性フェロモン生合成の制御機構

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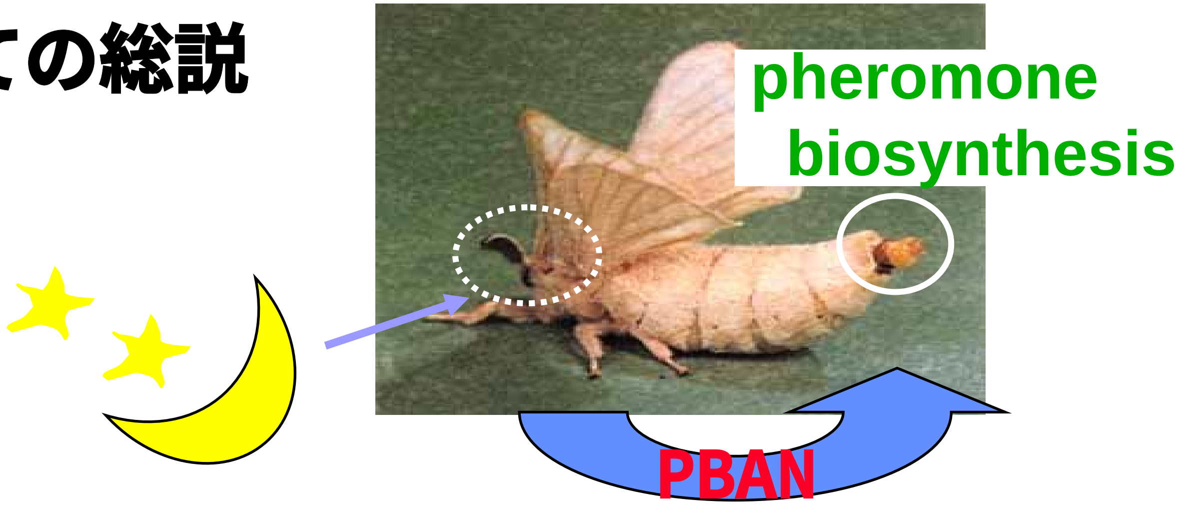
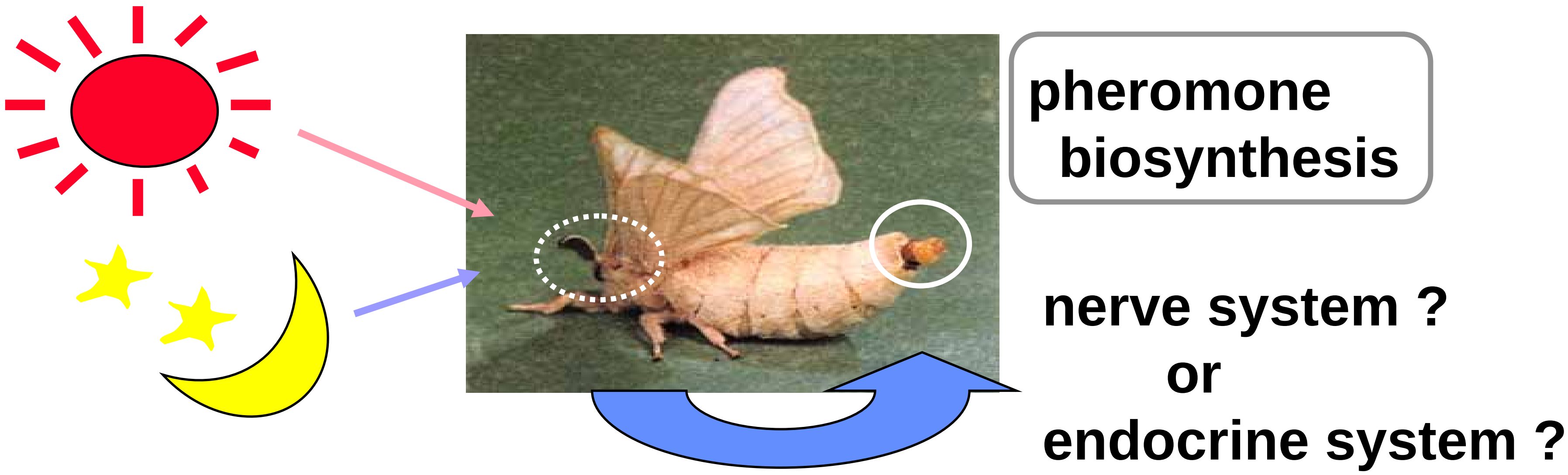
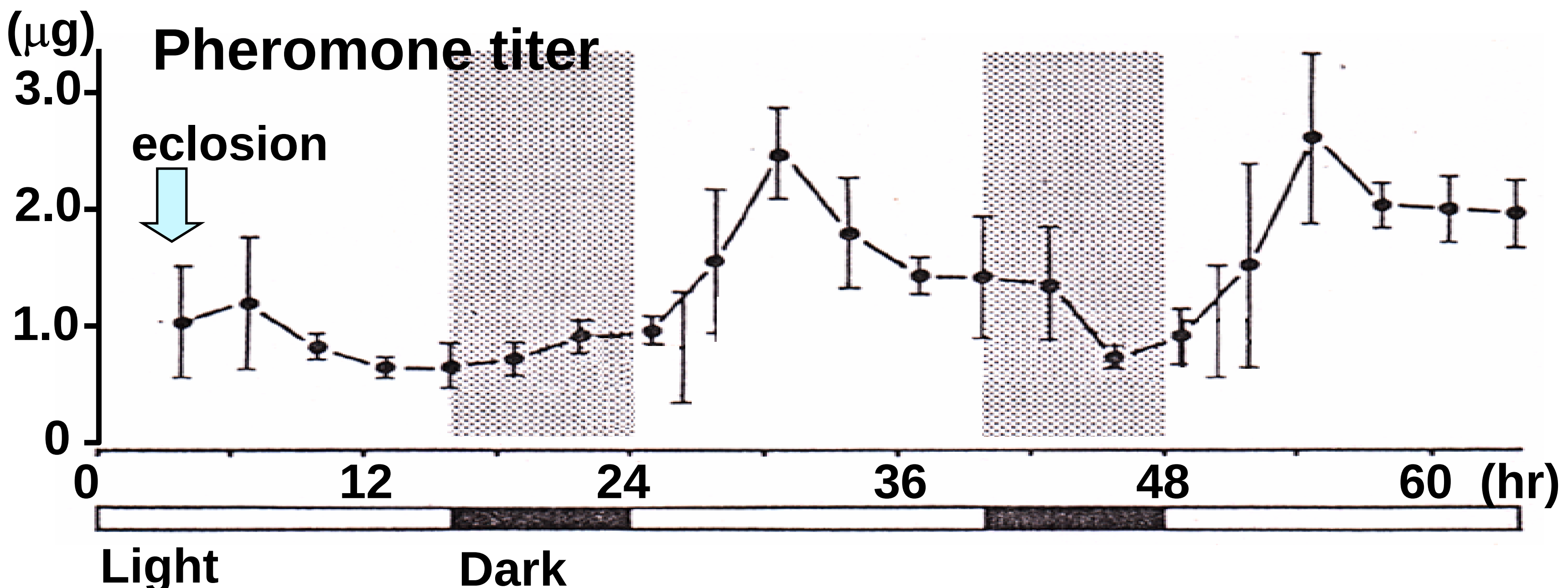


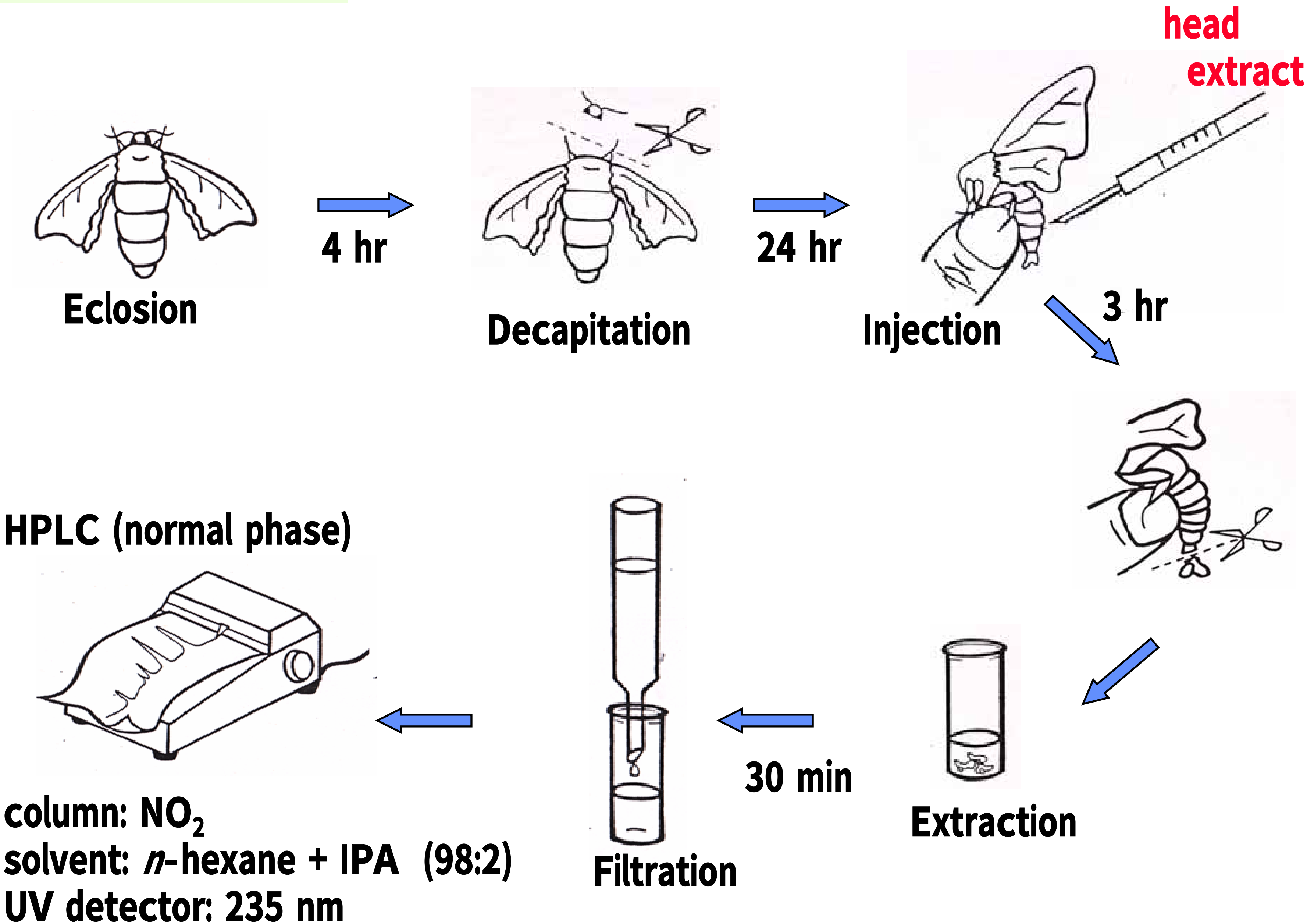
I. Introduction

a) Circadian rhythm





b) 断頭実験


head extract
Eclosion
4 hr
Decapitation
24 hr
Injection
3 hr
Extraction
30 min
Filtration
HPLC (normal phase)
column: NO_2
solvent: *n*-hexane + IPA (98:2)
UV detector: 235 nm

c) PBAN の発見

1. In Japan

Ohguchi et al., 1983.　日本応用動物昆虫学会　第27回大会

Ohguchi et al., 1985.　*Japan J. Appl. Entomol. Zool.*, 29: 265-269.
"Hormone-like substance present in the cephalic organs of the female moth, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae) and controlling sex pheromone production"

2. In USA

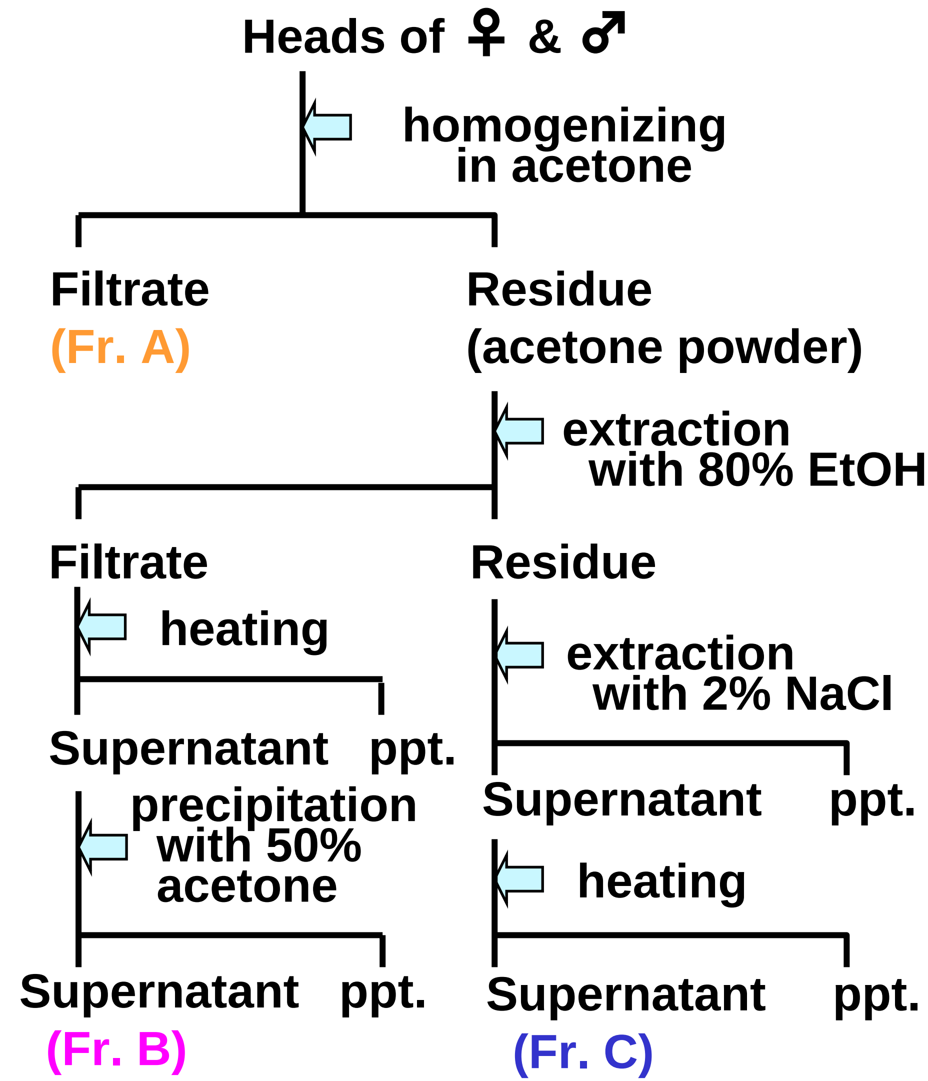
Raina & Klune, 1984.　*Science*, 225: 531-533.
"Brain factor control of sex pheromone production in the female corn earworm moth"

Pheromone **B**iosynthesis **A**ctivating **N**europeptide ⇨ **PBAN**

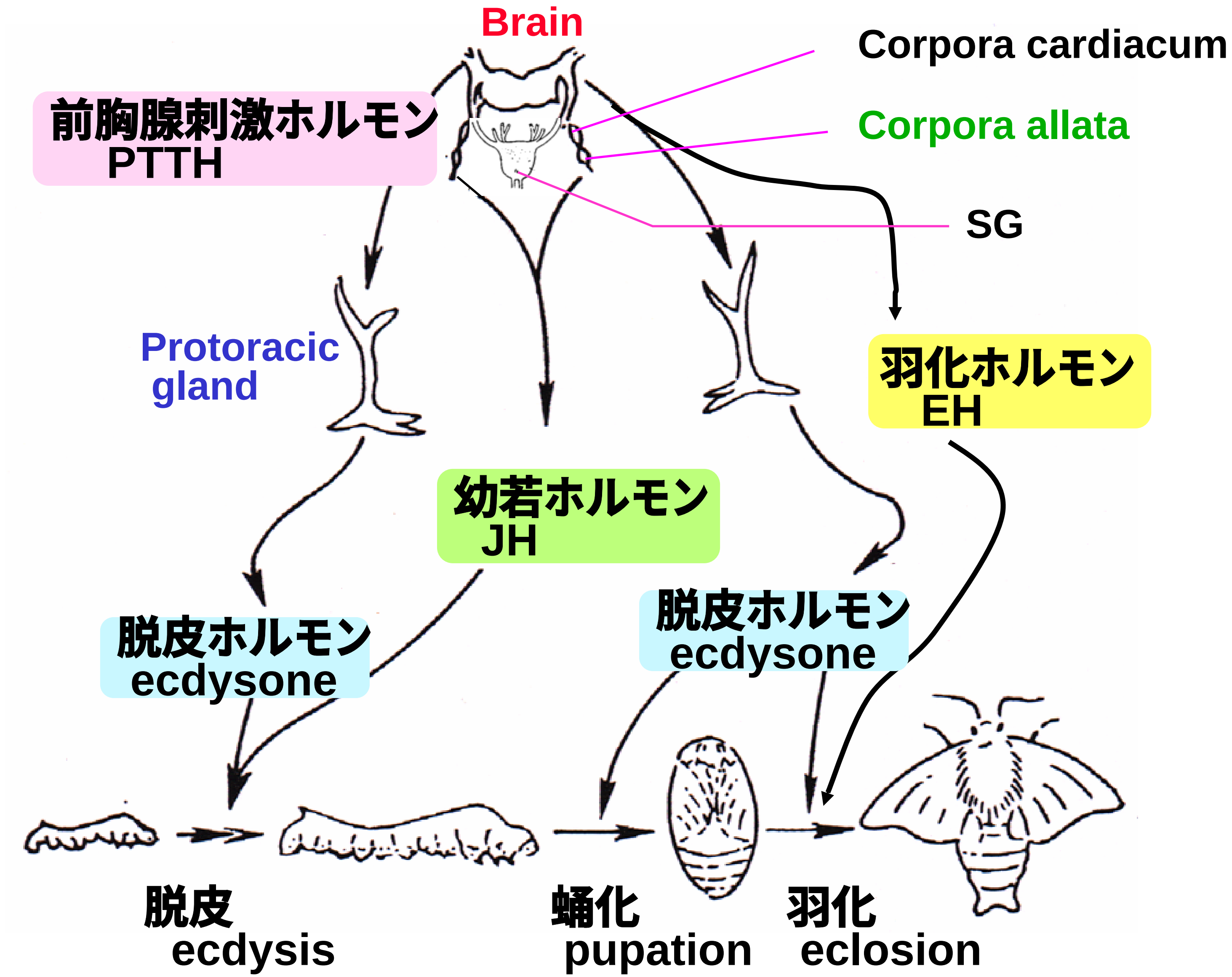
II. PBANの単離・同定

a) Preliminary experiments ①

Treatment	Bombykol (μg/♀)
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

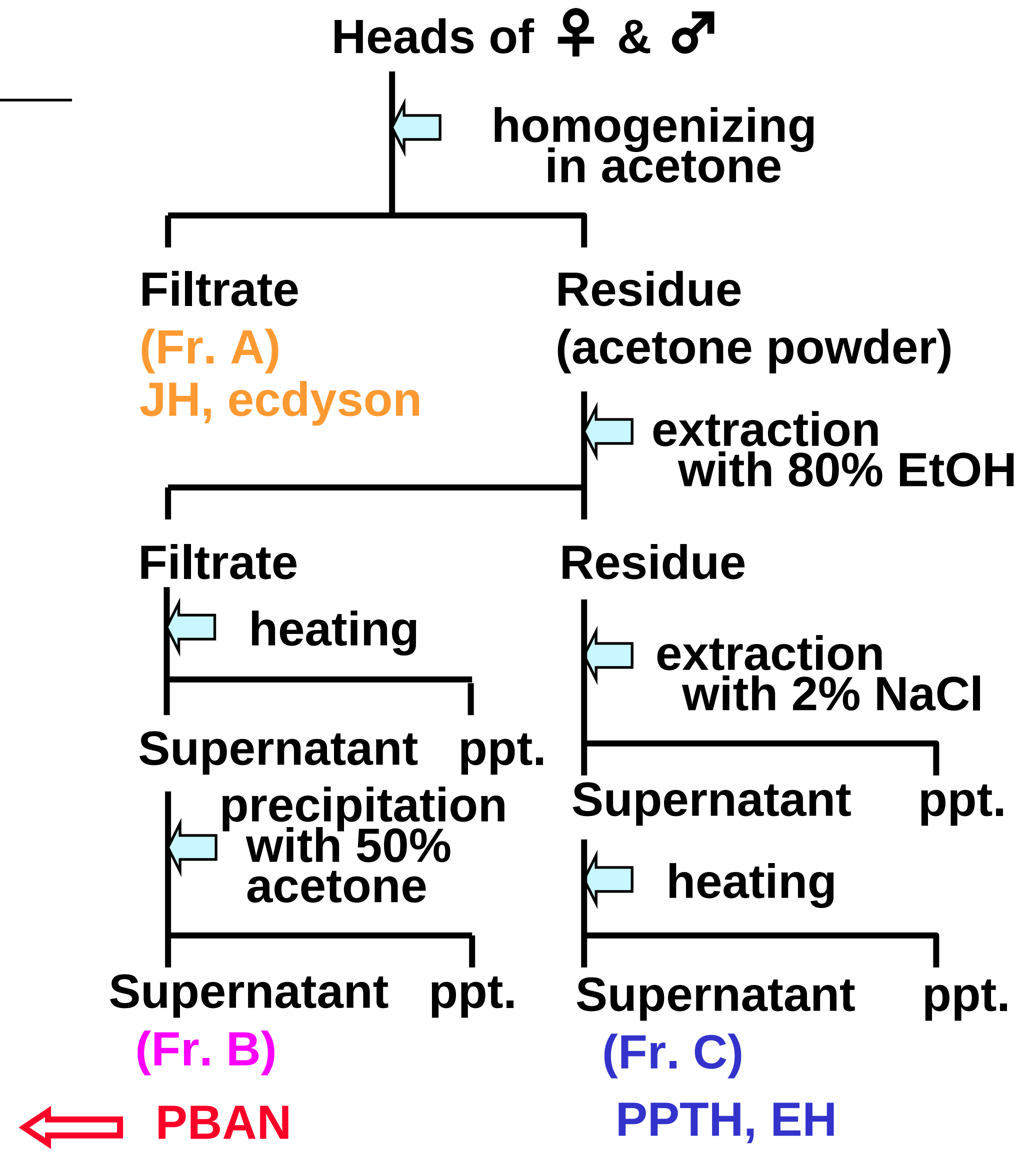


b) Endocrine system of insect



c) Preliminary experiments ②

Treatment	Bombykol (μg/♀)
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	0.24 ± 0.06
+ Fr. C	0.03 ± 0.02



d) Isolation of Bom-PBAN

aetone powder (75,000 ♂, 560 g)

↓ 80% EtOH extraction

↓ evaporation

aq. solution

↓ heating

↓ + acetone

supernatant

charcoal column

water | 25%EtOH | 80%EtOH | 25%Py

↓ Sephadex G-50 column

0.2 M $AcNH_4$ + 2% n-BuOH

(600 ml)

↓ ultradiltration

Amicon YM-2 membrane

(50 ml)

↓ QAE Sephadex column

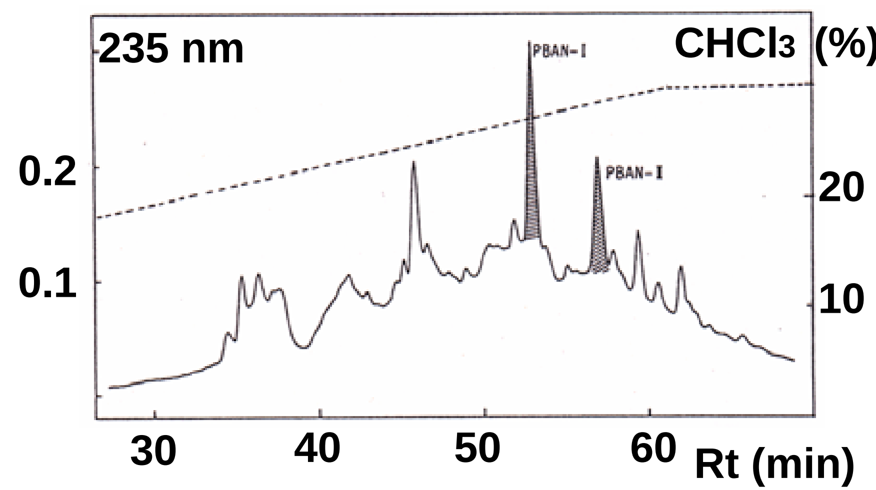
0.02M Tris-HCl + 0-0.5M NaCl

0.3 M NaCl fraction

↓ HPLC

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

1.5 μg (4700 unit ⇨ 6% yield)



III. PBANのアミノ酸配列

a) 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) PBANのアミノ酸配列の相同性 ①

a: 遺伝子解析からの同定

科名 種名	アミノ酸配列 (→ C-末側)	相同性 (%)
カイコガ科	1 10 20 30	
カイコ	LSEDMPATPADQEMYQPDPEEMESRTRYFSPRL-NH_2	
クワコ	∗∗∗∗∗∗∗∗∗∗∗∗∗I∗∗∗∗∗∗V∗∗∗∗∗∗∗∗∗∗∗∗-NH_2	94
ドクガ科		
マイマイガ	∗AD∗∗∗∗∗M∗∗∗∗V∗R∗E∗∗QID∗∗NK∗∗∗∗∗∗-NH_2	67
ヤガ科		
タバコガ☆	∗∗D∗∗∗∗∗∗∗∗∗∗∗∗RQ∗∗∗QID∗∗∗K∗∗∗∗∗∗-NH_2	79
オオタバコガ[a]	∗∗D∗∗∗∗∗∗∗∗∗∗∗∗RQ∗∗∗QID∗∗∗K∗∗∗∗∗∗-NH_2	79
ヨトウガ[a]	∗AD∗∗∗∗∗∗∗∗∗∗∗∗R∗∗∗∗QID∗∗∗K∗∗∗∗∗∗-NH_2	79
ハスモンヨトウ☆[a]	∗AD∗∗∗∗∗∗∗∗∗∗L∗R∗∗∗DQID∗∗∗K∗∗∗∗∗∗-NH_2	73
タマナヤガ[a]	∗AD∗T∗∗∗∗∗∗∗∗∗∗R∗∗∗∗QID∗∗∗K∗∗∗∗∗∗-NH2	76
ハマキガ科		
チャノコカクモンハマキ[a]	Q∗∗——AV∗SS∗EQV∗RQ∗MSPVDG∗LK∗∗∗∗∗∗-NH2	39

☆ は近縁な欧米種

∗ は カイコと同一のアミノ酸を、 (ピンク色の枠) は活性発現に必須の部分構造を示している。

b) PBANのアミノ酸配列の相同性 ② 文献

タバコガの近縁種 (*Helicoverpa zea*) Hez-PBAN (Raina, 1989)

カイコ (*Bombyx mori*) Bomo-PBAN I, II (Kitamura, 1989, 1990)

マイマイガ (*Lymantria disper*) Lyd-PBAN (Masler, 1994)

a: **遺伝子解析からの同定**

カイコ (*Bombyx mori)* Bomo-PBAN (Kawano,1992, 1997; Sato, 1993)

クワゴ (*Bombyx mandarina*) Boma-PBAN (Xu, 1999)

タバコガの近縁種 (*Helicoverpa zea*) Hez-PBAN (Davis, 1992)

(*Helicoverpa armigera*) Hear-PBAN (Zhang, 2004)

(*Heliothis virescens*) Hev-PBAN (Xu & Denlinqer, 2003)

オオタバコガ (*Helicoverpa assulta*) Heas-PBAN (Choi, 1998)

ヨトウガ (*Mamestra brassicae*) Mab-PBAN (Jacquin-Joly, 1998)

ハスモンヨトウの近縁種 (*Spodoptera littoralis*) Spl-PBAN (Iglesias, 2002)

タマナヤガ (*Agrotis ipsilon*) Agi-PBAN (Duportets, 1998,1999)

タバコスズメガ (*Manduca sexta*) Mas-PBAN (Xu & Denlinqer, 2004)

チャノコカクモンハマキ (*Adoxophyes honmai*) Adh-PBAN (Lee, 2001)

Amino Acid Sequences of FXPRLamides

(1) PBAN

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Bomo-PBAN I	H-	Leu	Ser	Glu	Asp	Met	Pro	Ala	Thr	Pro	Ala	Asp	Gln	Glu	Met	Tyr	Gln	Pro	Asp	Pro	Glu
Bomo-PBAN II	H- Arg	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Boma	H-	*	*	*	*	*	*	*	*	*	*	*	*	*	Ile	*	*	*	*	*	*
Lydi	H-	*	Ala	Asp	*	*	*	*	*	Met	*	*	*	*	Val	*	Arg	*	Glu	*	*
Agip	? H- Arg	*	Ala	Asp	*	Thr	*	*	*	*	*	*	*	*	*	*	Arg	*	*	*	*
Hear, Heas, Heze	H-	*	*	Asp	*	*	*	*	*	*	*	*	*	*	*	*	Arg	Gln	*	*	*
Hevi	H-	*	Ala	Asp	*	*	*	*	*	*	*	*	*	*	*	*	Arg	Gln	*	*	*
Mabr	H-	*	Ala	Asp	*	*	*	*	*	*	*	*	*	*	*	*	Arg	*	*	*	*
Spli	H-	*	Ala	Asp	*	*	*	*	*	*	*	*	*	*	Leu	*	Arg	*	*	*	Asp
Adho	H-	Gln	*	*	~	~	Ala	Val	*	Ser	Ser	*	Glu	Gln	Val	*	Arg	Gln	*	Met	Ser

21	22	23	24	25	25'	26	27	28	29	30	31	32	33		
Glu	Met	Glu	Ser	Arg	~	Thr	Arg	Tyr	Phe	Ser	Pro	Arg	Leu	NH2	Bomo-PBAI
*	*	*	*	*	~	*	*	*	*	*	*	*	*	NH2	Bomo-PBAI
Val	*	*	*	*	~	*	*	*	*	*	*	*	*		Boma
Gln	Ile	Asp	*	*	~	Asn	Lys	*	*	*	*	*	*	NH2	Lydi
Gln	Ile	Asp	*	*	~	*	Lys	*	*	*	*	*	*		Agip
Gln	Ile	Asp	*	*	~	*	Lys	*	*	*	*	*	*		Hear, Heas
Gln	Ile	Asp	*	*	Arg	*	Lys	*	*	*	*	*	*		Hevi
Gln	Ile	Asp	*	*	~	*	Lys	*	*	*	*	*	*		Mabr
Gln	Ile	Asp	*	*	~	*	Lys	*	*	*	*	*	*		Spli
Pro	Val	Asp	Gly	*	~	Leu	Lys	*	*	*	*	*	*		Adho

Bombyx mori	カイコ	Bomo-PBAN	Kitamura et al., 1989, 1990
Bombyx mandarina	クワゴ	Boma-cDNA	Xu et al., 1999
Limantria disper	マイマイガ	Lidi-PBAN	Masler et al., 1994
Agrotis ipsilon	タマナヤガ	Agip-cDNA	Duportets et al., 1998; 1999
Helicoverpa armigera	タバコガの近縁種	Hear-cDNA	Zhang et al., 2002 (GenBank #AAM43840)
Helicoverpa assulta	オオタバコガ	Heas-cDNA	Choi et al., 1998
Helicoverpa zea	タバコガの近縁種	Heze-PBAN	Raina et al., 1989
Heliothis virescens	タバコガの近縁種	Hevi-cDNA	Xu et al., 2003
Mamestra brassicae	ヨトウガ	Mabr-cDNA	Jacquin-Joly et al., 1998
Spodoptera littoralis	ハスモンヨトウの近縁種	Spli-cDNA	Iglesias et al., 2002
Adoxophyes honmai	チャノコカクモンハマキ	Adho-cDNA	Choi et al., 2004 (GenBank #AAK72980)

(2) Amino Acid Sequences of SGNPs Determined from PBAN/DH-cDNA

2a) SGNP I

	1				5					10					15					20					25	
Bomo-DH	Thr	Asp	Met	Lys	Asp	~	Glu	Ser	Asp	Arg	Gly	Ala	His	Ser	Glu	Arg	Gly	Ala	Leu	Trp	Phe	Gly	Pro	Arg	Leu	NH2
Boma	*	*	*	*	*	~	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	NH2
Agip	Asn	*	Val	*	*	Gly	Gly	Ala	*	*	*	*	*	*	Asp	*	*	Gly	Met	*	*	*	*	*	Ile	
Hear	Asn	*	Val	*	*	Gly	Gly	Ala	Ala	Ser	*	*	*	*	Asp	*	Leu	Gly	*	*	*	*	*	*	*	
Heas, Heze	Asn	*	Val	*	*	~	Gly	Ala	Ala	Ser	*	*	*	*	Asp	*	Leu	Gly	*	*	*	*	*	*	*	
Hevi	Asn	*	Asp	*	*	~	Gly	Ala	Ala	Ser	*	*	*	*	Asp	*	Leu	Gly	*	*	*	*	*	*	*	
Mabr																		Gly	*	*	*	*	*	*	Ile	
Spli	Asn	Glu	Ile	*	*	Gly	Gly	*	*	*	*	*	*	*	Asp	*	Ala	Gly	*	*	*	*	*	*	*	
Adho	Glu	Asn	Phe	*	Glu	Glu	Asn	Phe	*	*	Asn	Ile	Arg	*	Gly	*	Ala	Asn	Val	Val	*	Lys	*	Ile	*	

2b) SGNP II (α)

	1				5		7
Bomo	Ile	Ile	Phe	Thr	Pro	Lys	Leu
Boma	*	*	*	*	*	*	*
Agip	Val	*	*	*	*	*	*
Hear, Heas, Heze	Val	*	*	*	*	*	*
Hevi	Val	*	*	*	*	*	*
Mabr	Val	*	*	*	*	*	*
Spli	Val	*	*	*	*	*	*
Adho	Val	*	*	*	*	*	*

2c) SGNP III (β)

	1				5					10					15					20	
Bomo	Ser	Val	Ala	Lys	Pro	~	~	~	Gln	Thr	His	Glu	Ser	Leu	Glu	Phe	Ile	Pro	Arg	Leu	
Boma	*	*	*	*	*	~	~	~	~	~	~	*	*	*	*	*	*	*	*	*	
Agip	*	Lue	Ser	Tyr	Glu	Asp	~	~	Lys	Met	Phe	Asp	Asn	Val	*	*	Thr	*	*	*	
Hear, Heas, Heze	*	Lue	*	Tyr	Asp	Asp	~	~	Lys	Ser	Phe	*	Asn	Val	*	*	Thr	*	*	*	
Hevi	*	Lue	Ser	Tyr	Asp	Asp	~	~	Lys	Ser	Phe	*	Asn	Val	*	*	Thr	*	*	*	
Mabr	*	Lue	*	Tyr	Asp	Asp	~	~	Lys	Val	Phe	*	Asn	Val	*	*	Thr	*	*	*	
Psse-PT	Lys	Lue	Ser	Tyr	Asp	Asp	~	~	Lys	Val	Phe	*	Asn	Val	*	*	Thr	*	*	*	NH2
Spli	*	Lue	*	Tyr	Asp	Asp	~	~	Lys	Val	Phe	*	Asn	Val	*	*	Thr	*	*	*	
Adho	*	Met	Glu	Asp	*	Tyr	Glu	Glu	Lys	Arg	Ser	Tyr	Asp	Val	Asp	*	Thr	*	*	*	

2d) SGNP IV (γ)

	1				5			8
Bomo	Thr	Met	Ser	Phe	Ser	Pro	Arg	Leu
Boma	*	*	*	*	*	*	*	*
Hear, Heas, Heze	*	*	Asn	*	*	*	*	*
Hevi	*	*	Asn	*	*	*	*	*
Mabr	*	*	Asn	*	*	*	*	*
Spli	*	*	Asn	*	*	*	*	*
Agip	*	*	Asn	*	*	*	*	*
Adho	*	Val	Lys	Leu	Thr	*	*	*

Bombyx mori	カイコ	Bomo-DH	Imai et al., 1991
		Bomo-cDNA	Kawano et al., 1992; Sato et al.,1993
Bombyx mandarina	クワゴ	Boma-cDNA	Xu et al., 1999
Agrotis ipsilon	タマナヤガ	Agip-cDNA	Duportets et al., 1998; 1999
Helicoverpa armigera	タバコガの近縁種	Hear-cDNA	Zhang et al., 2002 (GenBank #AAM43840)
Helicoverpa assulta	オオタバコガ	Heas-cDNA	Choi et al., 1998
Helicoverpa zea	タバコガの近縁種	Heze-cDNA	Davis et al, 1992; Ma et al., 1994
Heliothis virescens	タバコガの近縁種	Hevi-cDNA	Xu et al., 2003
Mamestra brassicae	ヨトウガ	Mabr-cDNA	Jacquin-Joly et al., 1998
Spodoptera littoralis	ハスモンヨトウの近縁種	Spli-cDNA	Iglesias et al., 2002
Pseudaletia separata	アワヨトウ	Psse-PT	Matsumoto et al., 1992
Adoxophyes honmai	チャノコカクモンハマキ	Adho-cDNA	Choi et al., 2004 (GenBank #AAK72980)

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J. Seric. Sci. Jpn., **68**: 373-379
Xu, W.-H. and D. L. Denlinger, 2003
Molecular characterization of prothoracicotropic hormone and diapause hormone in *Heliothis virescens* during diapause, and a new role for diapause hormon
Insect Molec. Biol., **12**: 509-516

Ⅳ. PBANに関する研究

a) PBANの普遍性

20種以上の蛾類昆虫で存在が示唆

例外) イラクサギンウワバ *Trichoplusia ni*

Tang *et al.*, 1989. *PNAS*, 86: 1806.

b) 生産器官 ①

SG (SOG, suboesophageal ganglion、食道下神経球)

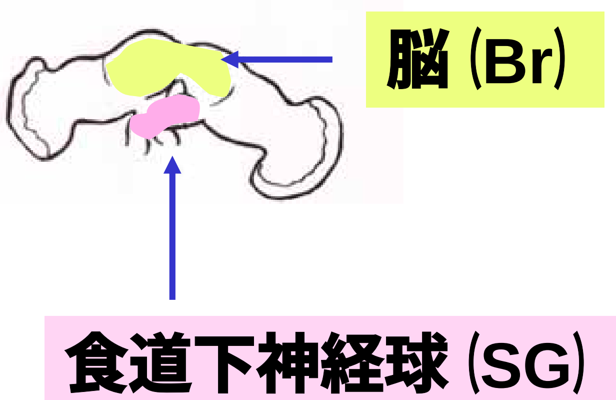
成虫では、脳とSGの分離は難しい

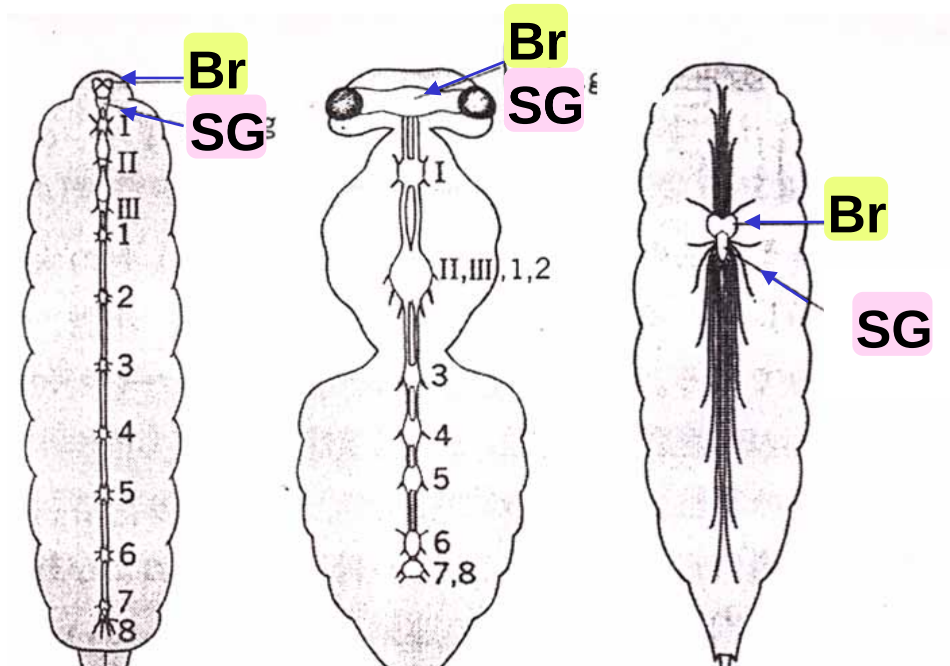
分泌細胞は、immunostain により決定

Kingan *et al.*, 1992. *Cell Tissue Res.*, 270: 229.

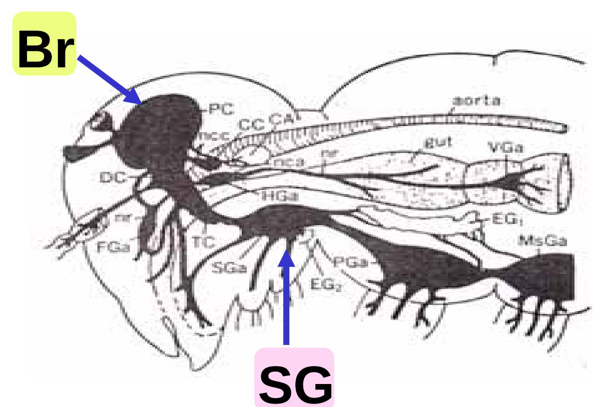
Ma & Roelofs, 1995. *J. Insect Physiol.*, 41: 339.

b) 生産器官 ② 食道下神経球





ガ (幼虫)　　ミツバチ (成虫)　　アブ (幼虫)



c) 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

カイコ: Arima *et al.*, 1991. *Appl. Entomol. Zool.*, 26: 137.
H. armigera: Rafaeli *et al.*,1990. *J. Insect Physiol.*, 36: 641.
S. littoralis: Fabrias *et al.*,1994. *Arch. Insect Biochem. Physiol.*, 27: 77

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. *PNAS*, 86: 2488.

d) ペプチドの活性発現部位

アミド化C-末端５残基 (FXPRL)　　← 活性部位

カイコ

Kuniyoshi *et al.*, 1991. *Peptide Chem.*, 1990: 251..

Kuniyoshi *et al.*, 1992. *Insect Biochem. Molec. Biol.*, 22: 399.

タバコヤガ.

Raina & Kempe, 1990. *Insect Biochem.*, 20: 849.

Raina & Kempe, 1992. *Insect Biochem. Molec. Biol.*, 22: 221.

FXPRLを含む環状ペプチド

アミド化C-末端を含まないが、１/１０の活性を示す

開環すると失活

Nachman et al., 1993. *Biochem. Biophys. Res. Comm.*, 193: 661.

e) レセプターたん白質

Choi *et al.*, 2003. *PNAS*, 100: 9721-9726.
"Identification of a G protein-coupled receptor for pheromone biosynthesis activating neuropeptide from pheromone glands of the moth *Helicoverpa zea*."

Drosophila のGPCRs (３４６アミノ酸残基、neuromedin U receptorと高い相同性) を参考に同定。昆虫細胞で発現させ、PBAN投与後のCaイオンの流入を確認。

neuromedin U： 哺乳動物の神経ペプチド、YFLFRPRNamide

Hull *et al.*, 2004. *J. Biol. Chem.*, in press.
"Cloning and characterization of the pheromone biosynthesis activating neuropeptide receptor from the silkmoth, *Bombyx mori*: significance of the carboxyl terminus in receptor internalization."

膜貫通部はHezPBAN-Rと高い相同性を有するが、C-末側に６７残基伸張し、機能も異なる。

f) PBANの活性化ステップ

Acetyl CoA

↓ ⇐ **PBAN**

PBAN	***A. velutinana***	Tang et al., *Proc. Natl. Acad. Sci. USA*, **86**: 1806 (1989)
	H. armigera	Rafaeli et al., *J. Insect Physiol.*, **36**: 641 (1990)
	H. zea	Jurenka et al., *Arch. Insect Biochem. Physiol.*, **17**: 81 (1991)
	M. brassicae	Jacquin et al., *Insect Biochem. Molec. Biol.*, **24**: 203 (1994)

Saturated Acid

↓ **Unsaturation** ⇐ **PBAN**

PBAN	***C. chalcites***	Altstein et al., *Insect Biochem.*, **19**: 645 (1989)
	M. brassicae	Bestmann et al., *Experientia*, **45**: 778 (1989)

↓ **Chain elongation or shortening**

Unsaturated Acid

↓ **Reduction** ⇐ **PBAN**

PBAN	***S. littoralis***	Martinez et al., *J. Biol. Chem.*, **265**: 1381 (1990)
	B. mori	Arima et al., *Appl. Entomol. Zool.*, **26**: 137 (1991)
		Ozawa et al., *Biosci. Biotech. Biochem.*, **57**: 2144 (1993)
	M. sexta	Fang et al., *Arch. Insect Biochem. Physiol.*, **29**: 35 (1995)
	T. pityocampa	Fabrias et al., *Insect Biochem. Molec. Biol.*, **25**: 655 (1995)

Unsaturated Alcohol

↓

Acetate, Aldehyde

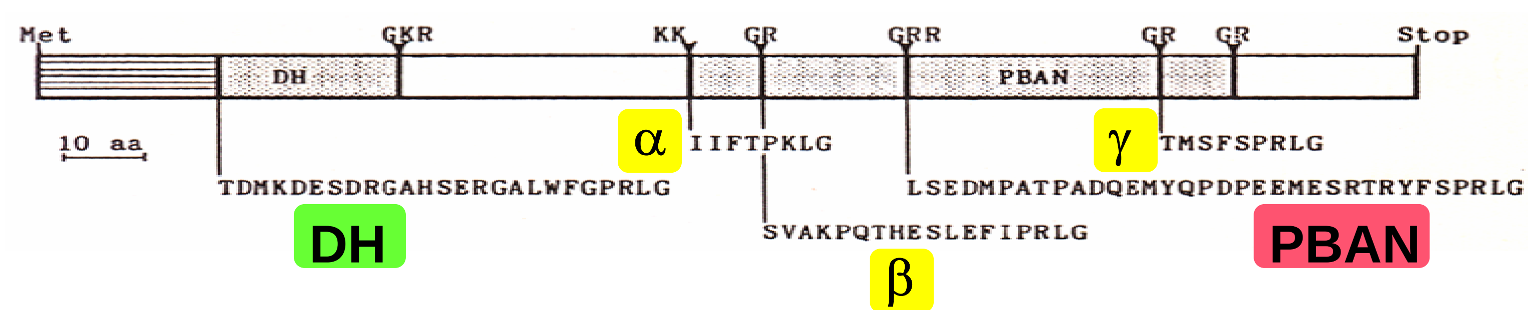
↓ **Emission** ⇐ **PBAN**

PBAN	***T. ni***	Zhao & Haynes, *J. Insect Physiol.*, **43**: 695 (1997)

g) 遺伝子（PBANcDNAの構造）①

カイコ　Kawano *et al*., 1992. *Biochem. Biophys. Res. Comm.*, 189: 221.

```
-40  ATCACTTCGCCCTCCAACCACTGAAGGGACAACAACAAAA 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

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 Lys Ile Ile Phe Thr Pro Lys Leu Gly Arg

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 Glu Phe Ile Pro Arg Leu Gly Arg Arg Leu Ser Glu

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

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

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  CTTGAGGTACCTCATTTGAGGTCTCGATCGACTCCGACGAACGGTTACGGTAAACGGCGACAATGTTAATGTTTTGGACGAAACAATTG
685  TTAATTAATAAATTCATGTGATTTTGTAATTGTAATTTATAAGTGAATAAAAAAATAAACTATTTAAAAT(polyA)
```



g) 遺伝子（PBANcDNAの構造）②

PBANとDHに対応する塩基配列は、同じcDNAにコードされている。さらに、FXPRLの配列を含む3つのペプチド (α, β, γ) が存在する。

カイコ　Kawano *et al*., 1992. *BBRC*, 189: 221.
タバコガ　Davis *et al*., 1992. *PNAS*, 89: 142.

カイコ 192残基を含む前駆体 —消化→ DH (24残基)、α-鎖（7残基）、β-鎖（17残基）、PBAN (33残基)、γ-鎖（8残基）

Mamestra brassicae（ヨトウガ） Jacquin-Joly *et al*.., 1998. *In. Bi. Mol Bi.*, 28: 251.
Helicoverpa assulta（オオタバコガ） Choi *et al*., 1998. *In. Bi. Mol Bi.*, 28: 759.
Agrotis ipsilon（タマナヤガ） Duportets *et al*., 1999. *Peptide*, 20: 899.
Bombyx mandarina（クワゴ） Xu *et al*., 1999. *J. Seric. Sci. Jpn.*, 68: 373.
Spodoptera littoralis Iglesias *et al*., 2002. *In. Bi. Mol Bi.*, 32: 901.
Heliothis virescens Xu & Denlinqer, 2003. *Insect Molec. Biol.*, 12: 509.
Manduca sexta (ﾀﾊﾞｺｽｽﾞﾒｶﾞ) Xu & Denlinqer, 2004. *Peptide*, 25: 1099.
Adoxophyes honmai Choi *et al*., 2004. *In. Bi. Mol Bi.*, 34: 927.
Helicoverpa armigera Zhang *et al*., 2004. *J. Insect Physiol.*, 50: 25.

h) DHとの関係

Bombyx mori ⟹ embryonic diapause の誘導

通常 の家蚕　SGからのDHの分泌 → 休眠卵
多化性品種N_4（非休眠卵を産卵）　DHの注射 → 休眠卵

Heliothis virescens
Xu & Denlinger, 2003. *Insect Mol. Biol*, 12: 509.

12L:12D, 20°C → 非休眠

		% termination (1 week 25 °C)
9L:15D, 18°C → 休眠		
	+ water injection	5 (*n* = 20)
	+ *Hvi*-DH injection	93 (*n* = 30)

⟹ *Hvi*-DHは成虫休眠を打破する

V. 交尾雌でのフェロモン生産の停止

a) PBAN と PSP

PBAN

pheromonotropicなペプチド、33アミノ酸残基、 MW 約4000

カイコガなど6種の蛾類昆虫から同定、80%以上の相同性

活性発現部位はアミド化されたC-末端5残基

⇨ FXPRLアミド

PSP

交尾後のフェロモン量の減少 （タバコヤガ）

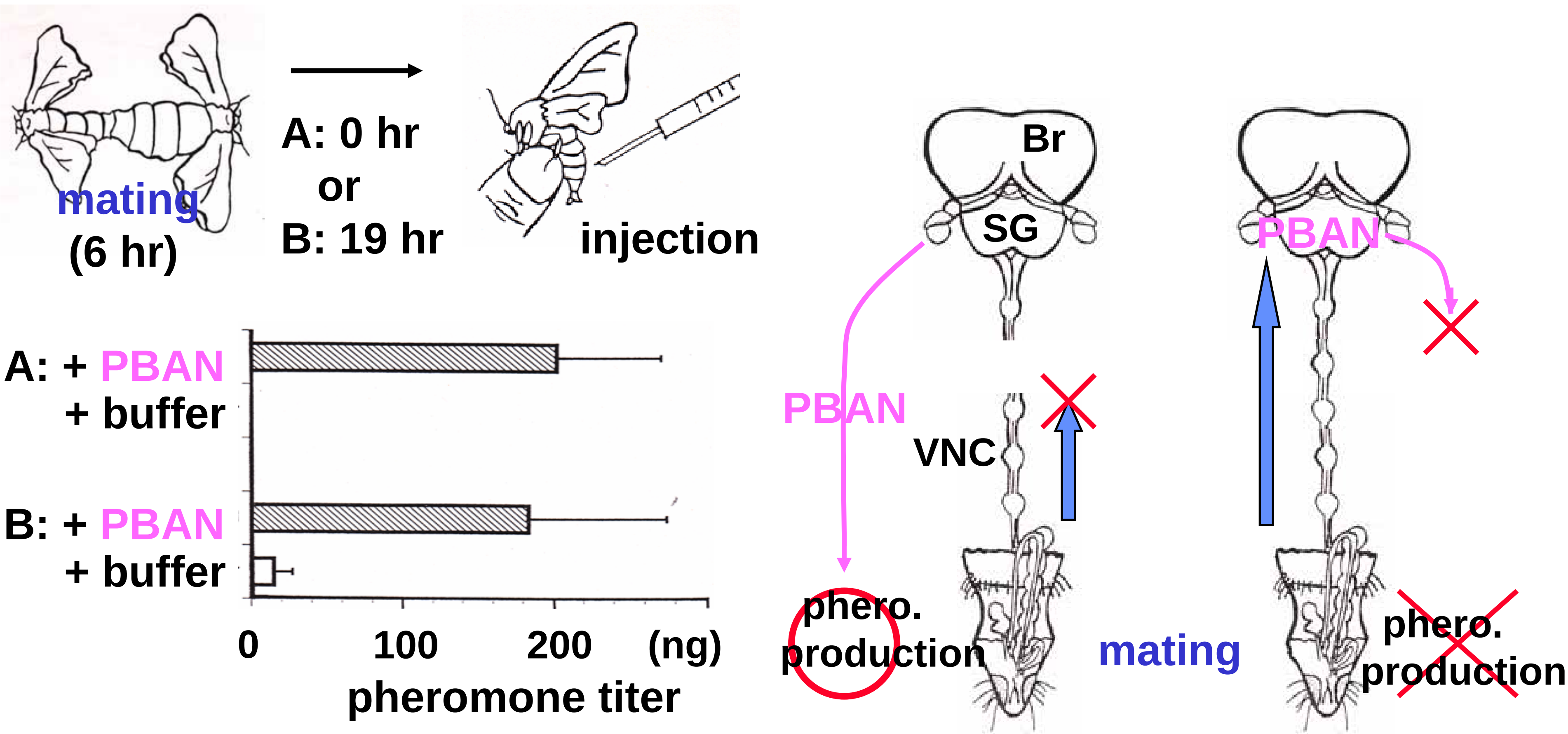
交尾の物理的刺激 → SGからPBANの分泌を抑制

雄からpheromonostatic peptite (PSP)の注入

57アミノ酸

（Kingan *et al.*, 1995. *PNAS*, 92: 5082. ）

b) カイコにおける交尾後のフェロモン消失機構



1. 交尾♀の頭部： phrononotropic活性あり
2. 交尾嚢： pheromonostatic活性なし
3. VNC切断♀の交尾： フェロモンは消失しない

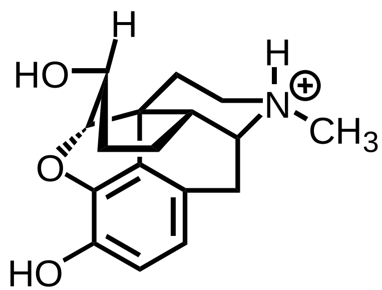
Ando *et al.*, 1996.
Arch. Insect Biochem. Physiol., 31: 207.

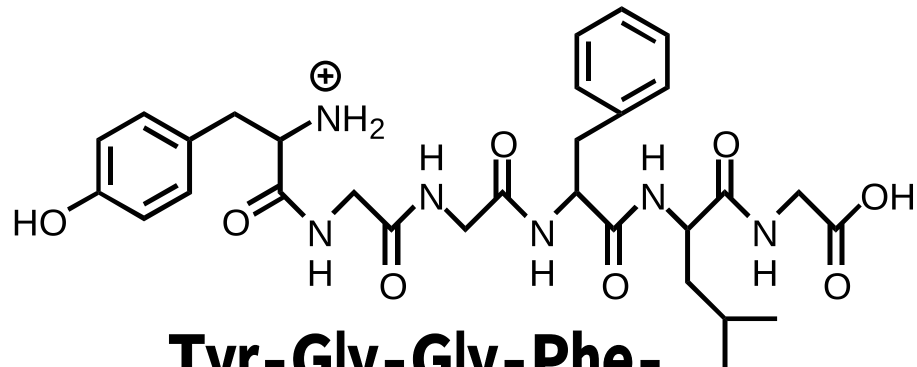
VI.　PBANの害虫防除への利用

ペプチドの活性発現部位：　アミド化C-末端5残基 (FXPRL)

→　低分子化合物での代用

例）　opioid（ morphine と enkephalin ）

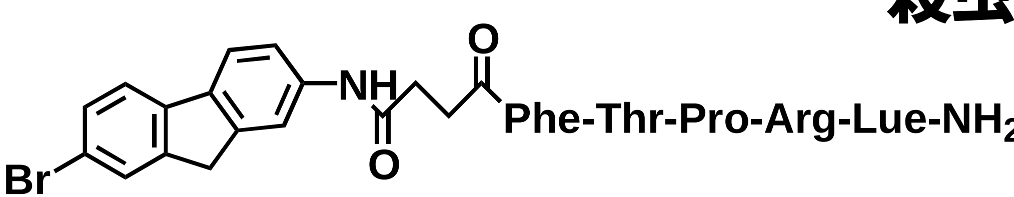




Tyr-Gly-Gly-Phe-

Teal & Nachman, 2002.　*Peptide*, 23: 801

親油性ペプチドの合成 → pheromonotropic
殺虫活性も確認



VII. PBANについての総説

Raina, 1993. *Ann. Rev. Entomol.*, 38: 329.
"Neuroendocrine control of sex pheromone biosynthesis in Lepidoptera."

Rafaeli, 2002. *Int. Rev. Cytol.*, 213: 49.
"Role of neuropeptides in sex pheromone production in moths."

Altstein, 2004. *Peptide*, 25: 1491.
"Role of neuropeptides in sex pheromone production in moths."

Application

Gäde & Goldsworthy, 2003. *Pest Manag. Sci.*, 59: 1063.
"Insect peptide hormones: a selective reiew of their physiology and potential application for pest control."

Altstein, 2003. *J. Mol. Neuroscience*, 22: 147.
"Novel insect control agents based on neuropeptide antagonists."