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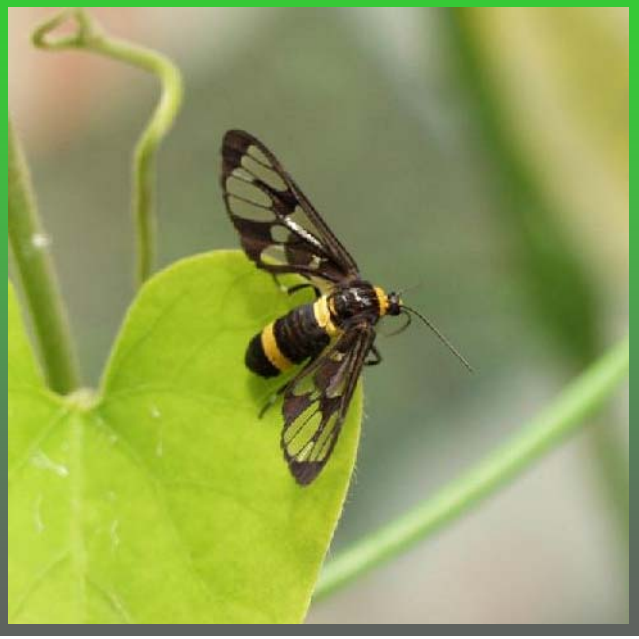
Identification of C_{21} Type II sex pheromone components and novel C_{20} and C_{22} trienyl biosynthetic precursors from a wasp moth, *Syntomoides imaon* (Arctiidae: Syntominae)

Tetsu ANDO et al.

Graduate School of BASE,
Tokyo University of
Agriculture and Technology,
Tokyo 184-8588, Japan
E-mail: antetsu@cc.tuat.ac.jp



Diurnal moths

Clearwing moth

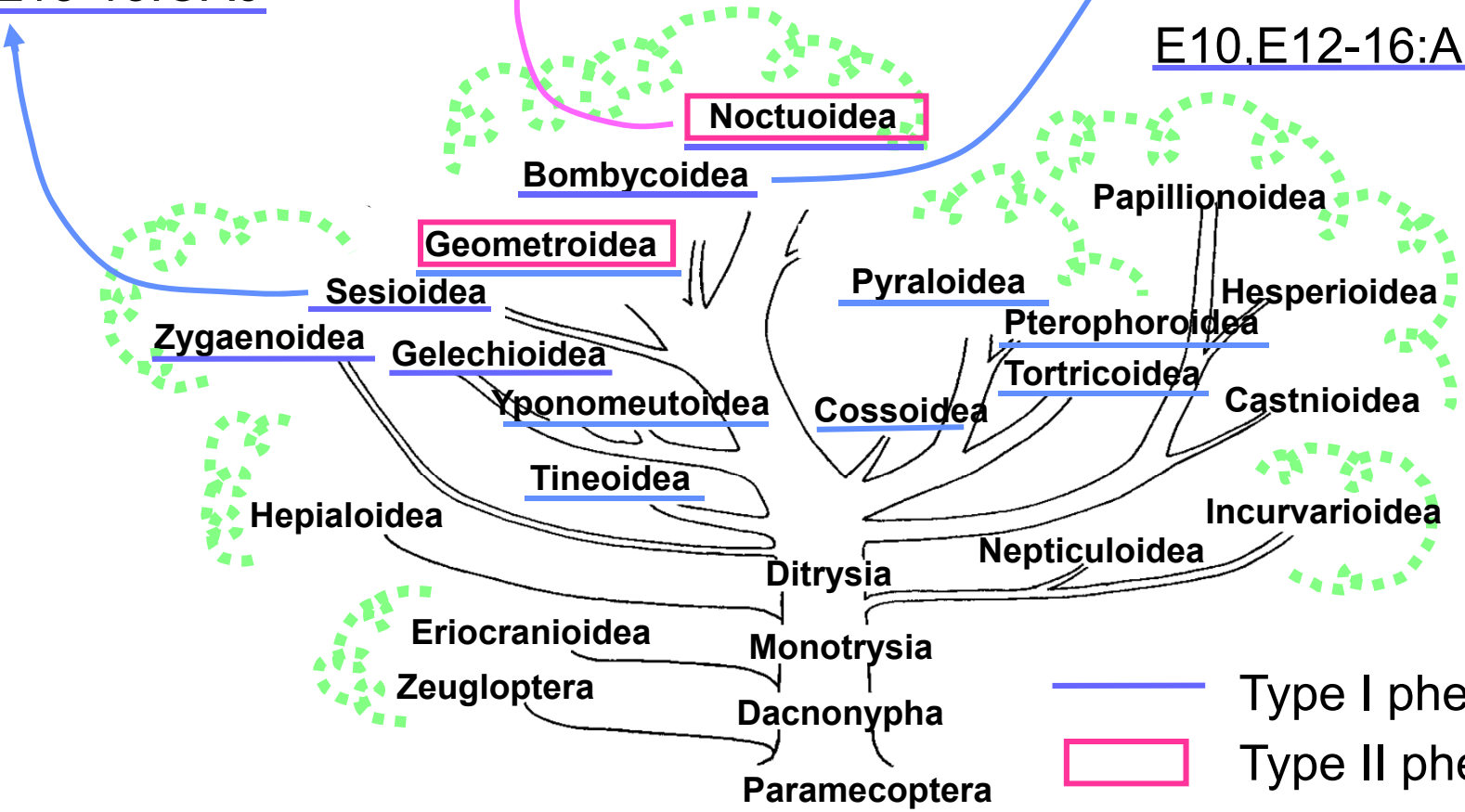
Z3,Z13-18:OAc

Wasp moth

Pheromone: Type II ?

Hawk moth

E10,E12-16:Ald



Arctiid species and pheromone study

Taxonomy			Japanese species	
Super-family	Family	Sub-family		
Noctuoidea	Notodontidae			
	Lymantriidae			
	Arctiidae	Lithosiinae	79	
	Nolidae	Syntominae	4	⇦ wasp moth
	Pantheidae	Arctiinae	51	
	Noctuidae			

In Syntominae, Type II pheromones (C21 triene and 9,10-epoxide) have been identified from two tropical species, *Empyreuma mucro* and *Syntomeida epilais* (no field data) (Descoins et al., 1989).

Insects used in this study

Syntomoides imaon Cramer (a wasp mimic species)

Distributed in Southeast Asia, such as India and Taiwan

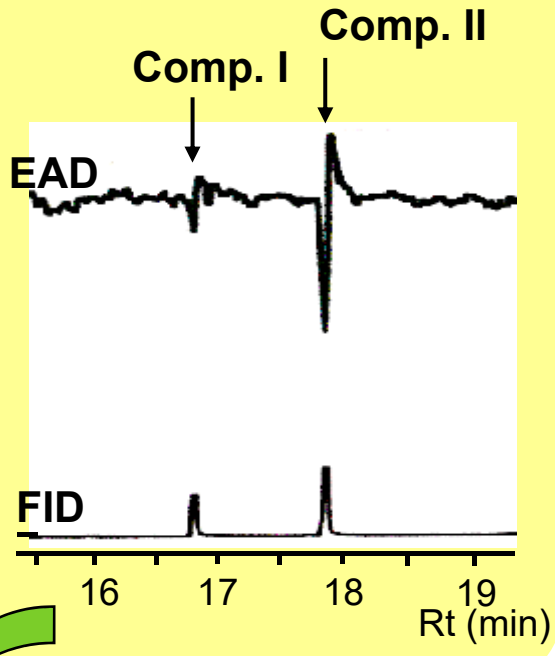
Found only in the Yonaguni-jima Island in Japan

Reared on a semisynthetic diet (16L-8D, 25ºC)

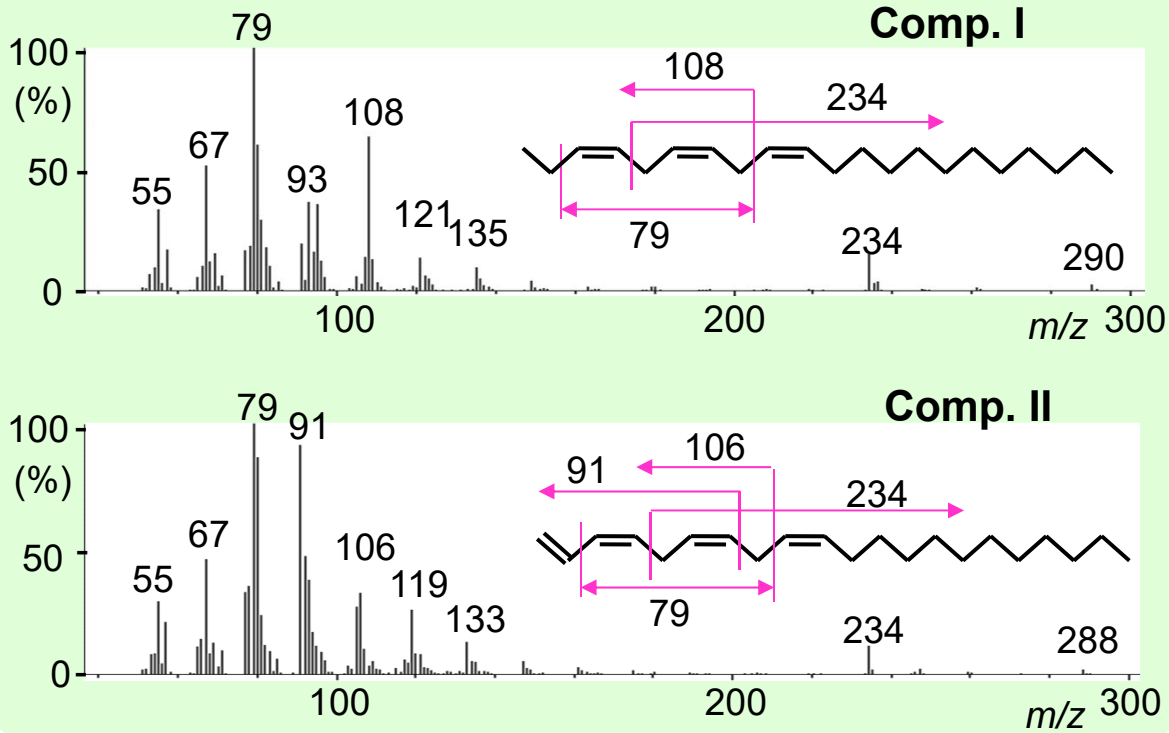
GC-EAD and GC-MS

The pheromone gland extract of *S. imaon* females was analyzed by GC-EAD and GC-MS equipped with a DB-23 column.

GC-EAD (0.5 FE)



Mass spectra (1 FE)



Comp. I (Z3,Z6,Z9-21:H)
Comp. II (1,Z3,Z6,Z9-21:H, 1.5 μg/female)
Comp. III (Z3,Z6,Z9-20:H)
Comp. IV (1,Z3,Z6,Z9-20:H)

I : II : III : IV = 32:67:0.6:0.7

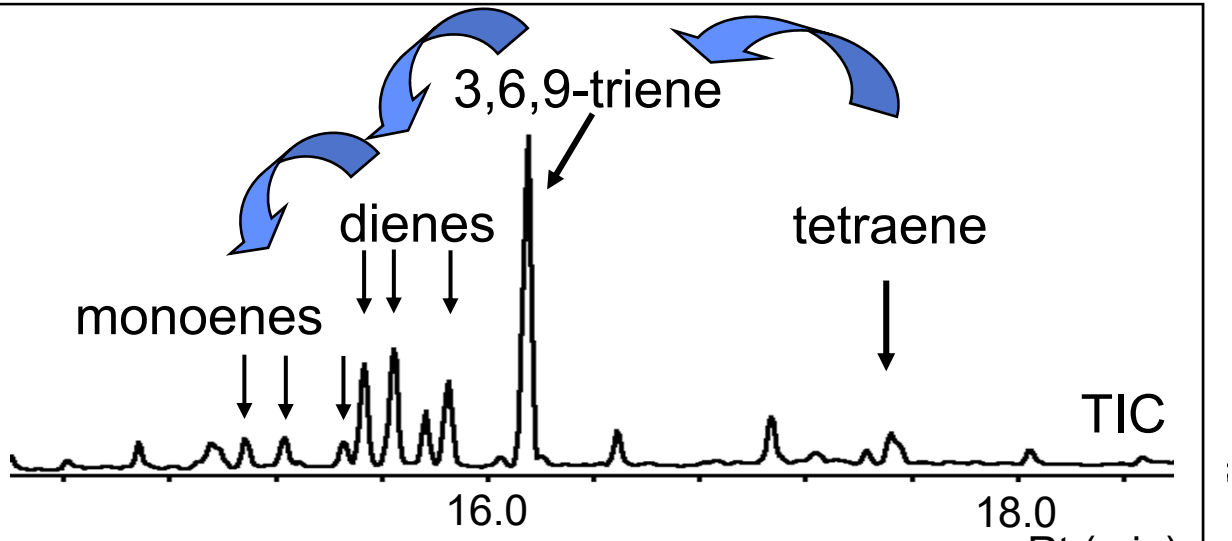
Chemical reactions with tetraene

Ex. 1)

Gland extract (20 FE)

↓ $N_2H_4 + H_2O_2$ / EtOH, 65°C, 2 hr

GC-MS analysis



Only one triene ⇒ Selective reduction at 1-position

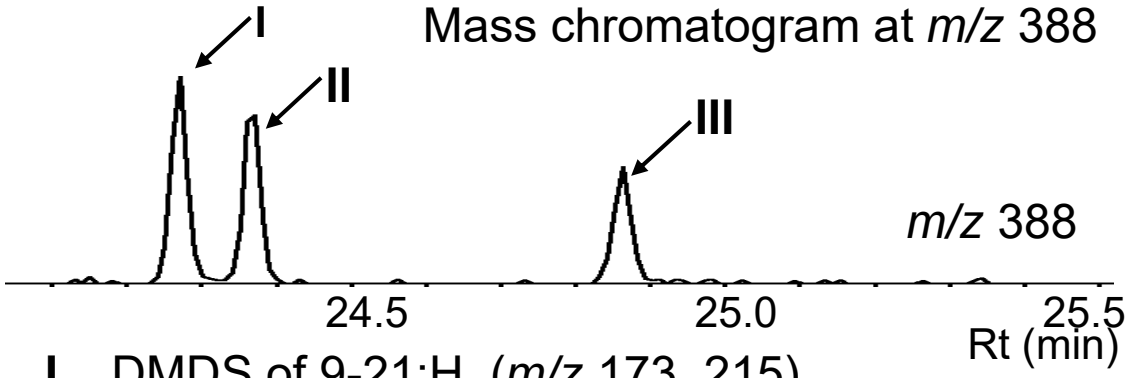
Ex. 2)

Gland extract (20 FE)

↓ $N_2H_4 + H_2O_2$ / EtOH, 65°C, 8 hr

↓ DMDS / I_2 , 40°C, 2 hr

GC-MS analysis



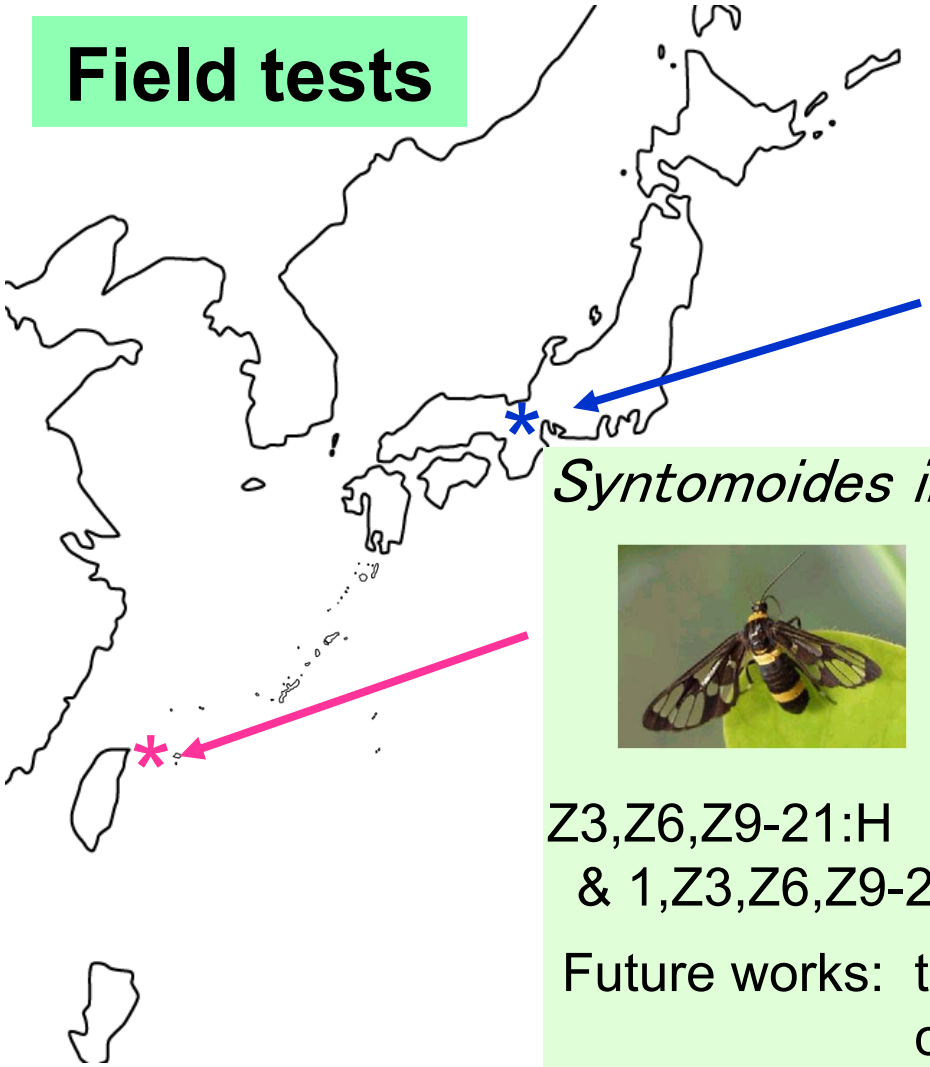
I DMDS of 9-21:H (*m/z* 173, 215)

II DMDS of 6-21:H (*m/z* 131, 257)

III DMDS of 3-21:H (*m/z* 89, 299)

⇒ 1,3,6,9-tetraene

Field tests



Ordinal wasp moth in Japan
Amata fortunei

Z3,Z6,Z9-21:H
& other components
(without tetraene)

Syntomoides imaon

Z3,Z6,Z9-21:H
& 1,Z3,Z6,Z9-21:H

Triene	Tetraene	Total males
0.5 mg	1.0 mg	31
1.0	0	0
0	1.0	0
0	0	0

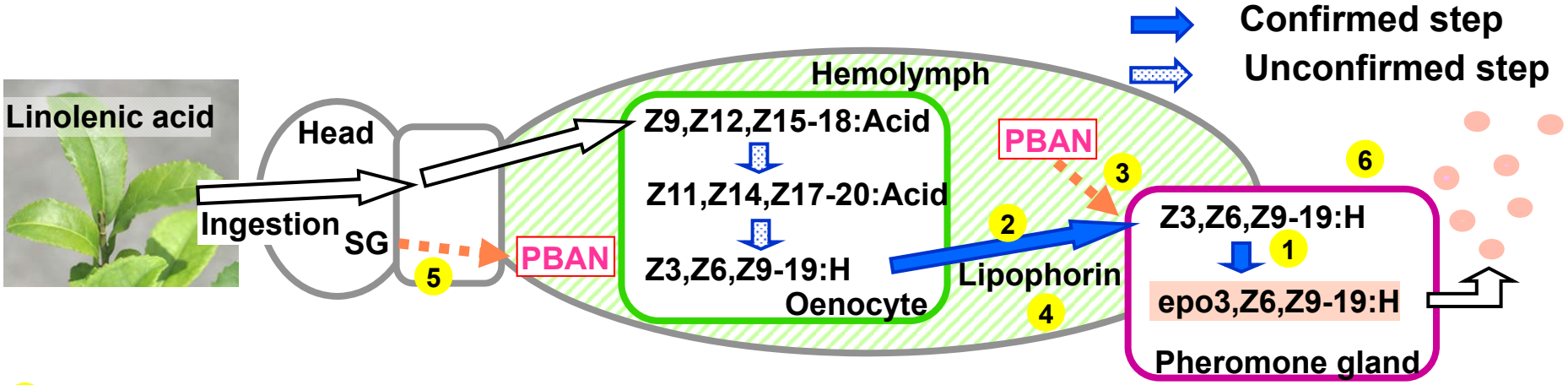
From 28 June to 5 July, 2007

Future works: the optical mixing ratio, roles of minor C20 compounds and visual cues, and etc.

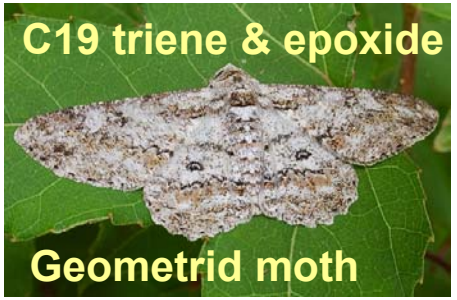
Identification of tetraenes

C21	C19
Epirrita autumnata (Geometridae)	*Operophtera brumata*
Arctia villica (Arctiidae)	*O. bruceata* (Geometridae)
Utetheisa ornatrix (Arctiidae)	

Biosynthesis of Type II pheromones



1. Specific epoxidation (Miyamoto et al., 1999. *IBMB*, 29: 63)
2. Transportation in hemolymph (Wei et al., 2003. *IBMB*, 33: 397)
3. Mode of action of PBAN (Wei et al., 2004. I*BMB*, 34: 1215)
4. Lipophorin association (Matsuoka et al., 2006. *IBMB*, 36: 576)
5. Identification of PBAN (Kawai et al., 2007. *IBMB*, 37: 330)
6. Structure of pheromone gland (Fujii et al., 2007. *JIP*, 53: 312)



C21 triene
+ tetraene

Wasp moth

desaturation

Z9,Z12,Z15-18:Acid	Z9,Z12,Z15,17-18:Acid
β-oxidation	
Z11,Z14,Z17-20:Acid	Z11,Z14,Z17,19-20:Acid
Z13,Z16,Z19-22:Acid	Z13,Z16,Z19,21-22:Acid
Z3,Z6,Z9-21:H	1,Z3,Z6,Z9-21:H

Oenocyte

New subject

Identification of these long-chain fatty acids, candidates of biosynthetic precursors

Analysis of fatty acids

Abdominal integument with oenocytes and peripheral fat bodies

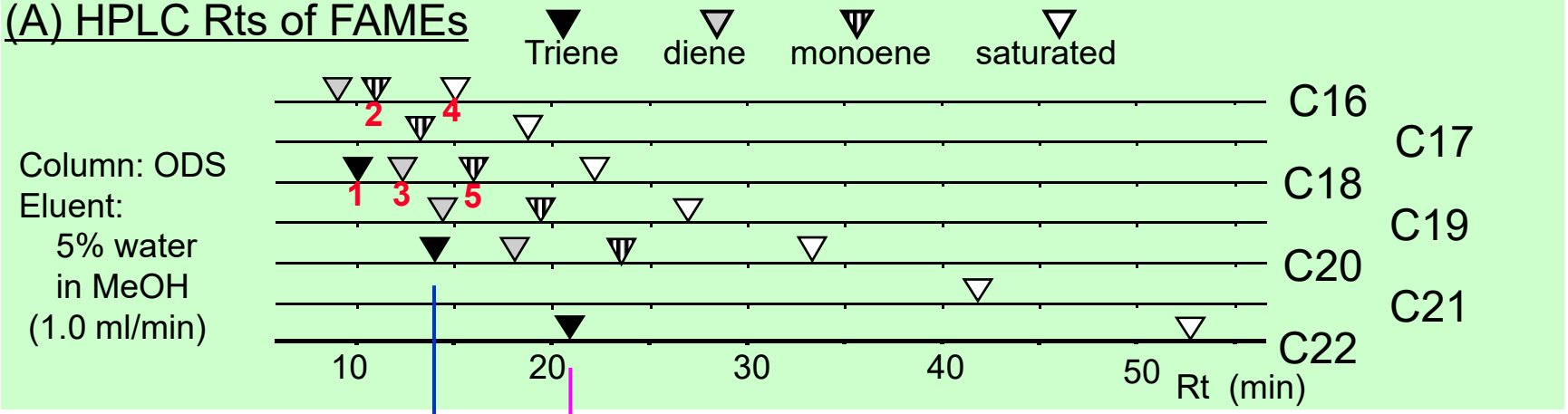
↓ Extraction, basic methanolysis, methylation with CH_2N_2

Mixture of fatty acid methyl esters (FAMEs) → GC-MS → biosynthetic precursors **X**

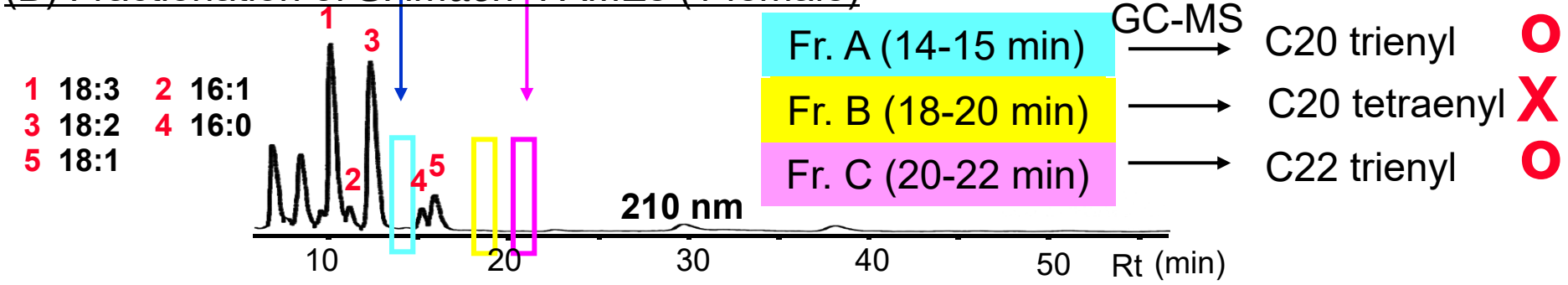
↓ HPLC separation (ODS column)

Fractions including a long-chain fatty acid ester

(A) HPLC Rts of FAMEs

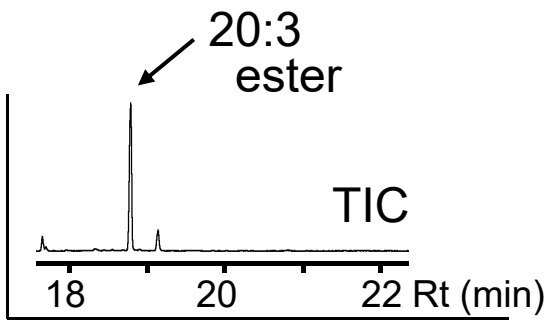


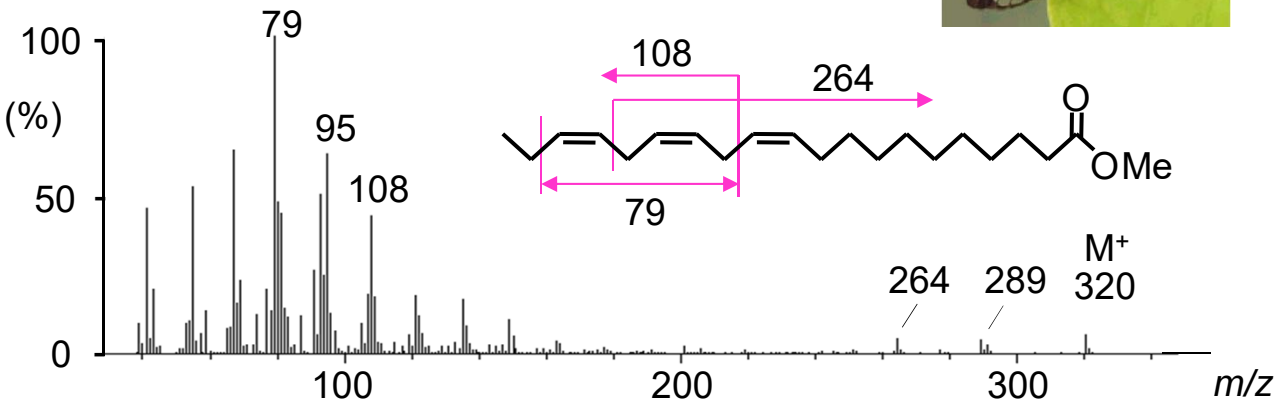
(B) Fractionation of *S. imaon* FAMEs (1 female)



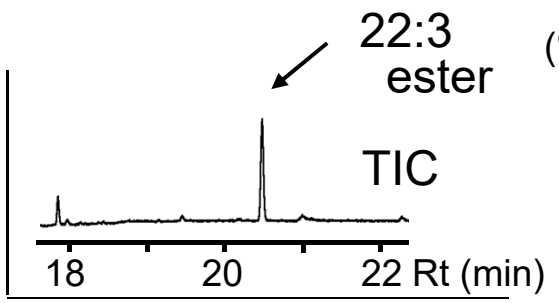
GC-MS of HPLC fractions

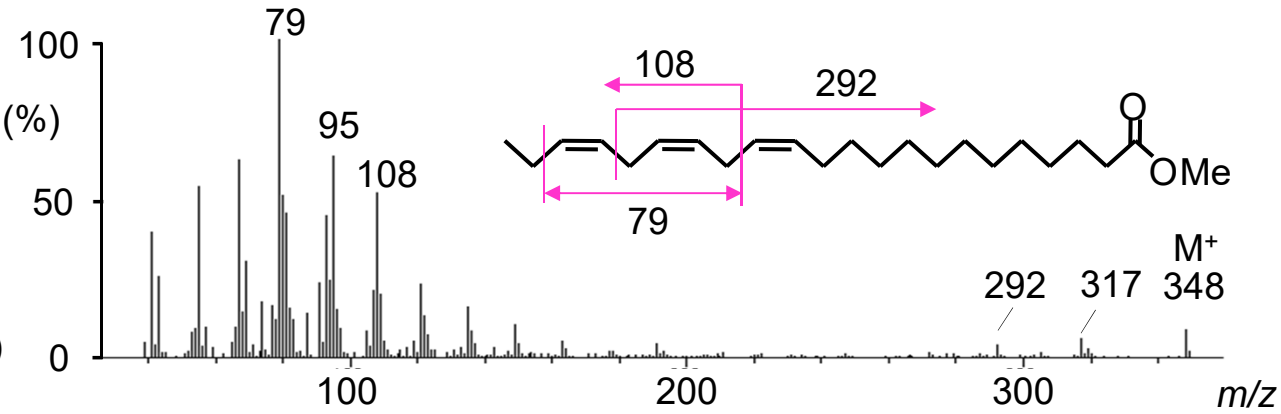
Fr. A (14-15 min)





Fr. C (20-22 min)





Ion fragments lower than *m/z* 150 in the spectra of the 20:3 and 22:3 esters were identical to those of the ester of linolenic acid (18:3), indicating the same trienyl structure.

Relative contents; 16:0 (0.3 mg/female, 100%), 20:3 (0.08%), 22:3 (0.03%)

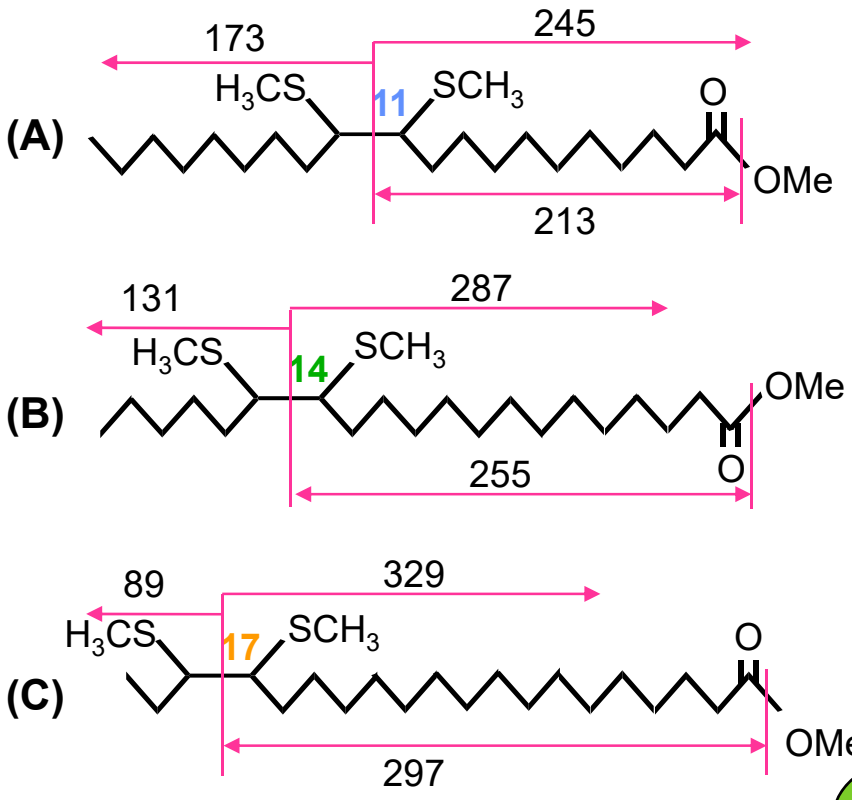
Unsaturated positions of C20 trienoate

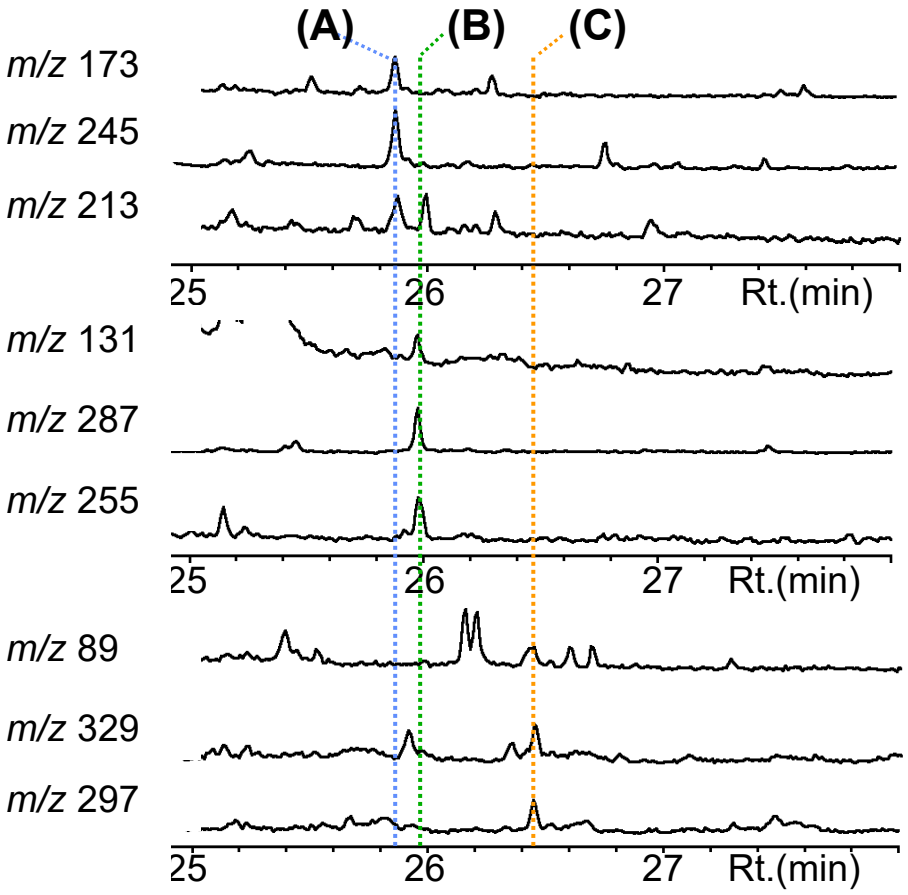
Fr. A (14-15 min)
20:3 ester

Diimide reduction → Mixture of monoenyl esters

DMDS / I_2 → GC-MS analysis

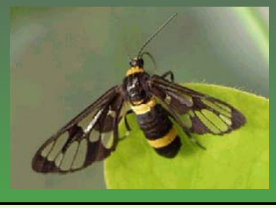
DMDS adducts of monoenyl esters





Methyl ester of 11,14,17-20:Acid

Summery



Female wasp moths produce Type II pheromones, a mixture of C21 triene and tetraene to attract males.

Fatty acid methyl esters (FAMEs) derived from lipids were fractionated by HPLC. GC-MS analysis revealed novel C20 and C22 trienoates, longer-chain analogs of linolenate.

In the another experiment with the geometrid moth, which produces C19 pheromone components, only C20 trienoate was detected.

Different systems of the chain elongation might play an important role in developing species-specific communication systems mediated with polyunsaturated hydrocarbons and derivatives, components of Type II lepidopteran sex pheromones.

Recent related studies

1) Biosynthesis of tetraene in *Utetheisa ornatrix* (Arctiidae: Arctiinae)

Choi et al., 2007. *JCE,* **33**: 1336

"D_4-Z3,Z6,Z9-21:H was injected into pupae. No label was incorporated into 1,Z3,Z6,Z9-21:H, indicating that the terminal double bond is introduced earlier in the biosynthetic pathway."

Tetraenyl acid intermediate was not detected in our study.

⇨ Is the step of desaturation linked effectively with decarboxylation?

2) Biosynthesis of even numbered triene in *Erannis bajaria* (Geometridae)

Goller et al., 2007. *JCE,* **33**: 1505

"D_4-Z3,Z6,Z9-18:H was detected after treatment of D_4-trienyl C19 or C20 acid. The result indicates that the C20 acid is shorted by α-oxidation to C19 acid, which can be reduced to an aldehyde and decarbonylated or decarboxylated to the pheromone component."

Biosynthesis of odd numbered triene has not been experimentally confirmed.

⇨ Where is decarboxylation carried out?

Acknowledgments

Co-workers

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Dr. H. Naka
JT Biohistory Research Hall, Osaka, Japan

Dr. Y. Kondo
Gifu University, Gifu, Japan

Chemical Ecology Laboratory of TUAT

Dr. M. Yamamoto

K. Matsuoka
MD. A. Islam
R. Yamakawa
N. D. Do
Y. Adachi
A. Yasmine
R. Kiyota