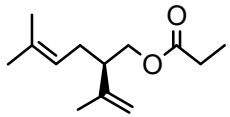


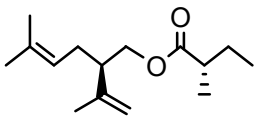
ant (alarm phe.)

lavandulyl acetate



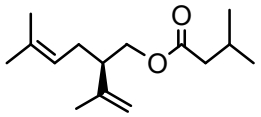
mealybug (F sex phe.)
thrips (aggregation phe.)

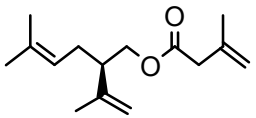




mealybug (F sex phe.)

fujikonyl butylate





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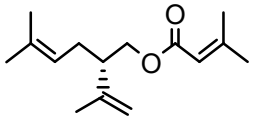
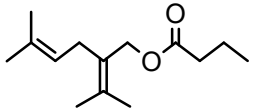
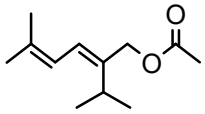
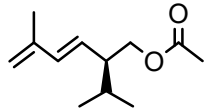




mealybug (F sex phe.)





mealybug (F sex phe.)

Sex pheromones of 35 scale insects have been reported.
In addition to the acyclic terpenes, scale insects secrete terpenes with a small ring and non-terpene methyl-branched compounds.

Since there are about 8,000 scale species in the world, various novel compounds will be discovered in the near future and used for pest control.

Thank you for your attention!!

Chemical Ecology Laboratory of TUAT (1982-2015)

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Dr. R. Yamakawa
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Dr. N. D. Do
Dr. T. Taguri
Dr . Q. Yan