

第4章 蛾類性フェロモンの生合成とその制御機構

(A) 生合成の研究法

A-1) 実験法

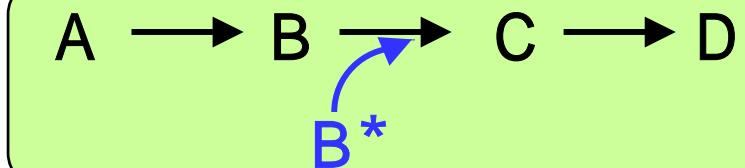
1. 突然変異株(種)の利用



2. 標識前駆体の投与実験

a) 安定同位元素 ^2H (D), ^{13}C

分析方法: GC-MS, NMR



フェロモン成分: 超微量物質

問題点

多量の前駆体の投与

本来の生合成系の搅乱の可能性

b) 放射性同位元素 ^3H (T), ^{14}C , ^{11}C

分析方法: オートラジオグラフィー

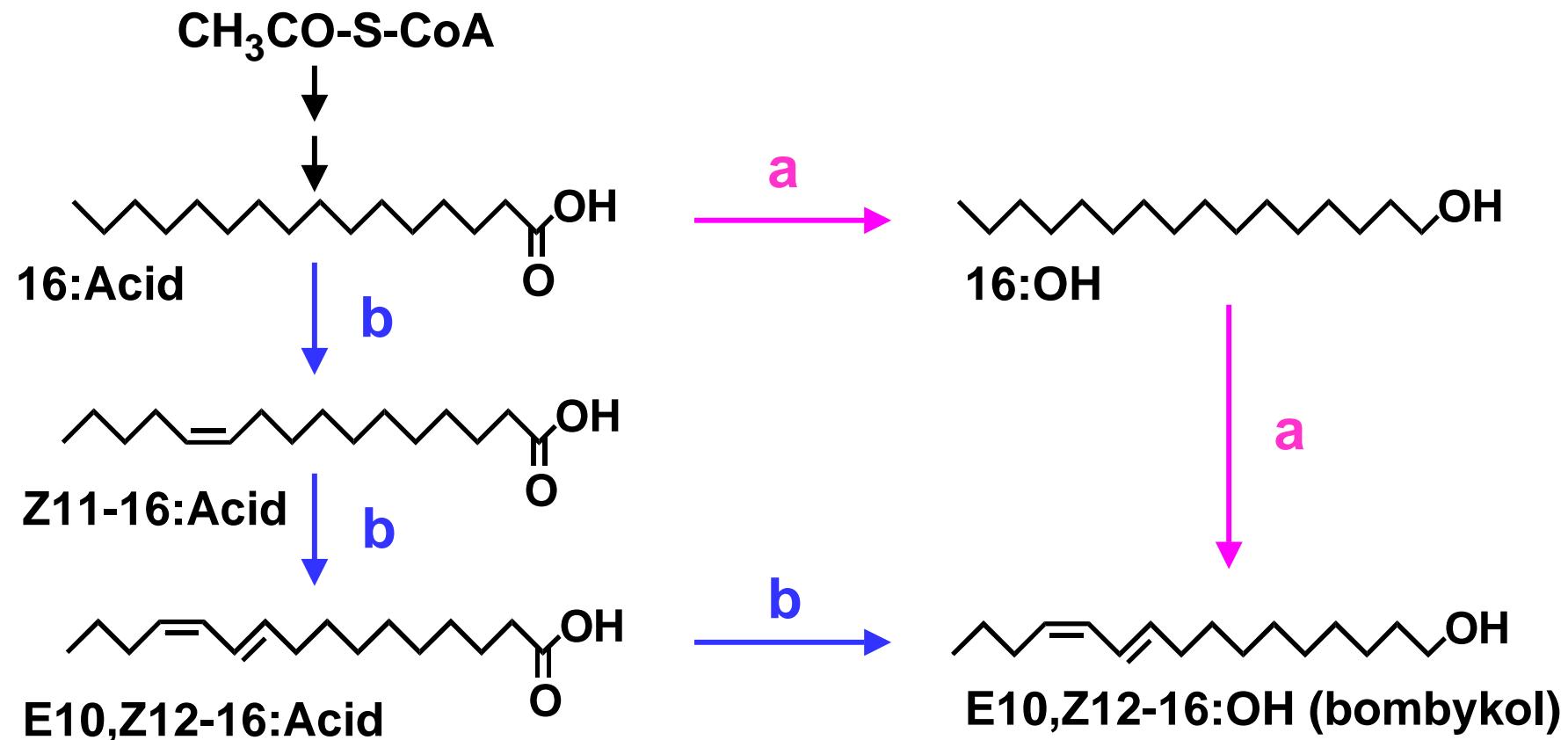
化学構造 OK?

問題点

個体:再結晶

液体:クロマトグラフィー

A-2) Bombykol (カイコの性フェロモン) の生合成経路



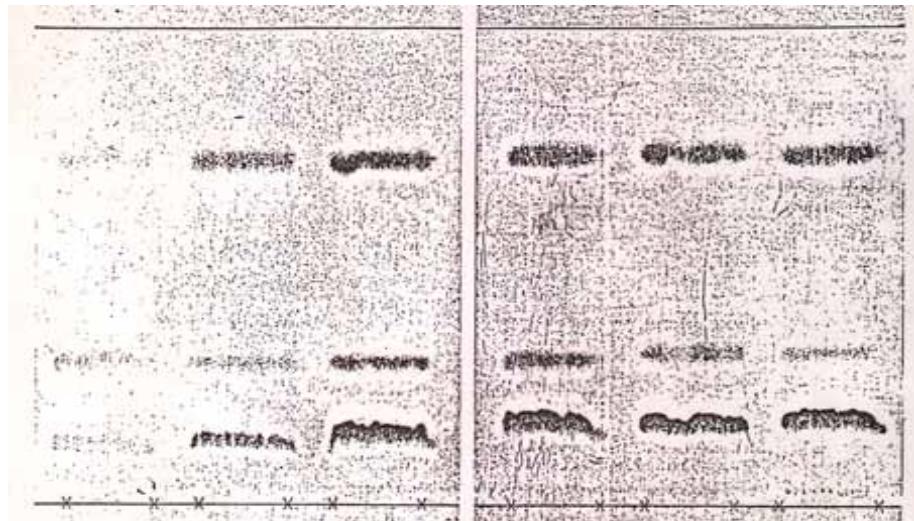
a Inoue & Hamamura, 1972. *Nippon Nogeikagaku Kaishi*, 46, 645

b Yamaoka et al., 1984. *Experientia*, 48, 80

Bjostad & Roelofs, 1984. *Insect Biochem.*, 14: 275

A-3) パルミチン酸 (16:Acid) の変換

TLC (オートラジオグラフィー)

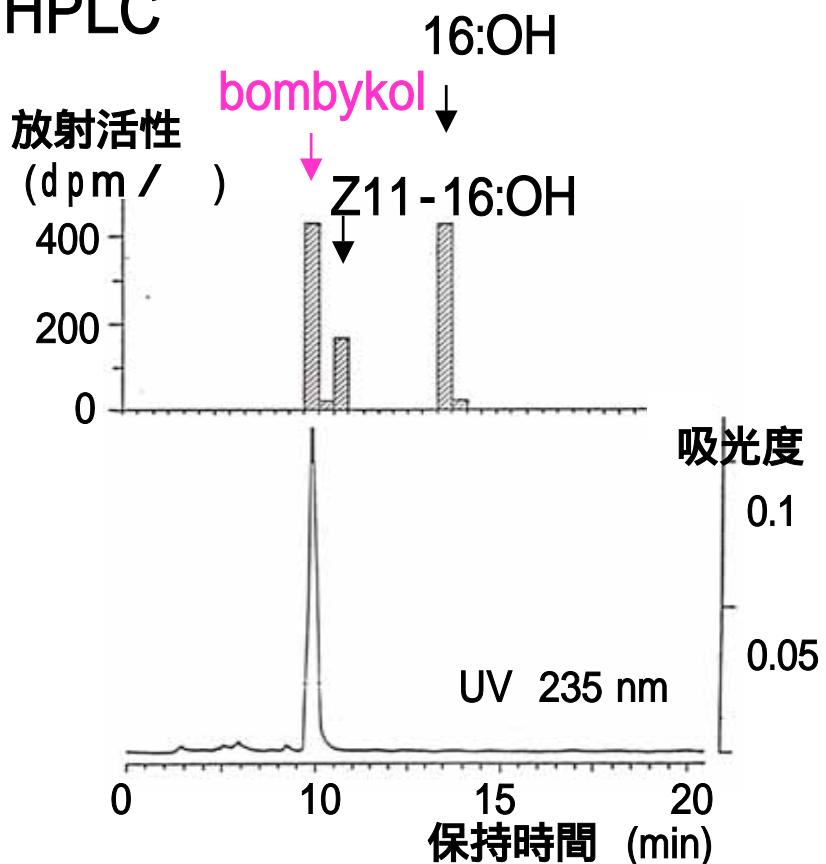


i ii iii iv v vi
solvent: benzene + ethyl acetate (4:1)

- i $[1\text{-}^{14}\text{C}]12\text{:Acid}$
- ii $[1\text{-}^{14}\text{C}]14\text{:Acid}$
- iii $[1\text{-}^{14}\text{C}]16\text{:Acid}$
- iv $[16\text{-}^{14}\text{C}]16\text{:Acid}$
- v $[1\text{-}^{14}\text{C}]18\text{:Acid}$
- vi $[18\text{-}^{14}\text{C}]18\text{:Acid}$

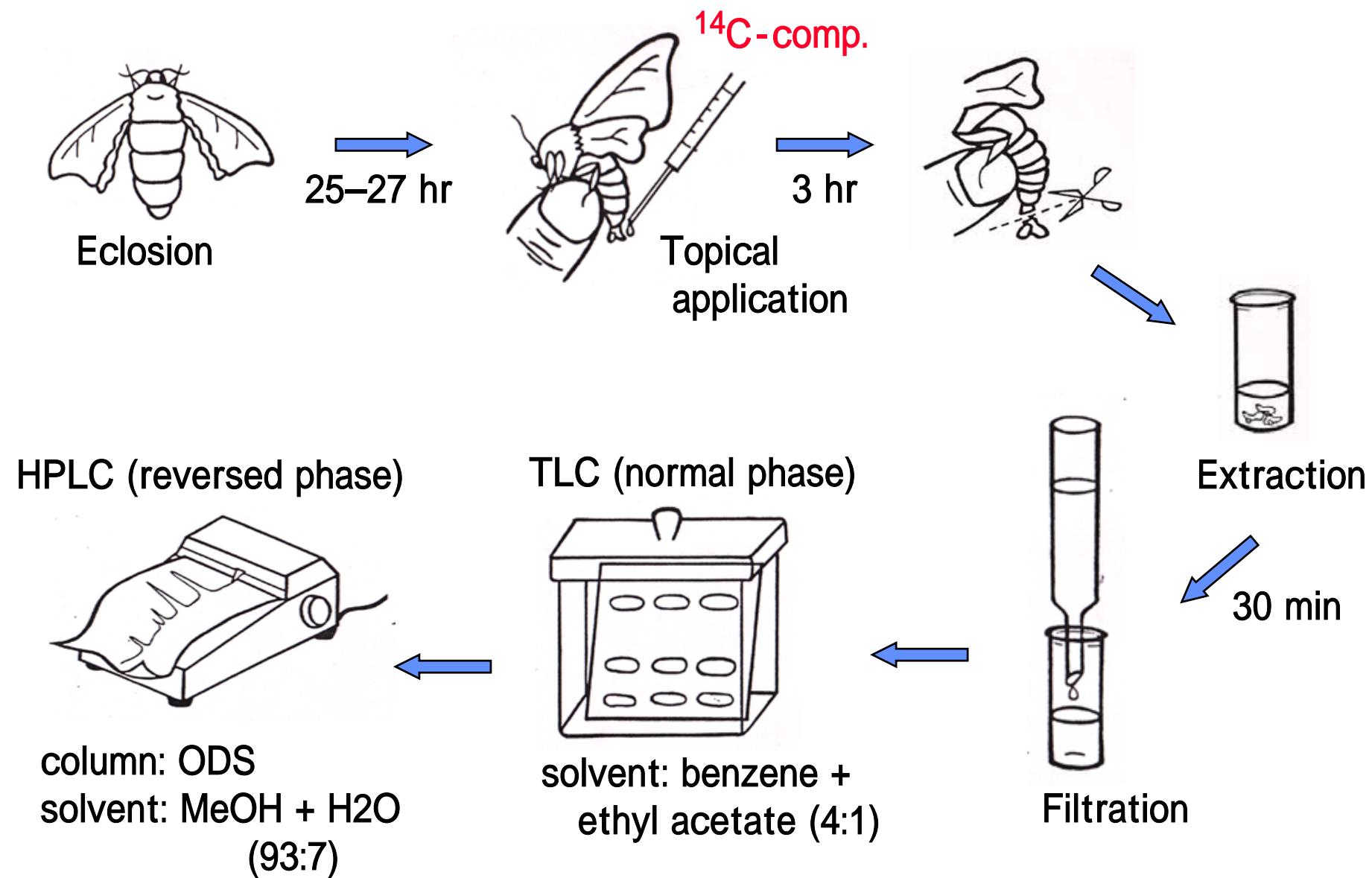
- A: triacylglycerols
- B: alcohols
- C: acids (recovery)

HPLC

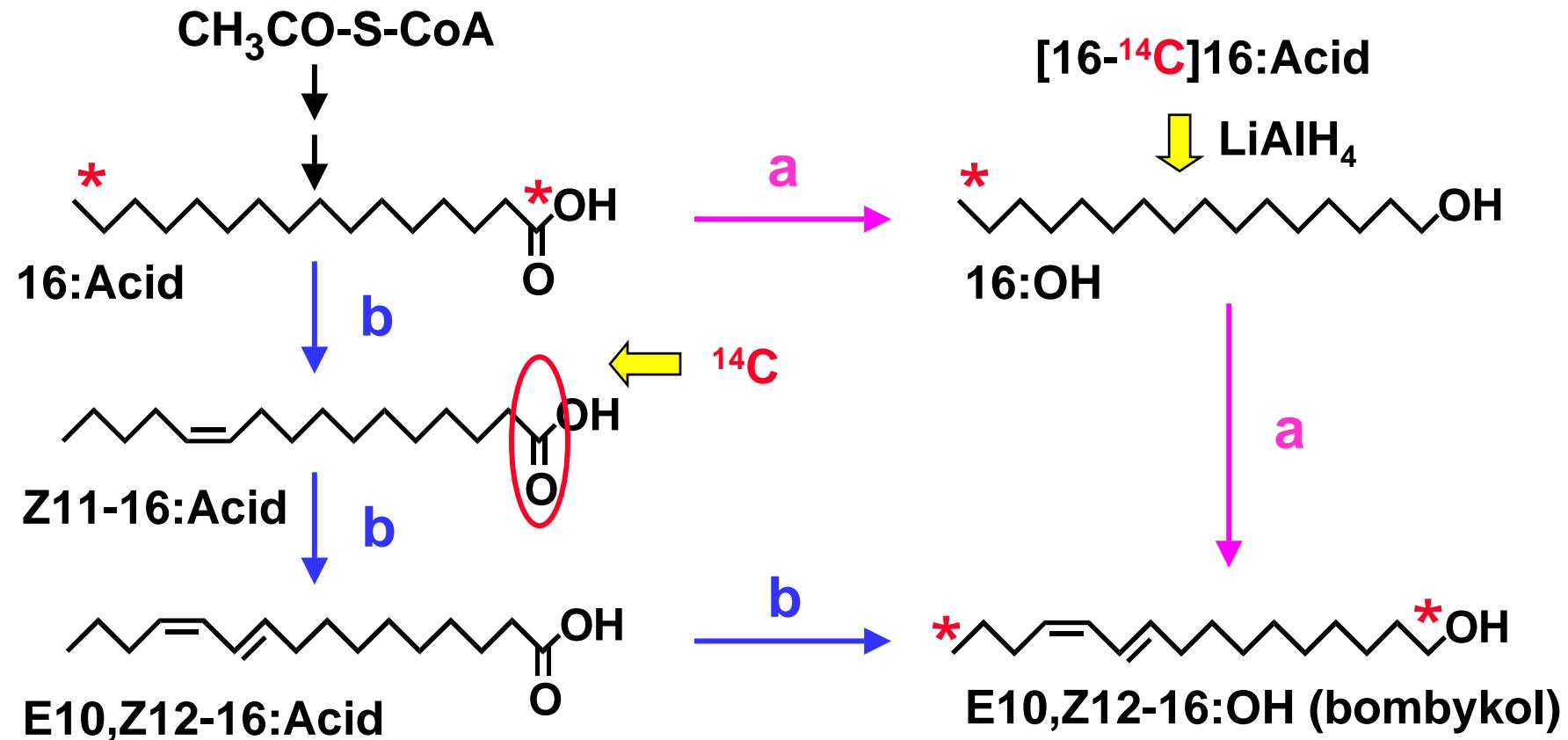


column: ODS (8 mm X 15 cm)
solvent: MeOH + H₂O (93:7)

A-4) 取込み実験法



A-2) Bombykol (カイコの性フェロモン) の生合成経路



a Inoue & Hamamura, 1972. *Nippon Nogeikagaku Kaishi*, 46, 645

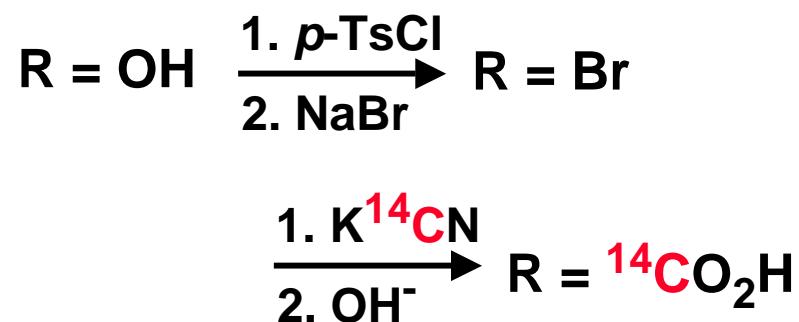
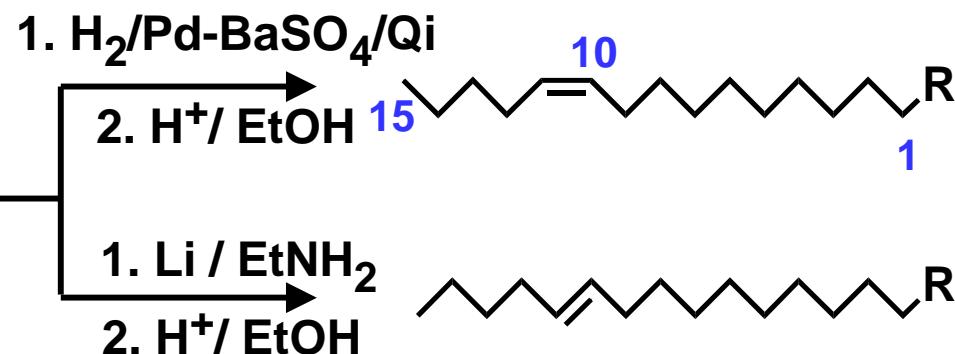
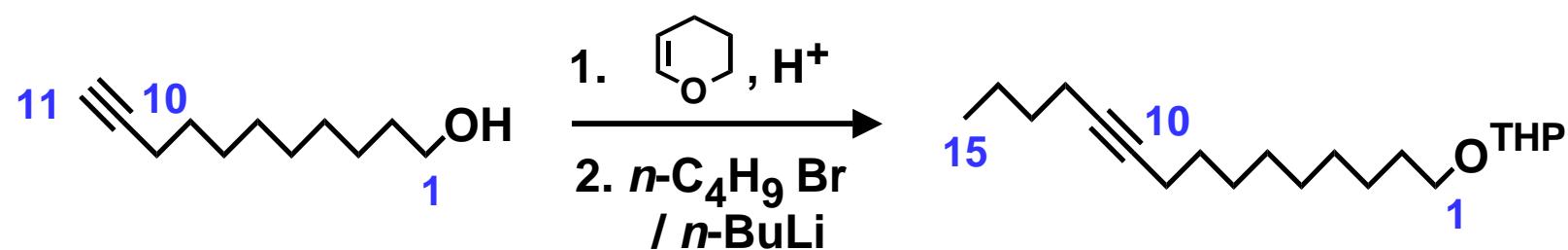
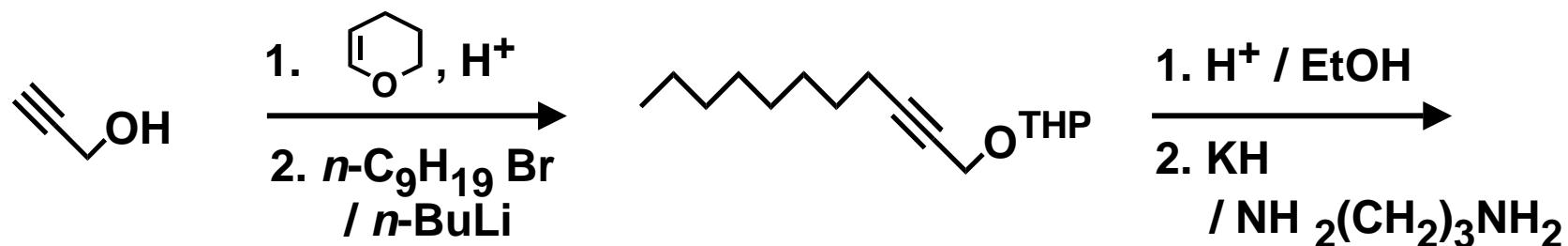
b Yamaoka et al., 1984. *Experientia*, 48, 80

Bjostad & Roelofs, 1984. *Insect Biochem.*, 14: 275

A-5) 標識化合物の合成

鉄則：アイソトープの導入は最終段階

Synthesis of [1-¹⁴C](Z)-11-hexadecenoic acid

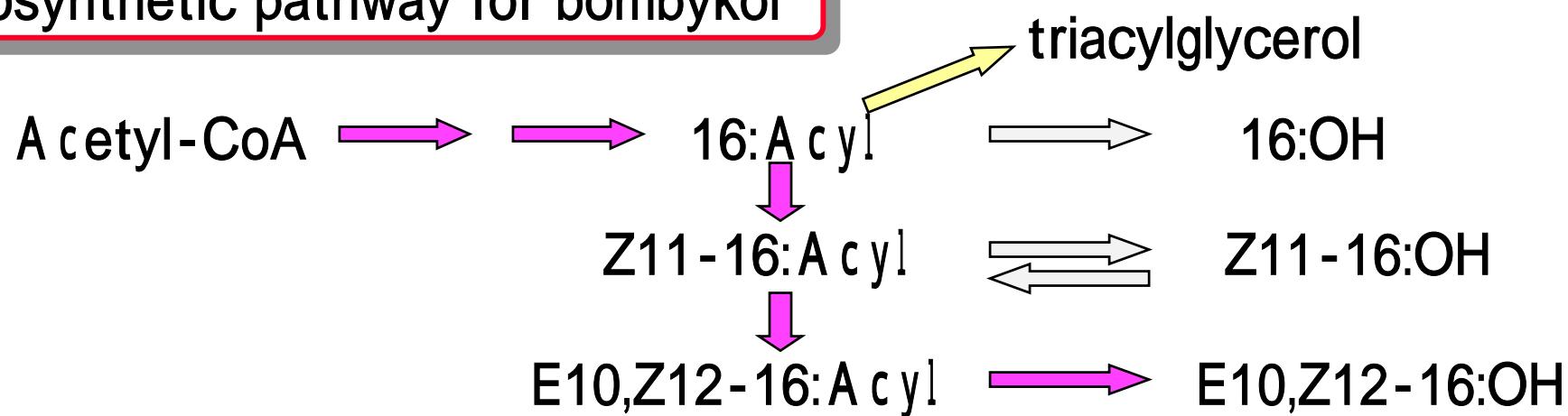


A-6) 取込み量の比較

	¹⁴ C-Labeled compound	Incorporation ratio (%)
[a]	12:Acid	0 . 2
	14:Acid	0 . 1
	16:Acid	1 . 7
	18:Acid	0 . 1
[b]	16:OH	0 . 2
	Z11-16:OH	0 . 9
	Z11-16:Acid	3 . 3

	¹⁴ C-Labeled compound	Incorporation ratio (%)
[c]	Z10-16:Acid	0 . 3
	E10-16:Acid	0 . 4
	E11-16:Acid	0 . 2
	Z12-16:Acid	0 . 4
	E12-16:Acid	0 . 5

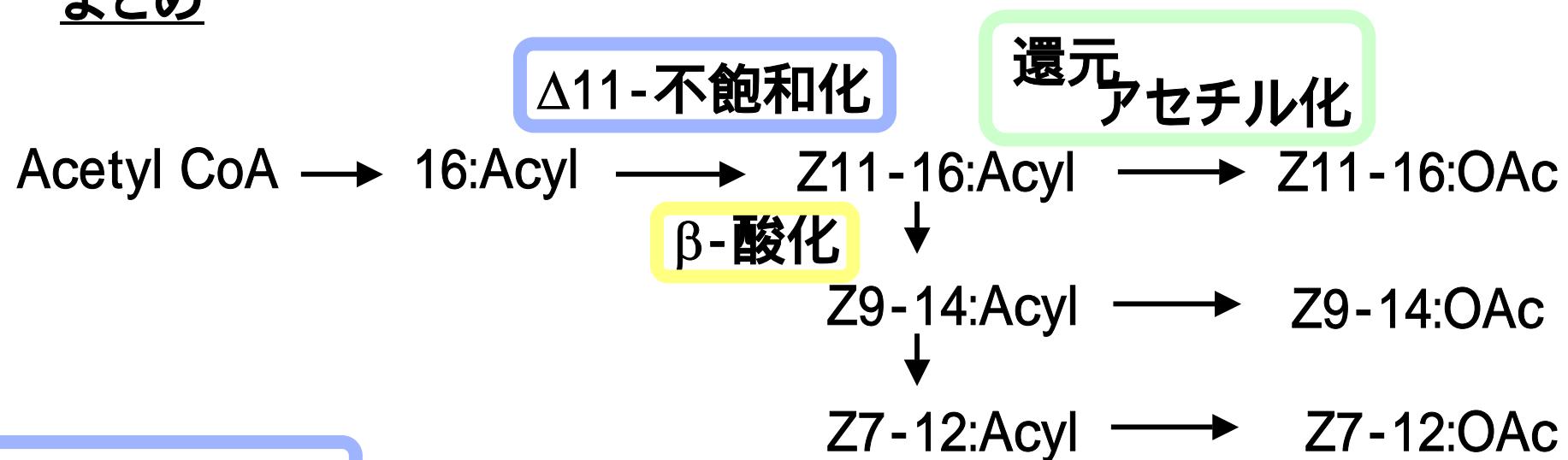
Biosynthetic pathway for bombykol



(B) 蛾類性フェロモン生合成

B-1) Type I のフェロモン

まとめ



△11-不飽和化

イラクサギンウワバにて遺伝子がクローニング

酵母内で発現 (Knipple et al., *ProNAS USA*, 95: 15287, 1998)

β-酸化酵素

?

還元、アセチル化酵素

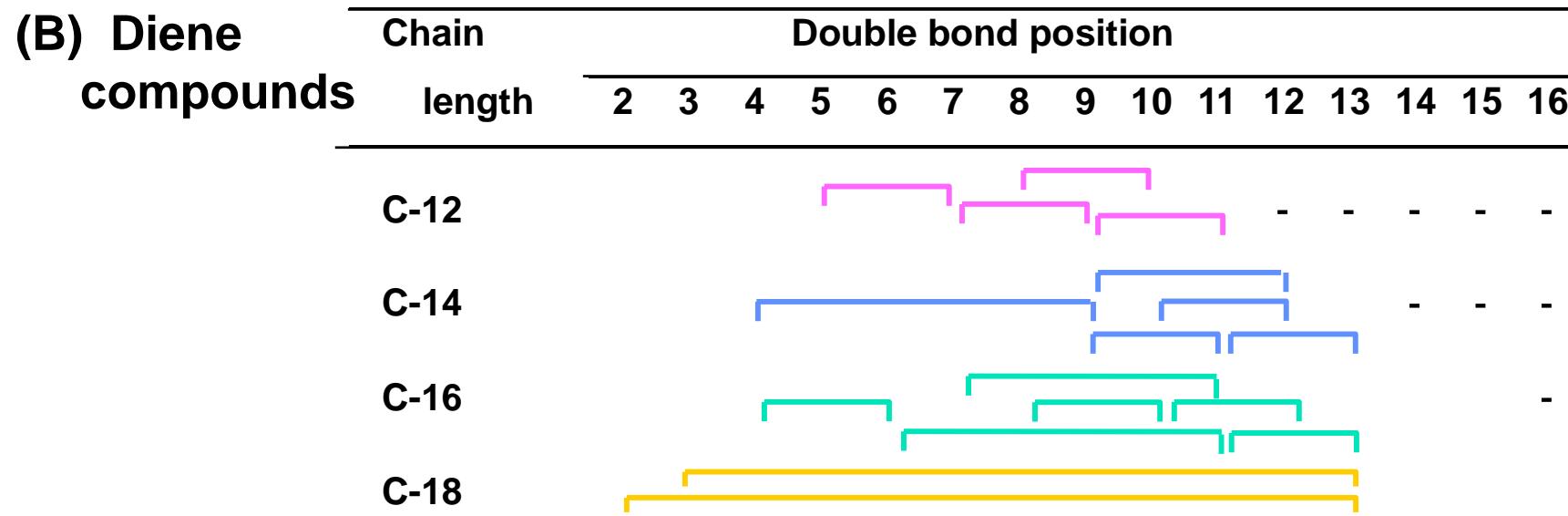
低い基質特異性

課題： 不飽和化の位置特異性のメカニズム、混合比の制御

(B-1) Type I のフェロモン

二重結合位置

(A) Monoene compounds	Chain length	Double bond position														
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C-10					●		●			-	-	-	-	-	-	-
C-12					●		●		●			-	-	-	-	-
C-14						●		●	●		●			-	-	-
C-16							●			●		●			-	-
C-18											●					



不飽和化酵素

遺伝子の単離（一覧、その1）

Z9	← 16	<i>Helicoverpa zea</i>	(Noct.)	Rosenfield <i>et al.</i> , 2001
		<i>Helicoverpa assulta</i>	(Noct.)	Jeong <i>et al.</i> , 2003
	← E11-14	<i>Spodoptera littoralis</i>	(Noct.)	Rosenfield <i>et al.</i> , 2004
Z10	← 14	<i>Planotortrix octo</i>	(Tort.)	Hao <i>et al.</i> , 2002(a)
Z11	← 16	<i>Trichoplusia ni</i>	(Noct.)	Knipple <i>et al.</i> , 2001
		<i>H. zea</i>	(Noct.)	Rosenfield <i>et al.</i> , 2001
		<i>H. assulta</i>	(Noct.)	Jeong <i>et al.</i> , 2003
		<i>Bombyx mori</i>	(Bomb.)	Moto <i>et al.</i> , 2004
		<i>Manduca sexta</i>	(Sphi.)	Matouskova <i>et al.</i> , 2007
E11	← 14,16	<i>Epiphyas postvittana</i>	(Tort.)	Liu <i>et al.</i> , 2002
	← E9-14	<i>E. postvittana</i>	(Tort.)	Liu <i>et al.</i> , 2002
	← 14	<i>Choristoneura parallela</i>	(Tort.)	Liu <i>et al.</i> , 2004

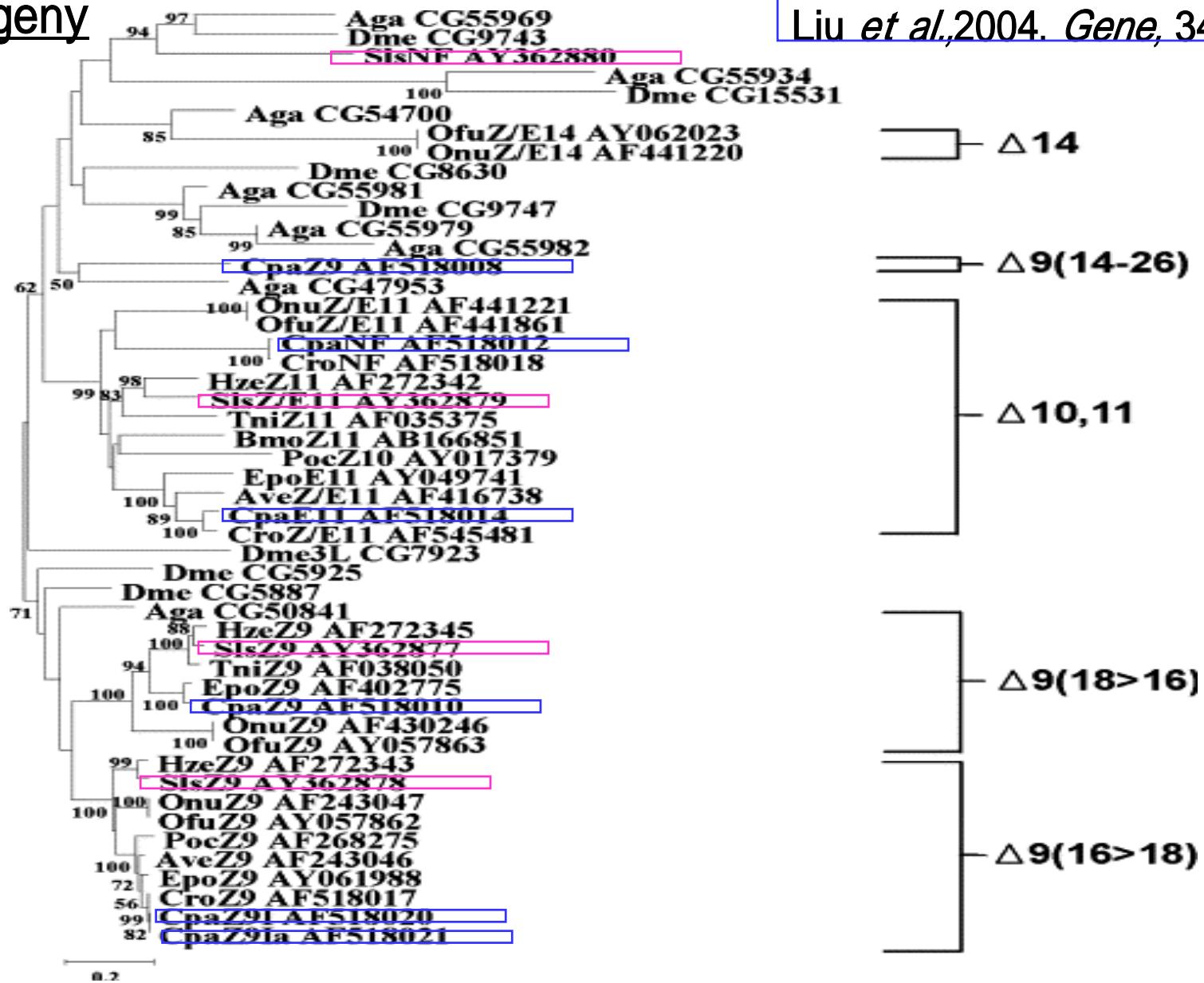
不飽和化酵素

遺伝子の単離（一覧、その2）

Z/E11	←14	<i>Argyrotaenia velutinana</i>	(Tort.)	Liu <i>et al.</i> , 2002
		<i>Choristoneura rosaceana</i>	(Tort.)	Hao <i>et al.</i> , 2002(b)
		<i>Ostrinia furnacalis</i>	(Pyra.)	Roelofs <i>et al.</i> , 2002
		<i>Ostrinia nubilalis</i>	(Pyra.)	Roelofs <i>et al.</i> , 2002
		<i>S. littoralis</i>	(Noct.)	Rodriguez <i>et al.</i> , 2004
		<i>Ostrinia scapulalis</i>	(Pyra.)	Fukuzawa <i>et al.</i> , 2006
Z/E14	←16	<i>O. furnacalis</i>	(Pyra.)	Roelofs <i>et al.</i> , 2002
		<i>O. nubilalis</i>	(Pyra.)	Roelofs <i>et al.</i> , 2002
E10,Z12	←16	<i>B. mori</i>	(Bomb.)	Moto <i>et al.</i> , 2004
	← Z11-16	<i>M. sexta</i>	(Sphi.)	Matouskova <i>et al.</i> , 2007

不飽和化酵素

Phylogeny

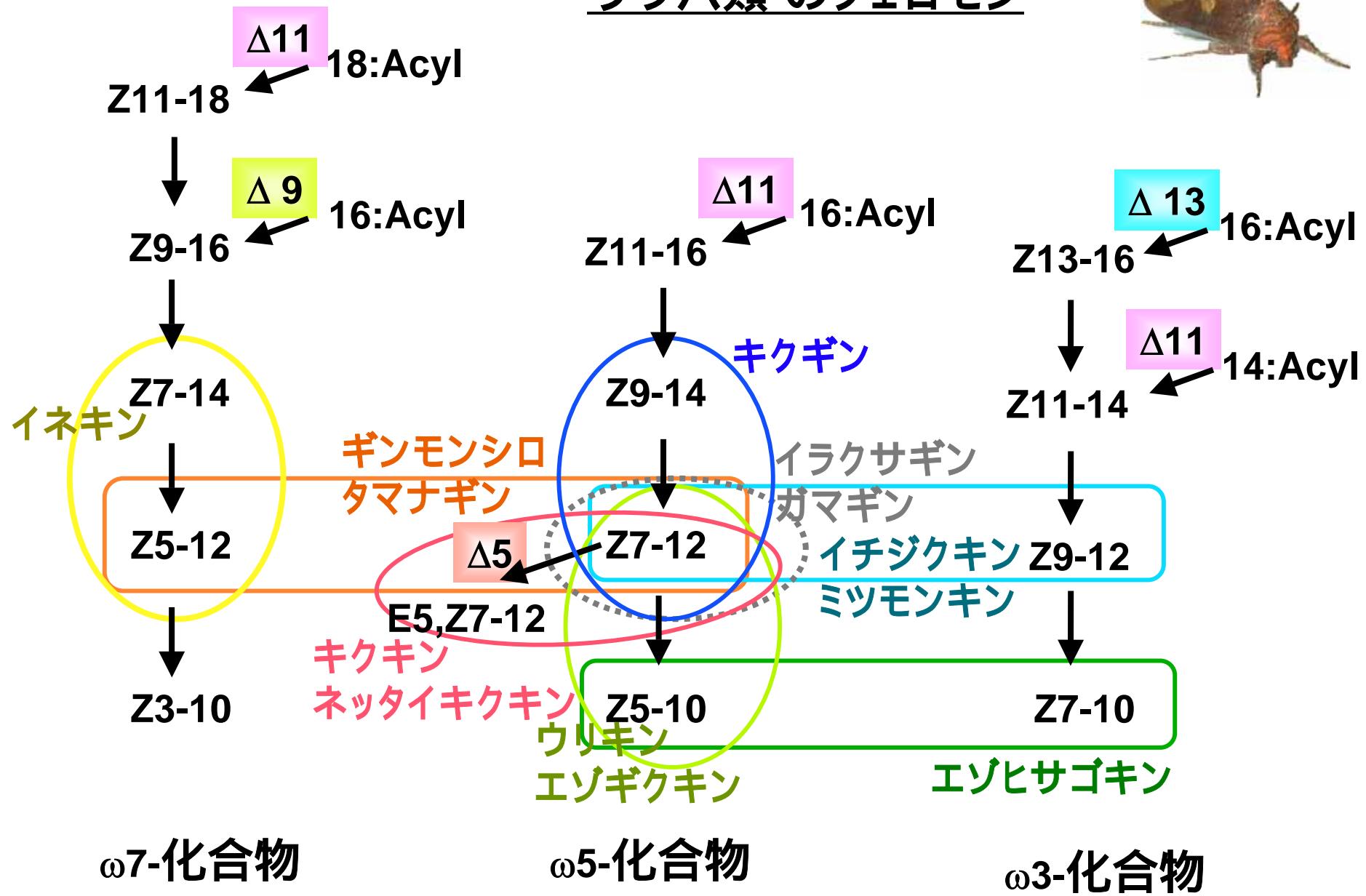


Rodriguez *et al.*, 2004. *IBMB* 34: 1315

Liu *et al.*, 2004. *Gene*, 342: 303.

B-1) Type I のフェロモン

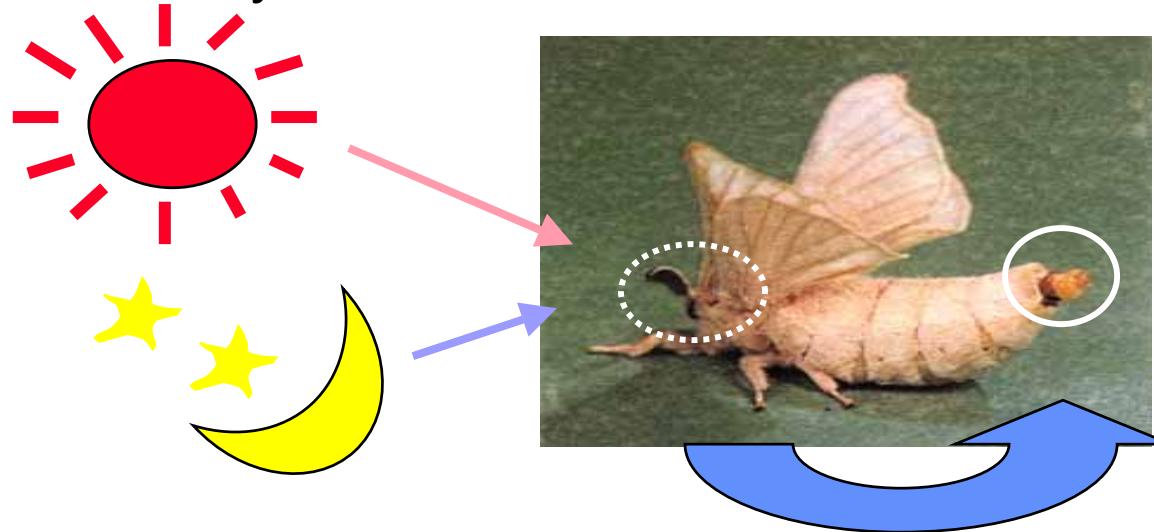
ウワバ類 のフェロモン



(B) 生合成の制御機構

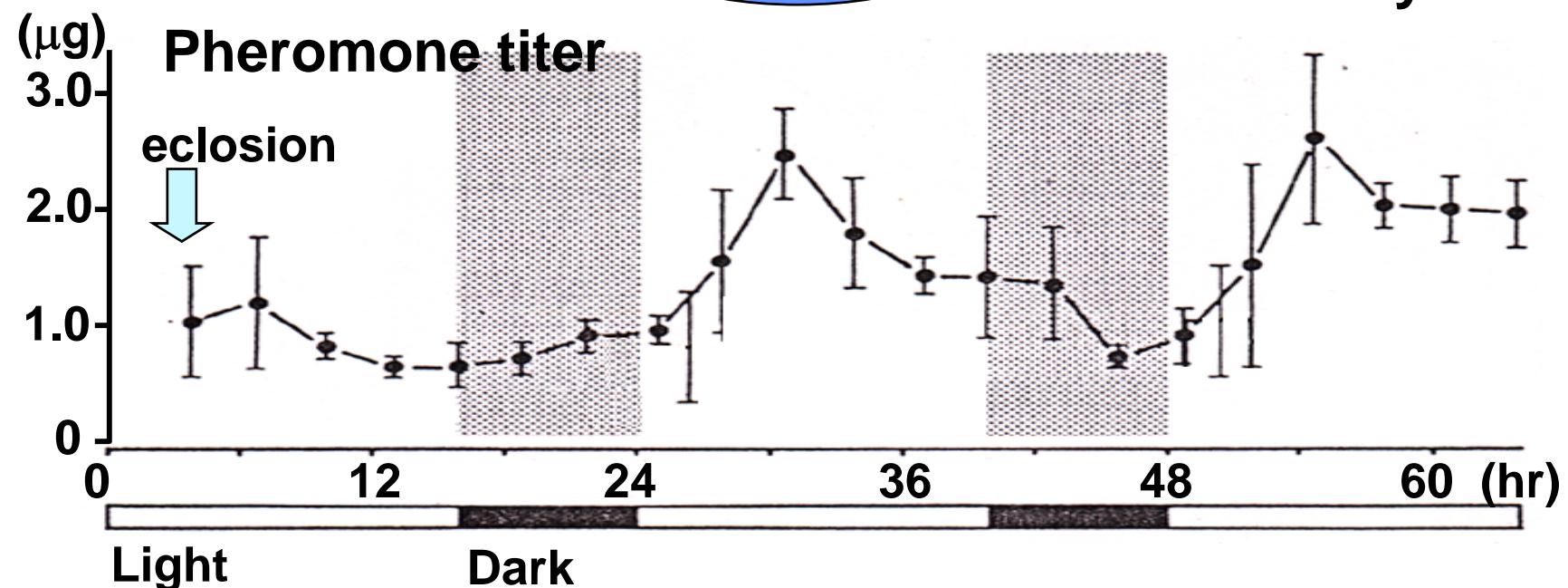
B-1) Introduction

Circadian rhythm



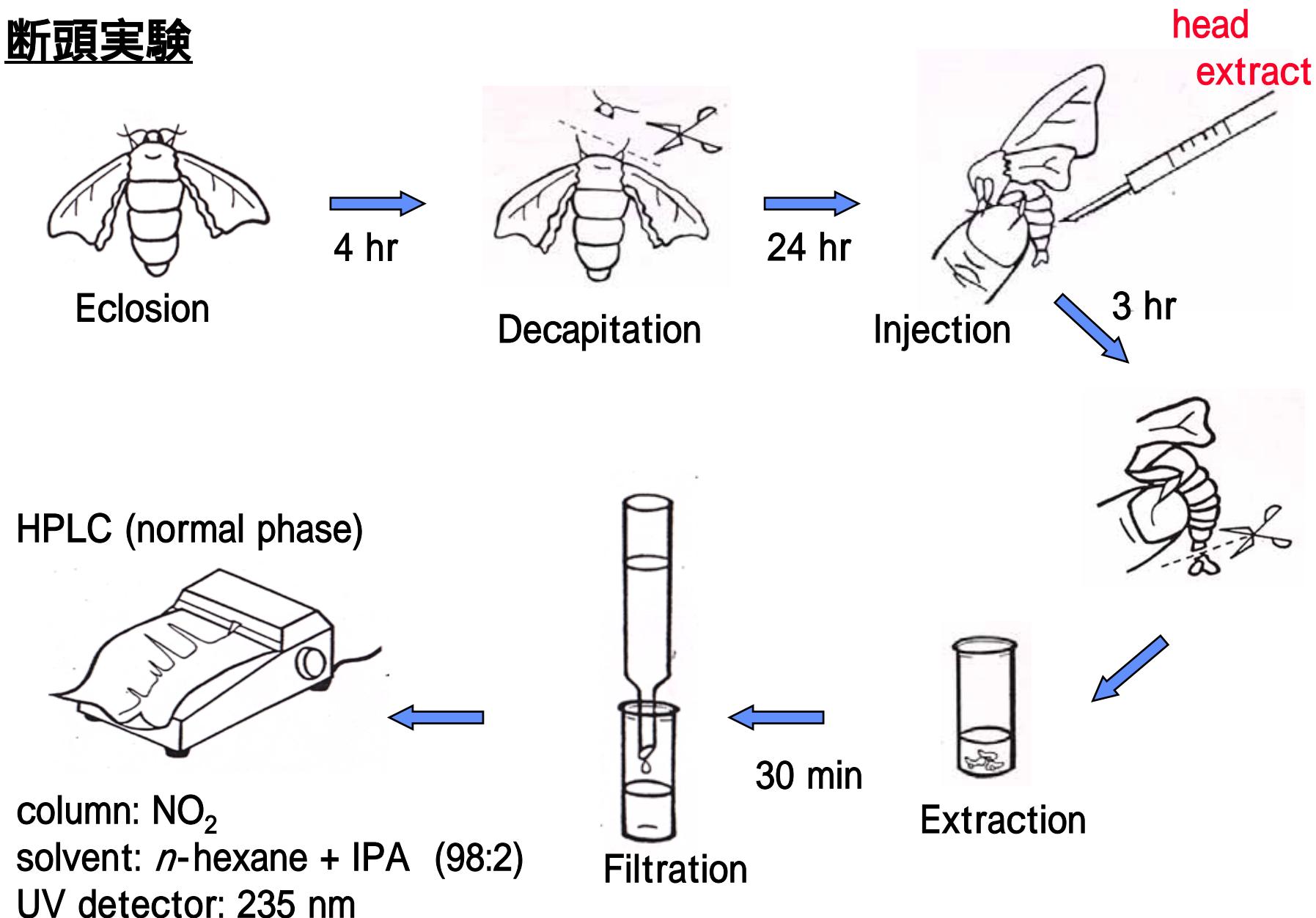
pheromone biosynthesis

nerve system ?
or
endocrine system ?



B-1) Introduction

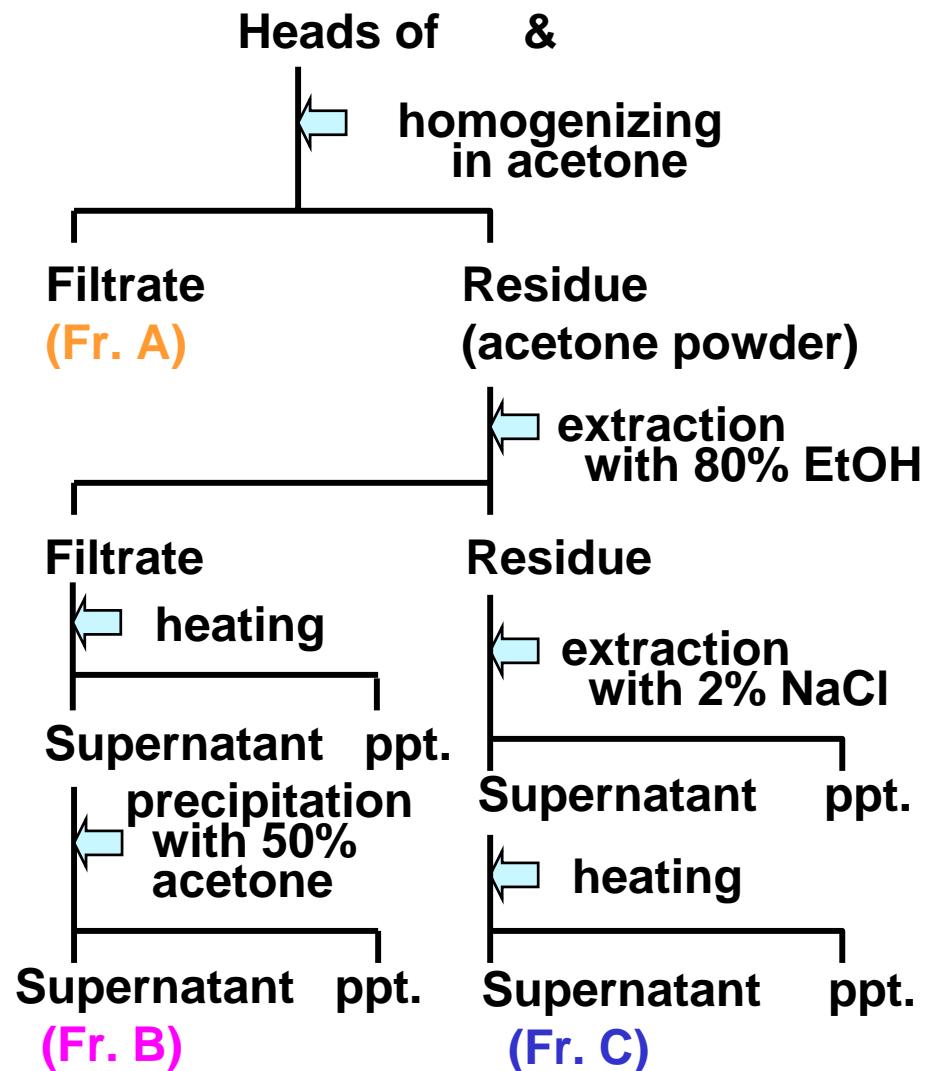
断頭実験



B-1) Introduction

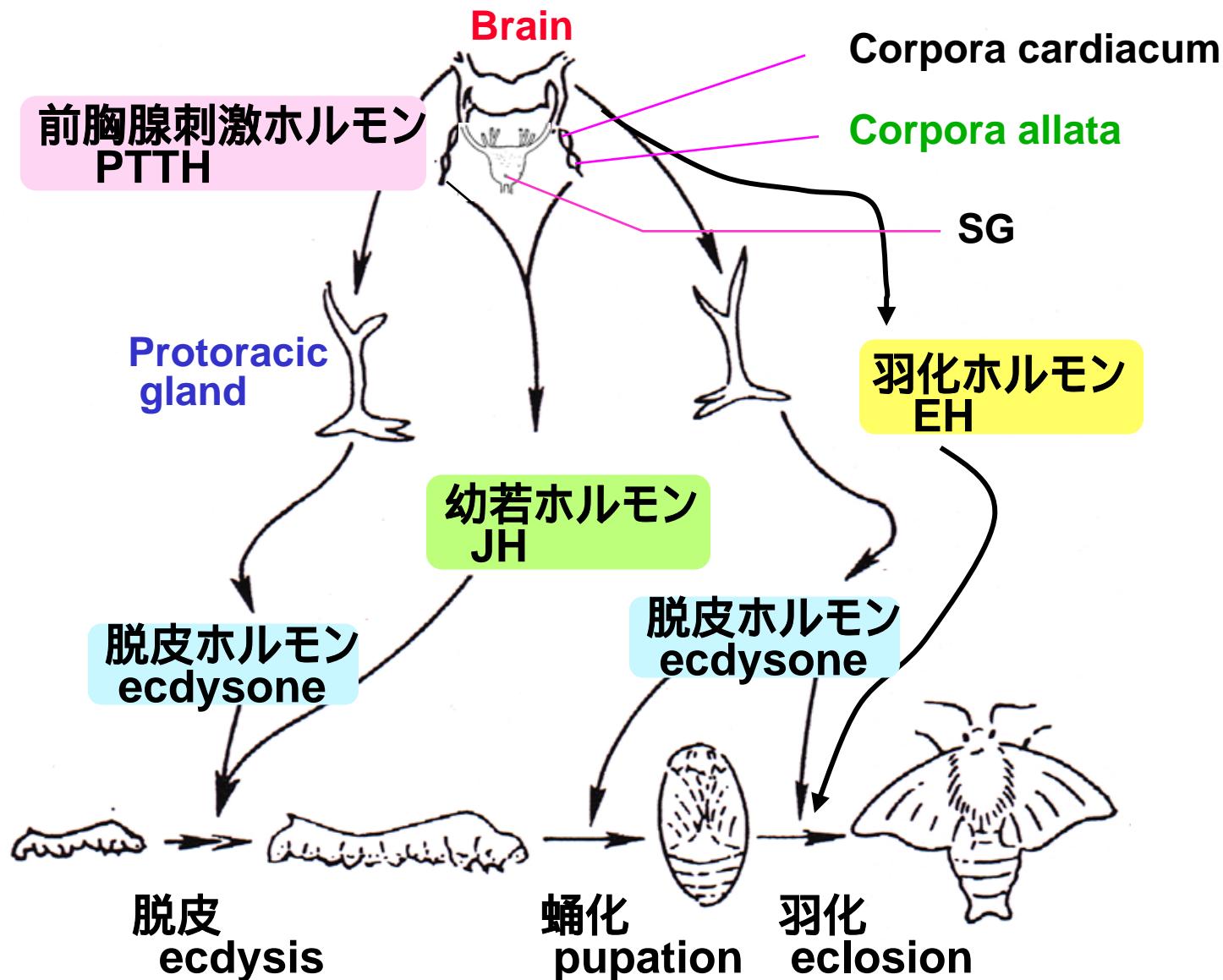
カイコでの予備実験

Treatment	Bombykol ($\mu\text{g}/\text{g}$)
Exp. 1)	
None	1.31 \pm 0.05
Decapitated	
+ Buffer	0.00 \pm 0.00
+ heads	0.19 \pm 0.07
Exp. 2)	
None	0.87 \pm 0.20
Decapitated	
+ Buffer	0.00 \pm 0.00
+ heads	0.15 \pm 0.05



B-1) Introduction

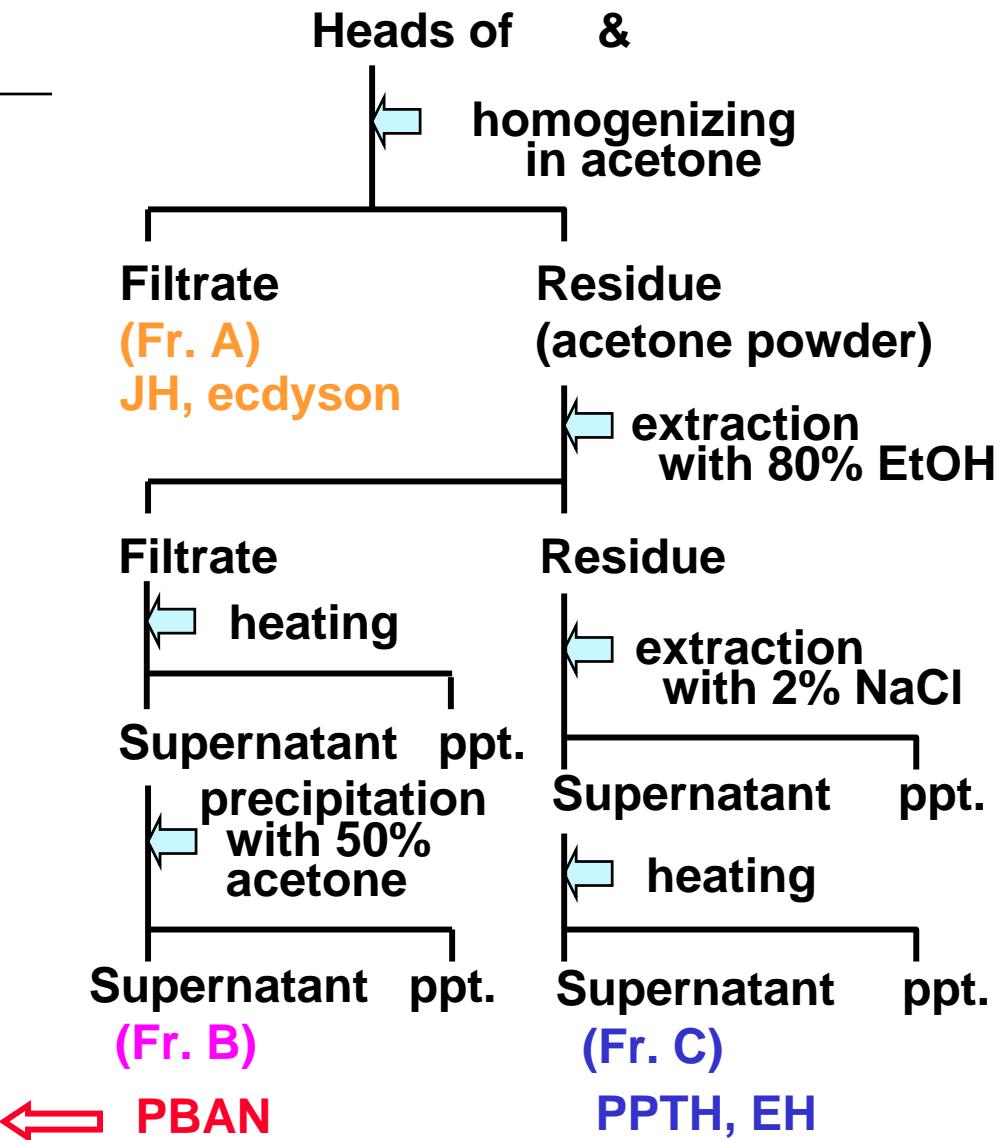
昆虫のホルモン



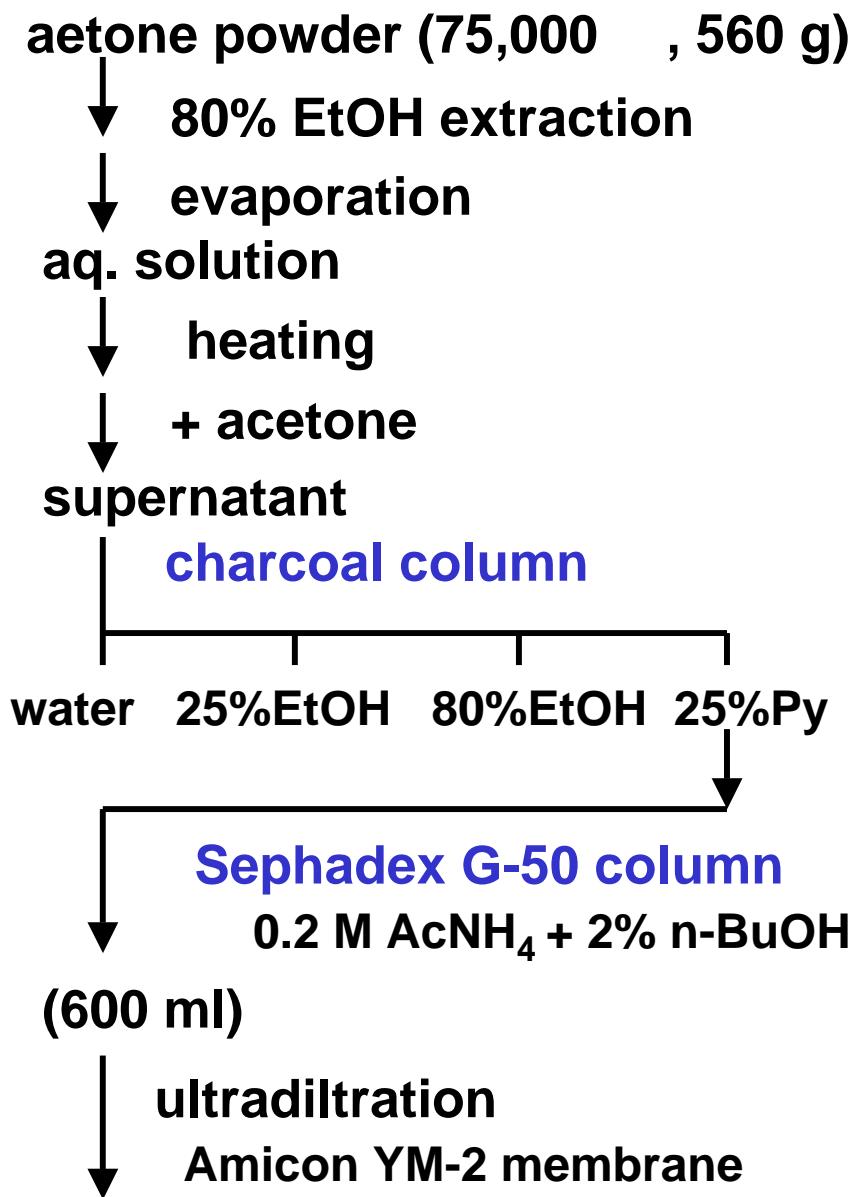
B-1) Introduction

カイコでの予備実験

Treatment	Bombykol ($\mu\text{g}/\text{g}$)
Exp. 1)	
None	1.31 ± 0.05
Decapitated	
+ Buffer	0.00 ± 0.00
+ heads	0.19 ± 0.07
Exp. 2)	
None	0.87 ± 0.20
Decapitated	
+ Buffer	0.00 ± 0.00
+ heads	0.15 ± 0.05
Exp. 3)	
None	0.69 ± 0.16
Decapitated	
+ Buffer	0.00 ± 0.00
+ Fr. A	0.06 ± 0.04
+ Fr. B	<u>0.24 ± 0.06</u>
+ Fr. C	0.03 ± 0.02



B-2) PBANの化学構造



(50 ml)

↓ QAE Sephadex column

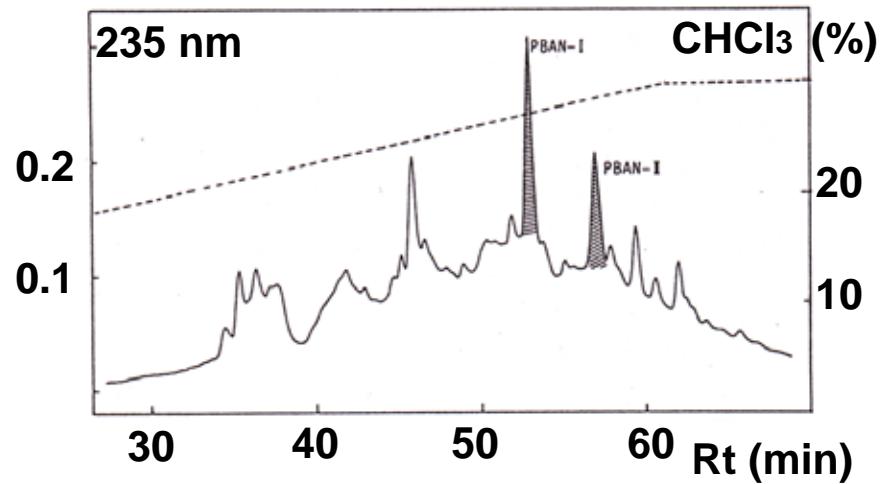
0.02M Tris-HCl + 0-0.5M NaCl

0.3 M NaCl fraction

HPLC

1. Develosil C8
2. VP-318
3. Vydac C18

1.5 µg (4700 unit ⇔ 6% yield)



B- 2) PBANの化学構造

FXPRL-amide

	1	5	10	15
Hez-PBAN	H-Leu Ser Asp Asp Met Pro Ala Thr Pro Ala Asp Gln Glu Met Tyr Arg Gln-			
Bom-PBAN I	H-Leu Ser Glu Asp Met Pro Ala Thr Pro Ala Asp Gln Glu Met Tyr Gln Pro-			
Bom-PBAN II	H-Arg Leu Ser Glu Asp Met Pro Ala Thr Pro Ala Asp Gln Glu Met Tyr Gln Pro-			
Lyd-PBAN	H-Leu Ala Asp Asp Met Pro Ala Thr Met Ala Asp Gln Glu Val Tyr Arg Pro-			
Bom-DH		H -Thr Asp Met Lys Asp Glu Ser Asp-		
Pss-MRCH			H-Lsy Leu-	

	20	25	30	33
Hez-PBAN	-Asp Pro Glu Gln Ile Asp Ser Arg Thr Lys Tyr Phe Ser Pro Arg Leu-NH ₂			
Bom-PBAN I	-Asp Pro Glu Glu Met Glu Ser Arg Thr Arg Tyr Phe Ser Pro Arg Leu-NH ₂			
Bom-PBAN II	-Asp Pro Glu Glu Met Glu Ser Arg Thr Arg Tyr Phe Ser Pro Arg Leu-NH ₂			
Lyd-PBAN	-Glu Pro Glu Gln Ile Asp Ser Arg Asn Lys Tyr Phe Ser Pro Arg Leu-NH ₂			
Bom-DH	-Arg Gly Ala His Ser Glu Arg Gly Ala Leu Trp Phe Gly Pro Arg Leu-NH ₂			
Pss-MRCH	-Ser Tyr Asp Asp Lys Val Phe Glu Asn Val Glu Phe Thr Pro Arg Leu-NH ₂			
Lom-MT I		H-Gly Ala Val Pro Ala Ala Gln Phe Ser Pro Arg Leu-NH ₂		
Lem-PK			pGlu Thr Ser Phe Thr Pro Arg Leu-NH ₂	

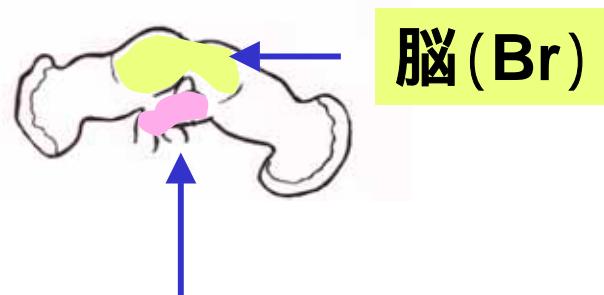
**Hez-PBAN (Raina et al., 1989), Bom-PBAN I and II (Kitamura et al., 1989, 1990),
Lyd-PBAN I (Masler et al., 1994), Bom-DH (Imai et al., 1991), Pss-MRCH (Matsumoto
et al., 1992), Lom-MT I and Lem-PK (Nachman and Holman, 1991)**

B-3) PBAN研究のまとめ

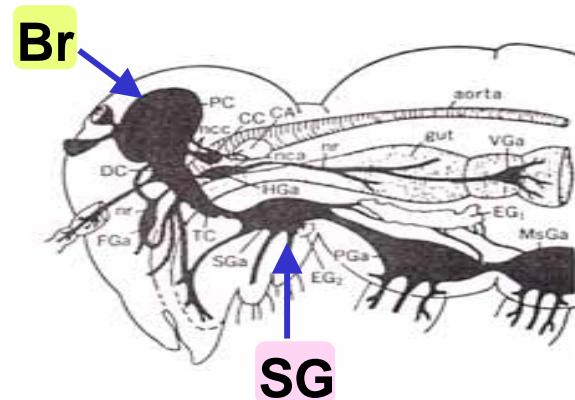
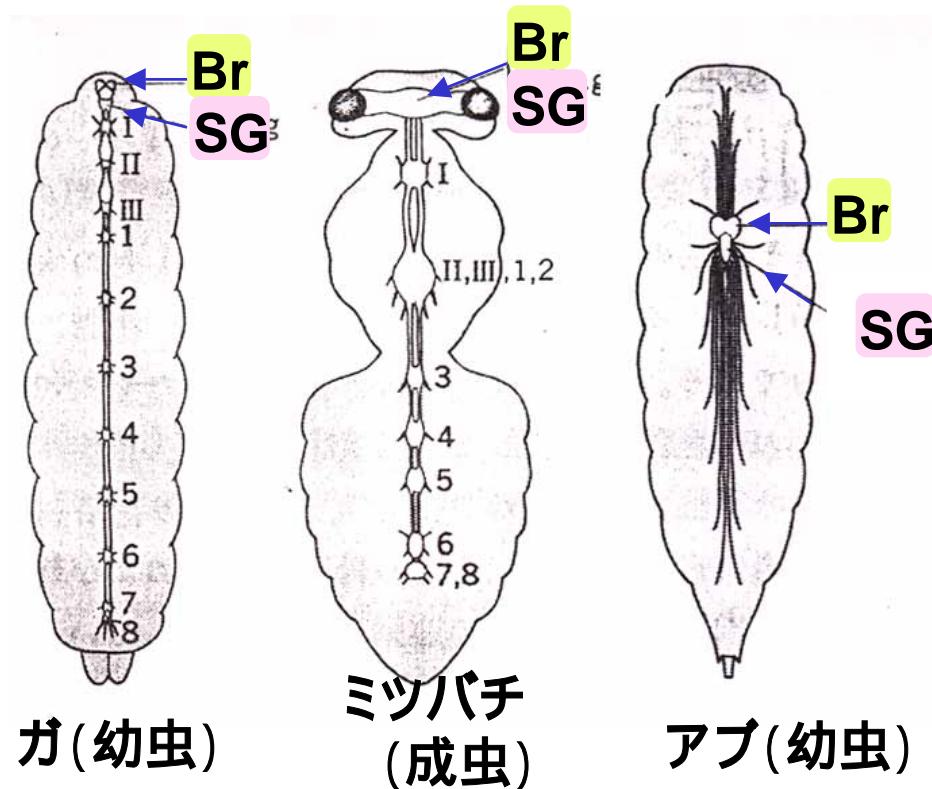
1. PBANの普遍性: 20種以上の蛾類昆虫で存在が示唆
2. 生産器官: SG (SOG, *suboesophageal ganglion*、食道下神経球)
3. 標的器官: フェロモン腺 (pheromone gland)
培養フェロモン腺を用いた実験により確認
例外) タバコヤガ *Helicoverpa zea*
 - VNC (ventral nerve cord)
 - TAG (terminal abdominal ganglion)
 - secondary messenger pheromone gland
 - Teal *et al.*, 1989. *ProNAS USA*, 86: 2488.
4. ペプチドの活性発現部位: アミド化C-末端5残基(FXPRL)
5. レセプターたん白質: マウスneuromedin U のレセプター
*Drosophila*の遺伝子
 - タバコヤガ Choi *et al.*, 2004. *ProNAS USA*, 100: 9721.
 - カイコ Hall *et al.*, 2004. *J. Biol. Chem.*, 279: 51500.
6. 活性化する生合成ステップ: いろいろ?
7. 遺伝子: PBAN と DH の関係?

B-3) PBAN研究のまとめ

食道下神経球

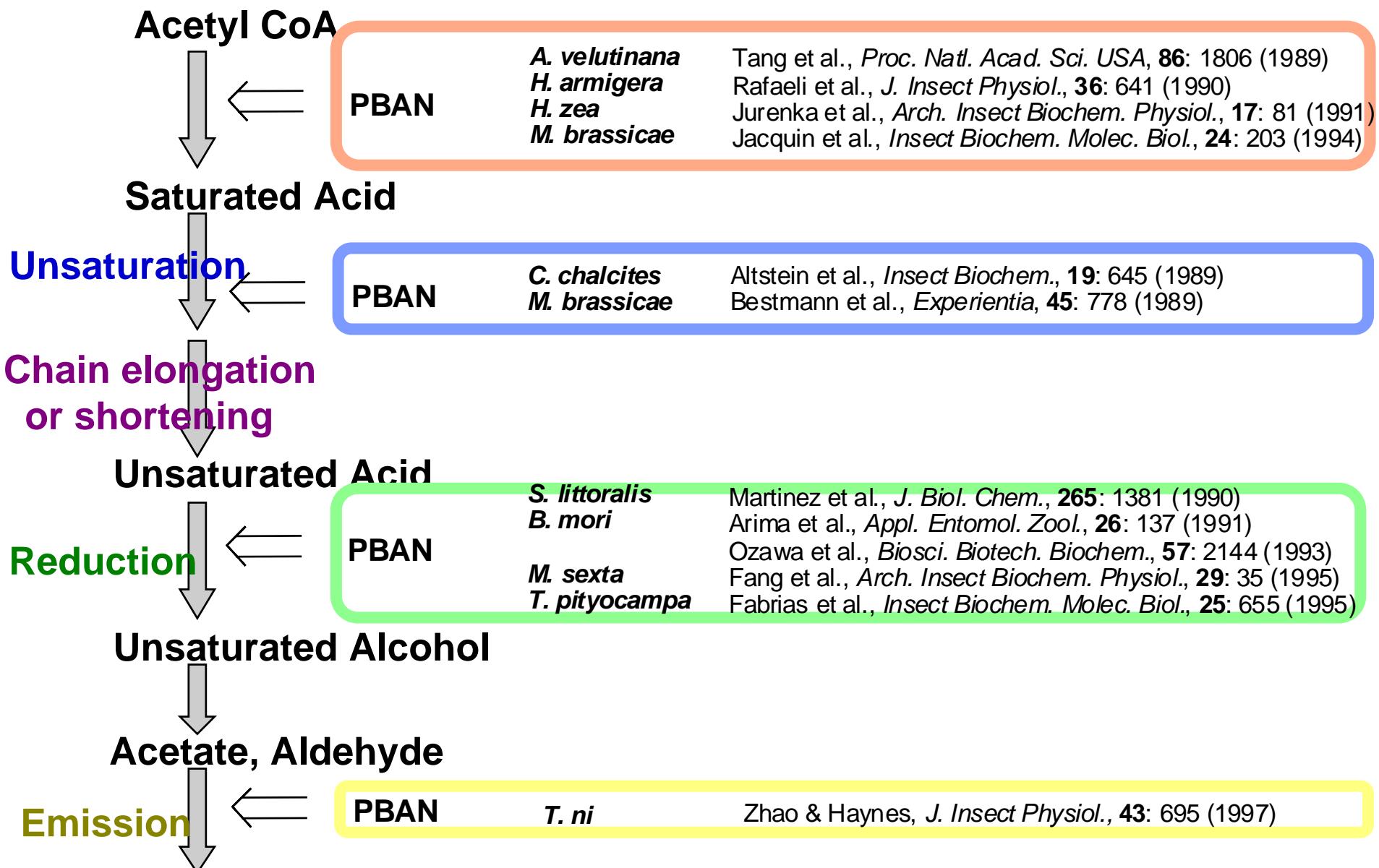


食道下神経球(SG)



B-3) PBAN研究のまとめ

PBANの活性化ステップ



B-3) PBAN研究のまとめ

カイコPBANcDNAの構造

Kawano *et al.*, 1992. *B. B. R. C.*, 189: 221.

-40 ATCACTTCGCCCTCCAACCAACTGAAGGGACAACAACAAAAA ATG TAT AAA ACC AAC ATT GTT TTC AAC GTT TTA GCT TTG
Met Tyr Lys Thr Asn Ile Val Phe Asn Val Leu Ala Leu

40 GCA TTG TTC AGT ATT TTC TTC GCG AGT TGC ACG GAT ATG AAG GAT GAA AGC GAC AGA GGA GCT CAC AGT
Ala Leu Phe Ser Ile Phe Phe Ala Ser Cys Thr Asp Met Lys Asp Glu Ser Asp Arg Gly Ala His Ser DH

109 GAG CGG GGC GCT CTC TGG TTC GGC CCC AGA CTC GGG AAG CGA TCA ATG AAG CCA TCC ACT GAA GAT AAC
Glu Arg Gly Ala Leu Trp Phe Gly Pro Arg Leu Gly Lys Arg Ser Met Lys Pro Ser Thr Glu Asp Asn

178 AGG CAA ACC TTC CTG AGG CTG CTC GAG GCG GCT GAT GCC CTC AAA TTT TAT TAC GAC CAG CTA CCT TAC
Arg Gln Thr Phe Leu Arg Leu Leu Glu Ala Ala Asp Ala Leu Lys Phe Tyr Tyr Asp Gln Leu Pro Tyr

247 GAG AGG CAA GCC GAT GAA CCG GAA ACC AAA GTA ACA AAG AAG ATC ATC TTC ACC CCC AAA CTC GGG AGG
Glu Arg Gln Ala Asp Glu Pro Glu Thr Lys Val Thr Lys Ile Ile Phe Thr Pro Lys Leu Gly Arg α

316 AGC GTC GCC AAA CCC CAG ACG CAT GAA AGC CTC GAA TTC ATC CCC CGG CTC GGA AGG CGG CTC TCT GAG
Ser Val Ala Lys Pro Gln Thr His Glu Ser Leu Phe Ile Pro Arg Leu Gly Arg Arg Leu Ser Glu β

385 GAC ATG CCT GCT ACG CCA GCT GAC CAG GAA ATG TAC CAA CCT GAC CCC GAA GAA ATG GAG TCA AGA ACA
Asp Met Pro Ala Thr Pro Ala Asp Gln Glu Met Tyr Gln Pro Asp Pro Glu Glu Met Glu Ser Arg Thr PBAN

454 AGA TAC TTC TCG CCC AGG CTG GGG CGC ACC ATG AGC TTT TCG CCC AGA CTG GGA AGG GAG CTT TCG TAC
Arg Tyr Phe Ser Pro Arg Leu Gly Arg Thr Met Ser Phe Ser Pro Arg Leu Gly Arg Glu Leu Ser Tyr γ

523 GAT TAC CCT ACA AAA TAT AGG GTT GCC AGA AGC GTT AAC AAG ACA ATG GAC AAC TAAACGAATTATGGTCCG
Asp Tyr Pro Thr Lys Tyr Arg Val Ala Arg Ser Val Asn Lys Thr Met Asp Asn ***

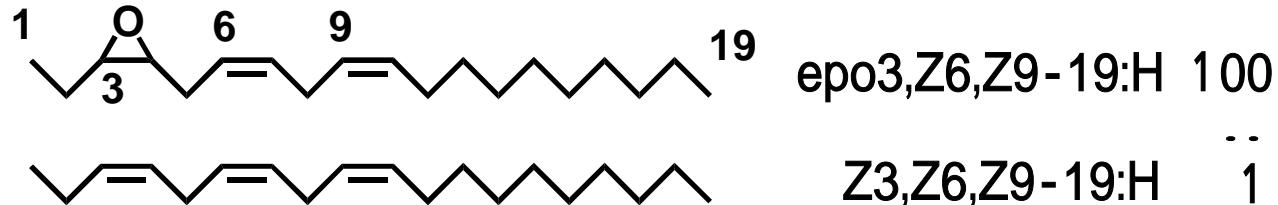
595 CTTGAGGTACCTCATTTGAGGTCTCGATCGACTCCGACGAACGGTTACGGTAAACGGCGACAATGTTAATGTTGGACGAAACAATTG
685 TTAATTAATAAATTCATGTGATTTGTAATTGTAATTAAAGTGAATAAAAAAATAAAACTATTAAAAT(polyA)

タバコガ Davis *et al.*, 1992. *ProNAS USA*, 89: 142.

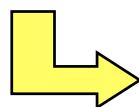
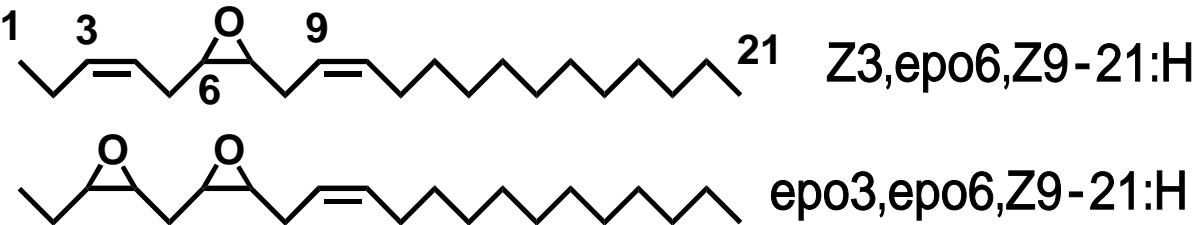
B- 4) シャクガでのPBAN研究

末端官能基がない (約 15 %)

ヨモギエダシャク : Japanese giant looper (*Ascotis selenaria cretacea*)

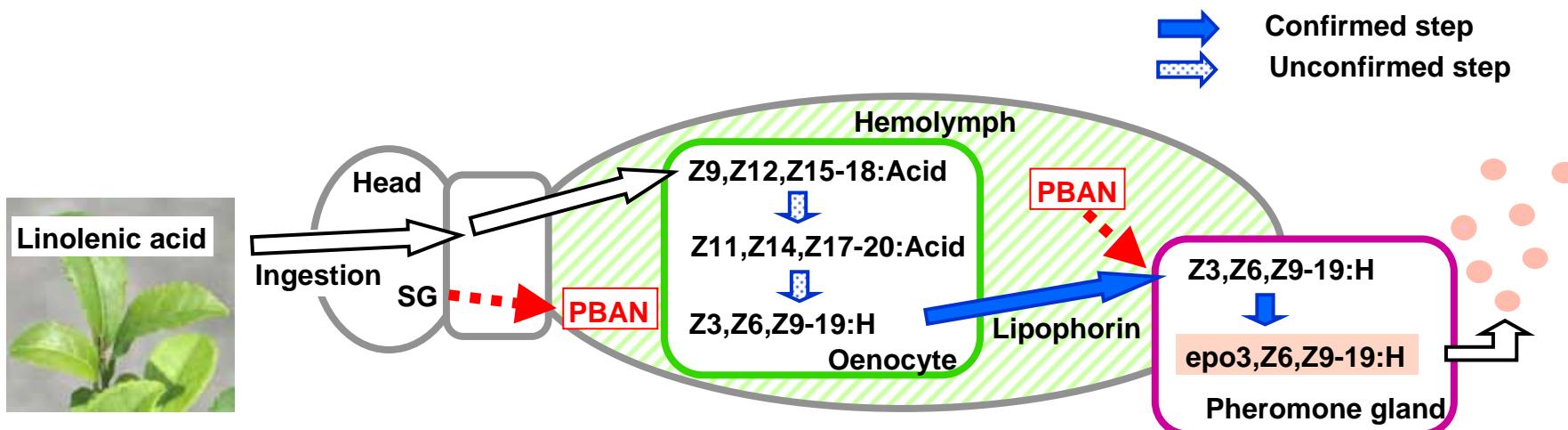
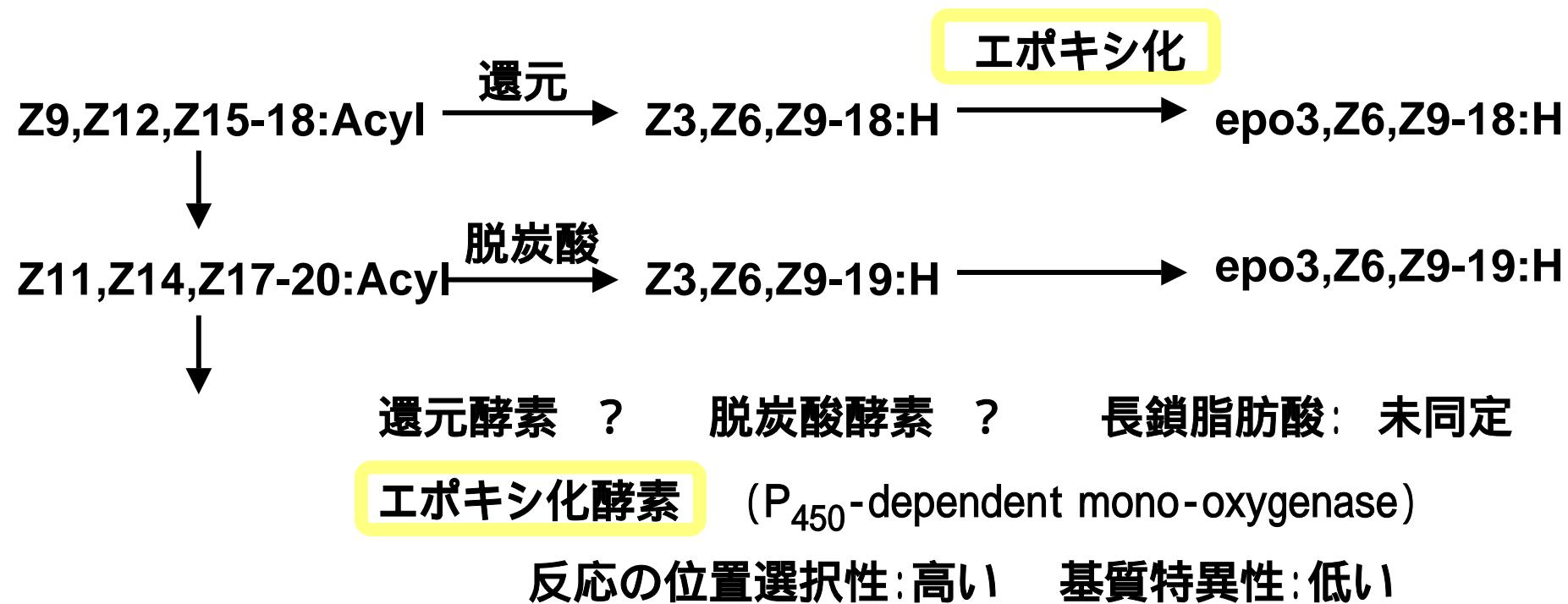


スキバドクガ : clear-winged tussock moth (*Perina nuda*)



- i 不飽和炭化水素とそのエポキシ化物
 - ii 直鎖の炭素数: 17 ~ 23 iii 二重結合数: 1 ~ 4
 - iv リノール酸、リノレン酸から生成される

B-4) シャクガでのPBAN研究



B-4) シャクガでのPBAN研究

Degenerateプライマーを用いたPCR

DH

MYKTNIVFNVLALALFSIFFASCTDMKDESDRGAHSERGALW**FGPRL**GKR

SMKPSTEDNRQTFRLLEAADALKFYYDQLPYERQADEPETK VTKK II **FT**

PKLGRSVAKPQT~~HESLE~~**FIPRL**GRRLSEDMPATPADQEMYQPDPEEMESR

PBAN

TRY**FSPRL**GRTMS**FSPRL**GRELSYDYPTKYRVARSVNKTMDN

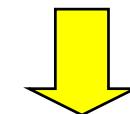
DH

	W	F	G	P	R	L
<i>B.mori</i>	T G G T T C G G G	C	C C C	A G A	C T	
<i>H.zea</i>	T G G T T C G G G	T	C C C	C G G	C T	
<i>H.armi</i>	T G G T T C G G G	T	C C C	A G A	C T	
		A		C	G	
	5'-T G G T T C G G G	[C]	C C C [A]	G [A]	C T-3'	
		T	C	C	G	

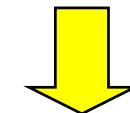
PBAN

	Y	F	S	P	R	L
<i>B.mori</i>	T A C T T C T C G	G	C C C	C A G G C T	G	
<i>H.zea</i>	T A C T T C T C C	C	C C A	A G G C T	C	
<i>H.armi</i>	T A C T T C T C C	C	C C A	A G G C T	C	
	T	G		G C		C
	3'-A T G A A G A G	[C]	G G [T]	T C C G A [C]	-5'	
	G	G	G	G	C	G

ヨモギエダシャク
100 のSG



PCR



3'RASE
5'RASE

B-4) シャクガでのPBAN研究

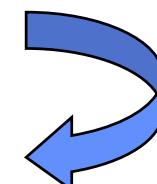
ヨモギエダシャクのPBANの構造

	DH homologue		α -SGNP		β -SGNP
Assc	NDLKEDG-EРЕANSDRQGLWFGPRL		VIFTPKL		S-----VDFTPRL
Bom	TDMKDES-DRGAHSDRGALWFGPRL	59%	IIFTPKL	85%	SVA-----KPQTHESELFIIPRL
Hez	NDVKDGA-ASGAHSDRLGLWFGPRL	68% (62%)	VIFTPKL	100% (85%)	SLA--YDDK-SF-ENVEFTPRL
Hev	NDDKDGA-ASGAHSDRLGLWFGPRL	68% (62%)	VIFTPKL	100% (85%)	SLS--YDDK-SF-ENVEFTPRL
Mas	NDIKDEG-DRGAHSDRGALWFGPRL	69% (83%)	VIFTPEL	85% (71%)	SLDDSTQEKRVFYENEFTPRL
Ads	N-FKEENFDRNIRSGRANVVFKPIL	37% (40%)	VIFTPKL	100% (85%)	SMEDPYEEKRSY-D-VDFTPRL

	PBAN homologue		γ -SGNP
Assc	QLVDDVPQRQQIEEDRL-----GSRTTRFFSPRL		TTIMNFSPRL
Bom	-LSEDMPATPADQE--IYQPDPEEMESR-TRYFSPRL	44%	T-MSFSPRL
Hez	-LSDDMPATPADQE--MYRQDP EQIDS R-TKYFSPRL	46% (76%)	T-MNFSPRL
Hev	-LADDMPATPADQE--MYRQDP EQIDS RRTKYFSPRL	45% (71%)	T-MNFSPRL
Mas	-ISEDMPATPSDQEYPMYHPDPEQIDS R-TTRYFSPRL	32% (71%)	T-HFSPRL
Ads	Q-----SEAVTSSDEQVYRQDMA SPV DGR-LKYFSPRL	40% (45%)	ANVFEKPI L

b-SGNP、PBAN homologue ともに異常に短い
両者の開裂部はGR (他種はすべてGRR)

	PBAN
Assc	SVDFTPRLGRQLVDDVPQRQQIEEDRLGSRTTRFFSPRL
Bom (PBAN I)	LSEDMP--ATPADQE--IYQPDPEEMESR-TRYFSPRL
Hez	LSDDMP--ATPADQE--MYRQDP EQIDS R-TKYFSPRL
Lyd	LADDMP--ATMADQE--VYRPEPEQIDS R-TKYFSPRL



B-4) シャクガでのPBAN研究

PBAN遺伝子に関する系統樹

