The 3rd International Symposium on Insect Pheromones

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Session III

Biosynthesis of Epoxyalkenyl Sex Pheromones: Biosynthetic Pathway, Substrate Specificity, and Endocrine Control

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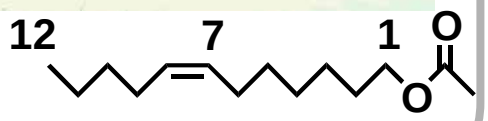
I. Biosynthetic Pathways for Lepidopteran Pheromones

Tipe I (Acetate of unsaturated fatty alcohol)

Acetyl CoA → 16:Acyl —Δ11-desaturation→ Z11-16:Acyl → Z9-14:Acyl —β-oxidation→ Z7-12:Acyl —reduction, acetylation→ Z7-12:OAc

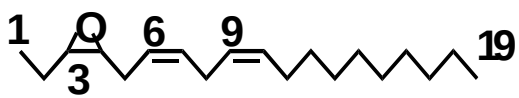
Anadevidia peponis



Type II (Epoxide of unsaturated hydrocabon)

Z9,Z12,Z15-18:Acyl → Z11,Z14,Z17-20:Acyl —decarboxylation→ Z3,Z6,Z9-19:H —epoxidation→ epo3,Z6,Z9-19:H

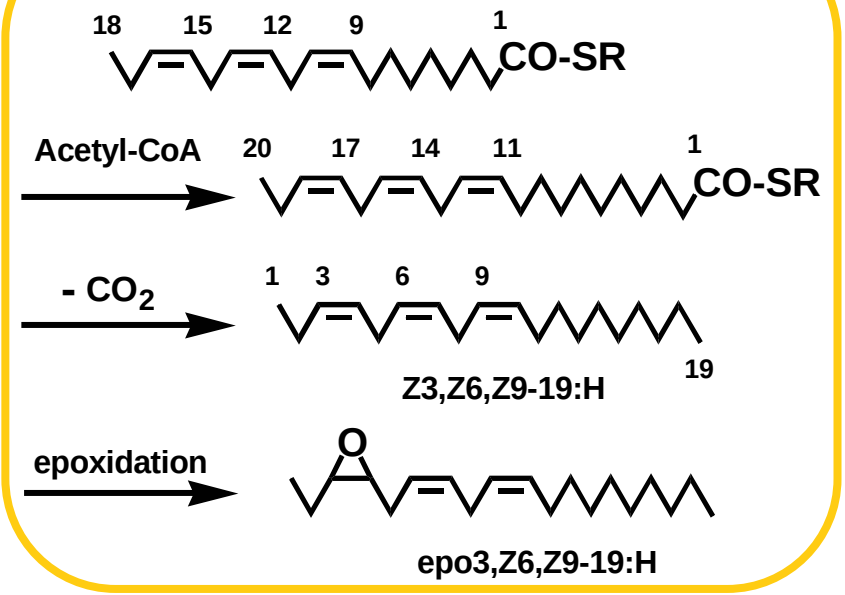
A. s. cretacea



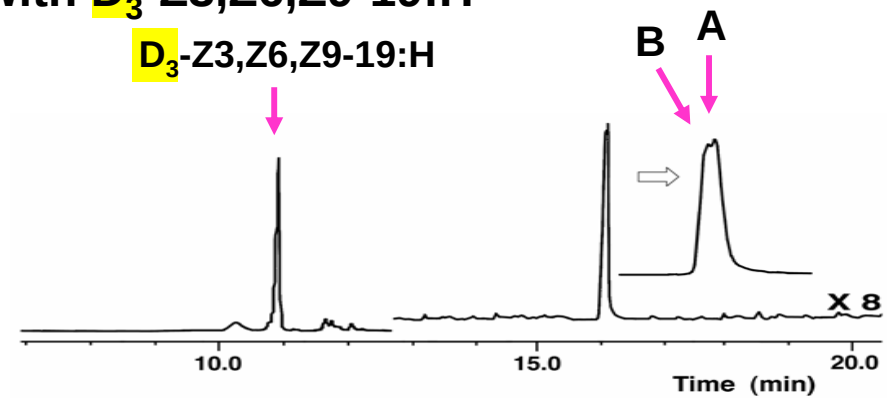
II. *In Vivo* Experiments with Geometrid Moth ①

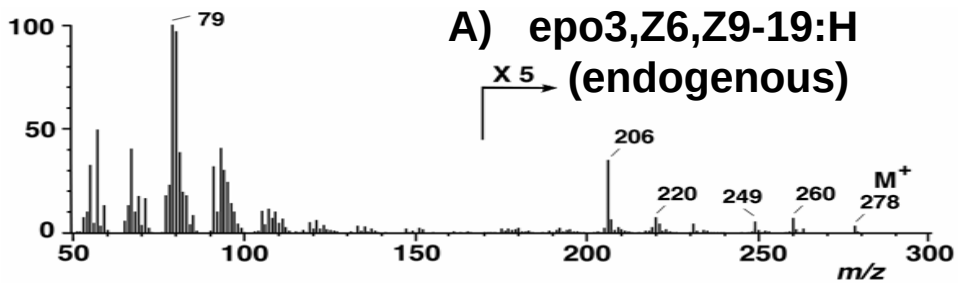
Ascotis selenaria cretacea
Japanese giant looper

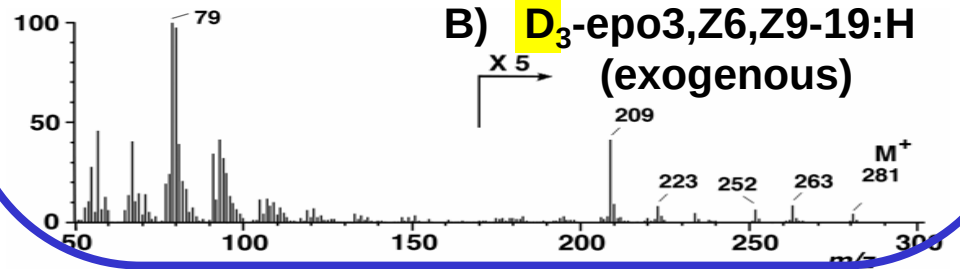
Proposed biosynthetic pathway



GC-MS analysis of the Pheromone Gland Extract from the Females Treated with D_3-Z3,Z6,Z9-19:H







II. *In Vivo* Experiments with Geometrid Moth ②

(A) Specificity of epoxidation in *A. s. cretacea*

Z3,Z6,Z9-19:H
Z3,Z6,Z9-17:H
Z3,Z6,Z9-22:H
Z3,Z6-19:H
Z3-19H, Z3-17:H

⇨ 3,4-epoxides

Z6,Z9-19:H
Z4-19:H
E3-19:H
Z2-19:H

⇨ no change

Epoxydase Attacked only (*Z*)-3-double bond without the substrate specificity on the carbon length and unsaturation degree

Strict formation of hydrocarbons ⇨ Species-specific pheromones

(B) Other steps

Identification of Z11,Z14,Z17-20:Acid
Incorporation of D_4-Z11,Z14,Z17-20:Acid

⇨ unsuccessful

Z9,Z12,Z15-18:Acyl —**Reductase**?→ **Z3,Z6,Z9-18:H** → **epo3,Z6,Z9-18:H**

↓ ?

Z11,Z14,Z17-20:Acyl —**Decarboxylase**?→ **Z3,Z6,Z9-19:H** —**Epoxydase**→ **epo3,Z6,Z9-19:H**

III. Analysis of Hemolymph Hydrocarbons

(A) Detection of pheromonal branched-hydrocarbons in hemolymph

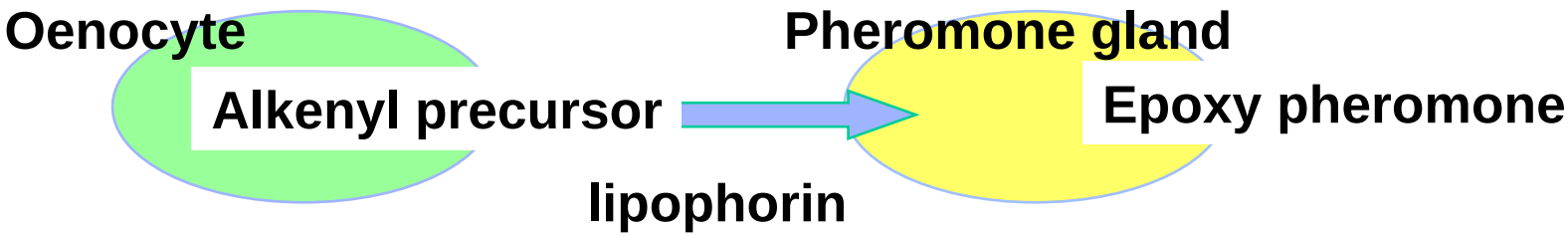
Homomelina aurantiaca (Arctuiidae)	**Me2-17:H**	Schal *et al*. (1998)
Scoliopteryx libatrix (Noctuidae)	**Z6,Me13-21:H**	Subchev & Jurenka (2001)
Limantria dispar (Lymantriidae)	**Me2,Z7-18:H** (precursor of disparlure)	Jurenka & Subchev (2000)

(B) GC-MS analysis of hemolymph from geometird females

IV. Biosynthetic Sites of Epoxyalkenyl Pheromones

(A) Study with the gypsy moth

Jurenka *et al*. (2003)
Proc. Natul. Acad. Sci. USA, **100:** 809



(B) Future works with geometrid moths

Role of Oenocyte and Mechanism of Polyalkene Biosynthesis

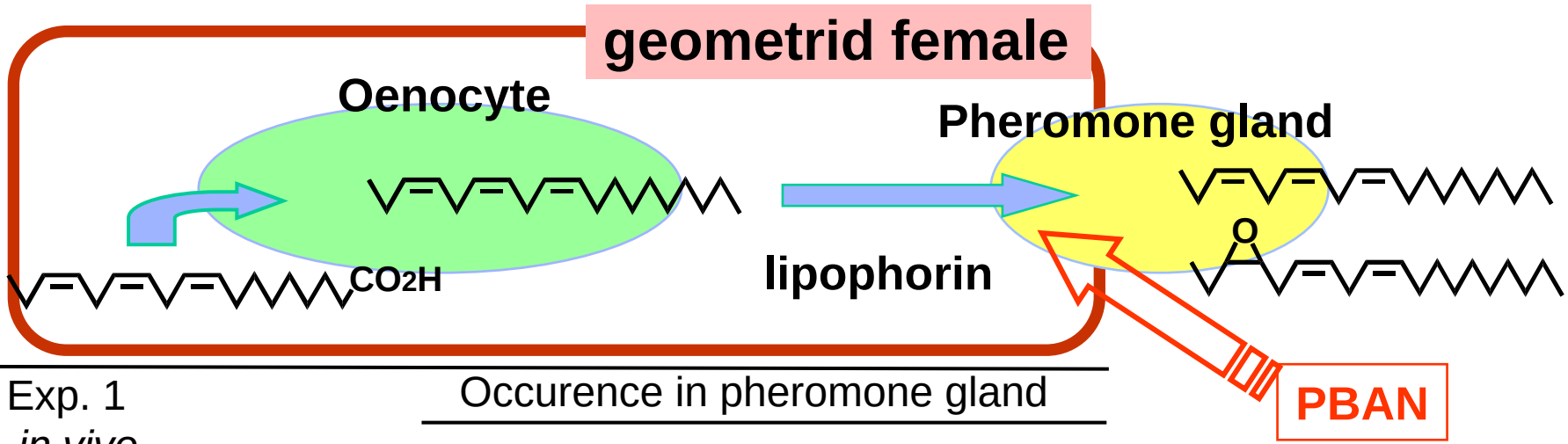
- Analysis of fatty acids and hydrocarbons in oenocytes
- Incorporation experiments with incubated oenocytes

Polyalkene Uptake by Pheromone Glands

- Analysis of lipophorin
- *In vivo* and *in vitro* experiments for uptake specificity

Endocrine Control System

V. Endocrine Control of Epoxyalkene Biosynthesis



Exp. 1 *in vivo*	Occurence in pheromone gland	
	Z3,Z6,Z9-19:H	epo3,Z6,Z9-19:H
Decapitaion	○	×
Bom-PBAN injection	○	○
Head-extract injection	○	○

⇒ Activation of epoxidation

Exp. 2 *in vitro*	Pheromone gland from decapitated female		
	Treatment		Production of
	Z3,Z6,Z9-19:H	PBAN	epo3,Z6,Z9-19:H
	+ **(topical)**	+	○
	+ **(topical)**	−	○

⇒ ~~Activation of epoxidation~~

Exp. 3 *in vivo*	Decapitated female		
	Treatment		Production of
	Z3,Z6,Z9-19:H	PBAN	epo3,Z6,Z9-19:H
	+ **(injection)**	+	○
	+ **(injection)**	−	×

⇒ Activation of uptake by gland

VI. Evolution of Biosynthetic Systems

Hybrids of Type I and Type II Pheromones

Estigmene acrea (Arctiidae)

Z3,Z6,epo9-21:H
+ Z9,Z12,Z15-18:Ald
+ Z9,Z12-18:Ald (27:6:1)

Achaea janata (Noctuidae)

Z3,Z6,Z9-21:H
+ Z6,Z9-21:H + 21:H
+ Z9,Z12-18:Ald

Neoleucinodes elegantalis (Pyralidae)

E11-16:OH
+ Z3,Z6,Z9-23:H (20:1)

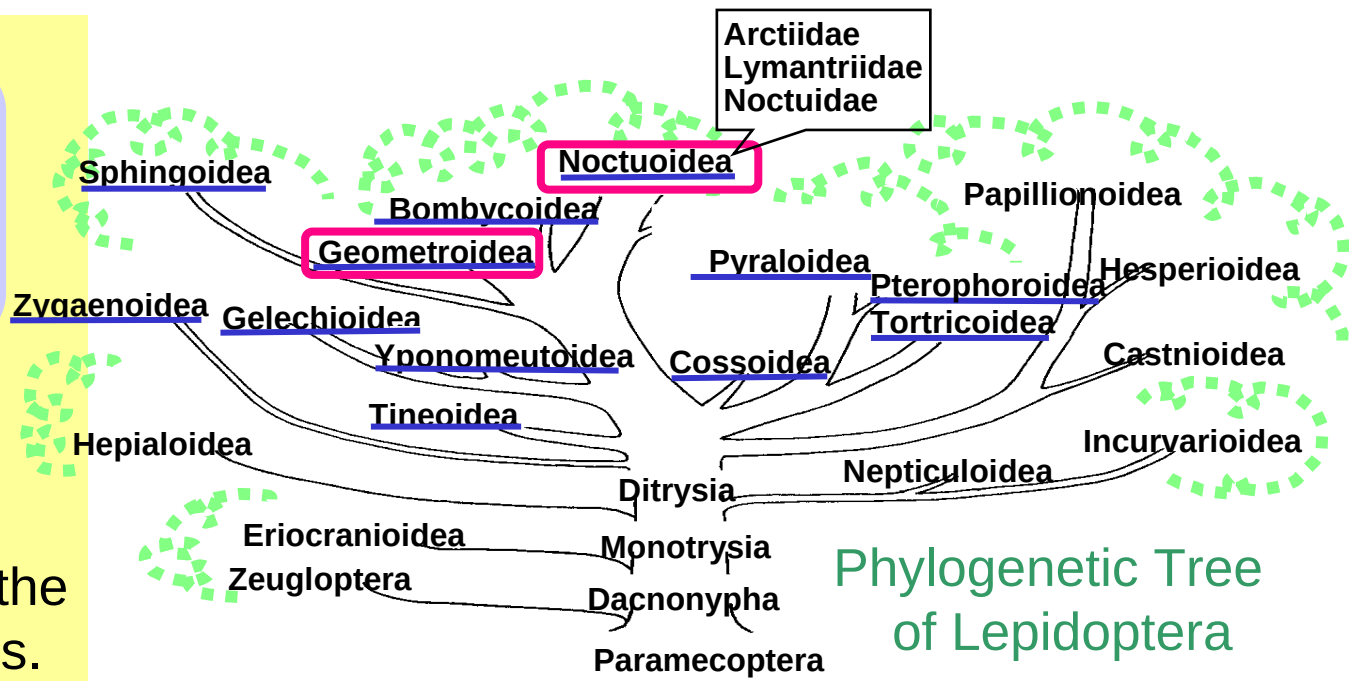
Euproctis pulverea (Lymantriidae)

Z11,Z14,Z17-20:OisoBut
+ Z11,Z14,Z17-20:OMe4-Val (2:1)

epo9,Z12,Z15-18:Ald
epo8-12:OH
epo11-16:OAc

Undiscovered

Epoxydase is a young enzyme achieved by the highly evolved species.



Phylogenetic Tree of Lepidoptera

Acknowledgments

Chemical Ecology Laboratory of TUAT

1) Study on substrate specificity of epoxidation
T. MIYAMOTO, Dr. M. YAMAMOTO, A. ONO, K. OHTANI

2) Study on polyunsaturated hydrocarbons in hemolymph
W. WEI, T. MIYAMOTO, M. ENDO, T. MURAKAWA
G.-Q. PU