The dominant expression of AP-AChE gene in the body and similarity in substrate specificity and sensitivity to inhibitors between AO-CxT and AP-CxTS seem to be the reasons why the biochemical properties of AChE mixture of the resistant mosquito [23] resemble those of AP-AChE produced in baculovirus insect cell system. Even if AO-AChE is functioning in the synaps, mutations responsible to the insensitivity for predominant AP-AChE are selected in the population by insecticide control. Considering the fact that resistant insects live normally in the exposure of high concentration of AChE inhibitors which completely suppress the activity of AO-AChE, it is concluded that AP-AChE has a main role of AChE in the synaps of the insect nerve. At last, the function of AO-AChE remains to be elucidated.

Acknowledgement

We thank Dr. DeMar Taylor, Graduate School of Life and Environmental Sciences, University of Tsukuba for critical reading of the manuscript. We thank also Dr. T. Kozaki, Mr. H. Mizuno, Mr. S.-H. Oh and Mr. M. Terada for their assistance in arranging data.

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Figure legends

Fig. 1. Schematic drawing of the active site of *Torpedo* AChE with the substrate, acetylcholine. 3-Dementional positions of amino acids composing catalytic triad (Ser200, Glu327, His440), oxyanion hole (Gly118, Gly119, Ala201), acyl pocket (Trp233, Phe288, Phe290) and anionic binding site (Trp84) are presented. Hydrogen bond (dotted line) and covalent dond (dotted box) among amino acids and the substrate are also presented. An amino acid (Phe331) composing acyl pocket of invertevrate AChE was added in the scheme.

Fig. 2. Alignment of AChE protein sequences of AP-AChE of *M. persicae* (myspe-p) and *Cx. tritaeniorhynchus* (cultr-p), AO-AChE of *Cx. tritaeniorhynchus* (cultr-o), and *D. melanogaster* (drome) and *Torpedo* AChE (torca). 1, 2, 3: cysteine pair forming intra-subunit disulfide bond, a: tryptophan of anionic binding site, d: cysteine forming inter-subunit bond, o: amino acid of oxyanion hole, p: amino acid of acyl pocket, t: amino acid of catalytic triad.

Fig. 3. Susceptibility of AChEs in resistant (Toyama) and susceptible (Taiwan) strains of *Cx. tritaeniorhynchus* to inhibitors. NK-2 – NK-8 are alkylsulfonylphenyl methansulfonates, having ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl and *n*-pentyl, respectively.

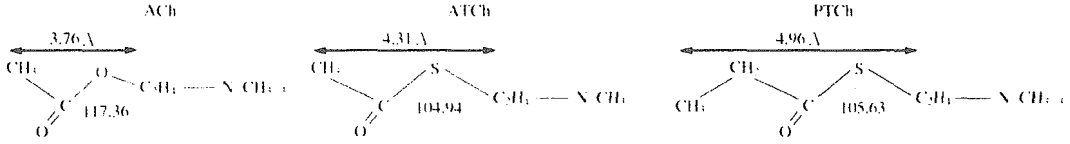
Fig. 4. Relationship between the susceptibility of AChE to alkylsulfonylphenyl methansulfonates and the length of their alky moiety in resistant (Toyama) and susceptible (Taiwan) strains of *Cx. tritaeniorhynchus*. 2 – 8 indicate alkylsulfonylphenyl methansulfonates NK-2 – NK-8, shown in Fig. 3. Parabolic relationships were obtained: $Y=-0.86(X-4.55)^2+3.66$ for resistant strain and $Y=-0.88(X-4.55)^2+5.41$ for susceptible strain.

Fig. 5. Linkage map of *Cx. tritaeniorhynchus* showing loci for AO-AChE (AChE1), AP-AChE (AChE2), and insecticide-insensitivity of AChE ($AChE^R$). Other labels on the chromosome show molecular markers. Map distances are listed in Kosambi centiMorgans.

Fig. 6. Phylogenetic tree of AChE in insects and mites. The scale bar represents 5 percent divergence. p or o shows homology to *Drosophila Ace*-paralogous or -orthologous, respectively. Sequence data are derived from GenBank or publications in the parenthesis. *Aedes aegypti* (p, AJ621915; o, G1245693); *Aedes albopictus* (p and o, unpublished); *Anopheles gambiae* (p, AJ488492; o, AAAB01008846); *Apis mellifera* (p, XP-393751; o, AAG43568); *Bactrocera oleae* (AAM69920); *Bombyx mori* (p and o, unpublished); *Culex pipiens* (p, AJ489456); *Culex tritaeniorhynchus* (p, AB122152; o, AB122151); *Drosophila melanogaster* (X05893); *Heliothis armigera* (AAM90333); *Leptinotarsa decemlineata* (L41180); *Lucilia cuprina* (AAC02779); *Musca domestica* (AJ310134); *Myzus persicae* (p, AY147797; o, AF287291); *Nephotettix cincticeps* (p, unpublished ; o, AF145235); *Pediculus humanus* (p and o, unpublished); *Plutella xylostella* (p, AAV65825; o, AY061975); *Schizaphis graminum* (AF321574); Aphis gossypii (p, AF502082; o, AF502081); *Tetranychus kanzawai* [57]; *Tetranychus urticae* (AY188448); *Torpedo californica* (X56517).

Table 1. *Km* values of AChE in resistant Toyama and susceptible Taiwan strains for various substrates

	Substrate		
	Acetylcholine (ACh)	Acetylthiocholine (ATCh)	Propionylthiocholine (PTCh)
Toyama	4.68×10^{-5}	6.79×10^{-4}	8.08×10^{-3}
Taiwan	7.19×10^{-5}	1.18×10^{-4}	1.01×10^{-3}
Toyama/Taiwan	0.65	5.75	7.77



Structure of substrates. Distance between methyl carbon of acyl moiety and first methylene carbon of choline moiety, and the angle at the oxygen atom or sulfur atom are shown. Structures of the substrates were estimated by the energy minimizing method of BIOSYM.

Table 2. Amino acid substitutions in the active site of AChE and insecticide insensitivity.

Substitution	Species	Insecticide	Insensitivity
AO-AChE			
Acyl pocket			
Phe290Tyr	*D. melanogaster* [15]	paraoxon	80 [62]
(with Phe78Ser, Ile129Val, Gly227Ala)			
	M. domestica [17,18]	fenitroxon	20 [17]
(with Gly227Ala or Val)			
other part			
Ile129Val	*B. oleae* [20]	omethoate	16 [63]
(with Gly396Ser)			
AP-AChE			
Oxyanion hole			
Gly119Ser	*Cx. pipiens* [52]	propoxur	30000 [29]
	An. gambiae [51]		
	An. albimanus [58]	propoxur	1500 [64]
Ala201Ser	*A. gossypii* [42,43,59]	omethoate	150 [34]
(with Ser331Phe)			
Acyl pocket			
Phe290Val	*N. cincticeps* [a]	propoxur	115 [32]
Phe331Trp	*Cx. tritaeniorhynchus* [40]	fenitroxon	2000 [26]
	T. kanzawai [57]	Phenthoateoxon	1000 [28]
Phe331Cys	*T. urticae* [56]	DDVP	1000 [56]
Ser331Phe	*A. gossypii* [42,43]	pirimicarb	650 [37]
	M. persicae [41]	pirimicarb	100 [33]
Other part			
Gly227Ala	*P. xylostella* [54]	prothiophos	26 [54]
(with heterozygous Ala201Ser and Ala441Gly)			

[a]: Terada, unpublished

Table 2, by Kono and Tomita

Table 3. K_m values of *Cx. tritaeniorhynchus* AChEs expressed in baculovirus-insect cultured cell system.

AChE	*Km* (mM)		
	ACh	ATCh	PTCh
AO-CxT	0.036	0.034	0.031
AP-CxTS	0.013	0.036	0.032
AP-CxTI	0.011	0.256	0.342
AP-CxTS/AO-CxT	0.36	1.07	1.04
AP-CxTI/AP-CxTS	0.83	7.06	10.52

AO-CxT, AP-CxTS, and AP-CxTI : AO-AChE, sensitive AP-AChE, and insensitive AP-AChE with Phe455Trp substitution, respectively.

Table 2, by Kono and Tomita

Table 4. I_{50} values for various inhibitors of *Cx. tritaeniorhynchus* AChEs expressed in baculovirus-insect cultured cell system.

AChE	I_{50} (M)				
	Fenitroxon	DDVP	Carbaryl	Eserin	Pirimicarb
AO-CxT	$1.8x10^{-7}$	$9.7x10^{-9}$	$1.3x10^{-7}$	$7.1x10^{-10}$	$8.6x10^{-6}$
AP-CxTS	$1.4x10^{-7}$	$3.1x10^{-7}$	$2.0x10^{-7}$	$9.5x10^{-10}$	$1.1x10^{-5}$
AP-CxTI	$1.8x10^{-3}$	$1.0x10^{-3}$	$3.7x10^{-5}$	$7.1x10^{-7}$	$1.0x10^{-1}$
AP-CxTS/AO-CxT	0.77	31.95	1.53	1.33	1.27
AP-CxTI/AP-CxTS	12860	3225	185	747	9090

AO-CxT, AP-CxTS, and AP-CxTI : AO-AChE, sensitive AP-AChE, and insensitive AP-AChE with Phe455Trp substitution, respectively.

Table 3, by Kono and Tomita

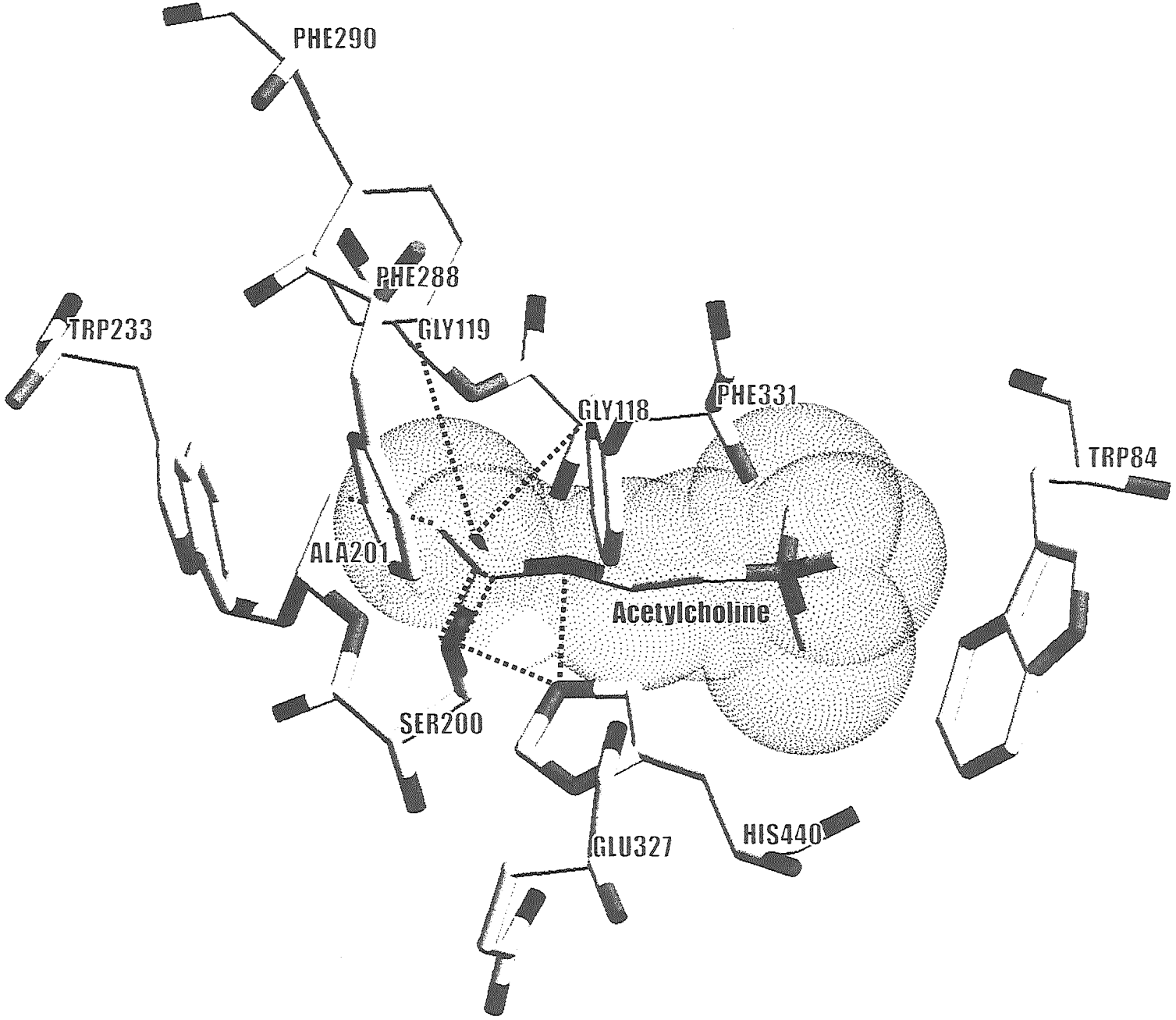


Fig.1 by Kono & Tomita

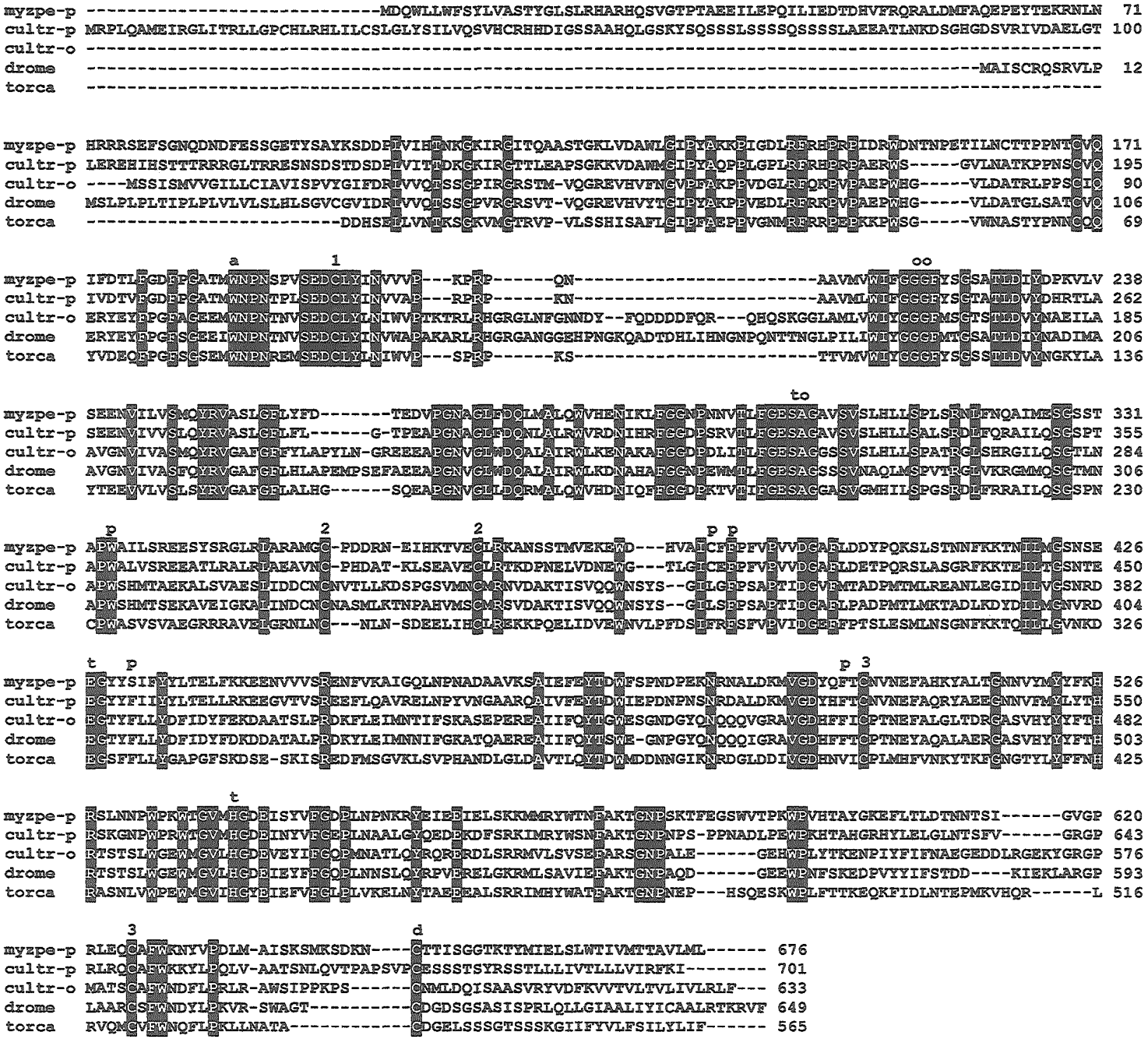


Fig.2 by Kono & Tomita

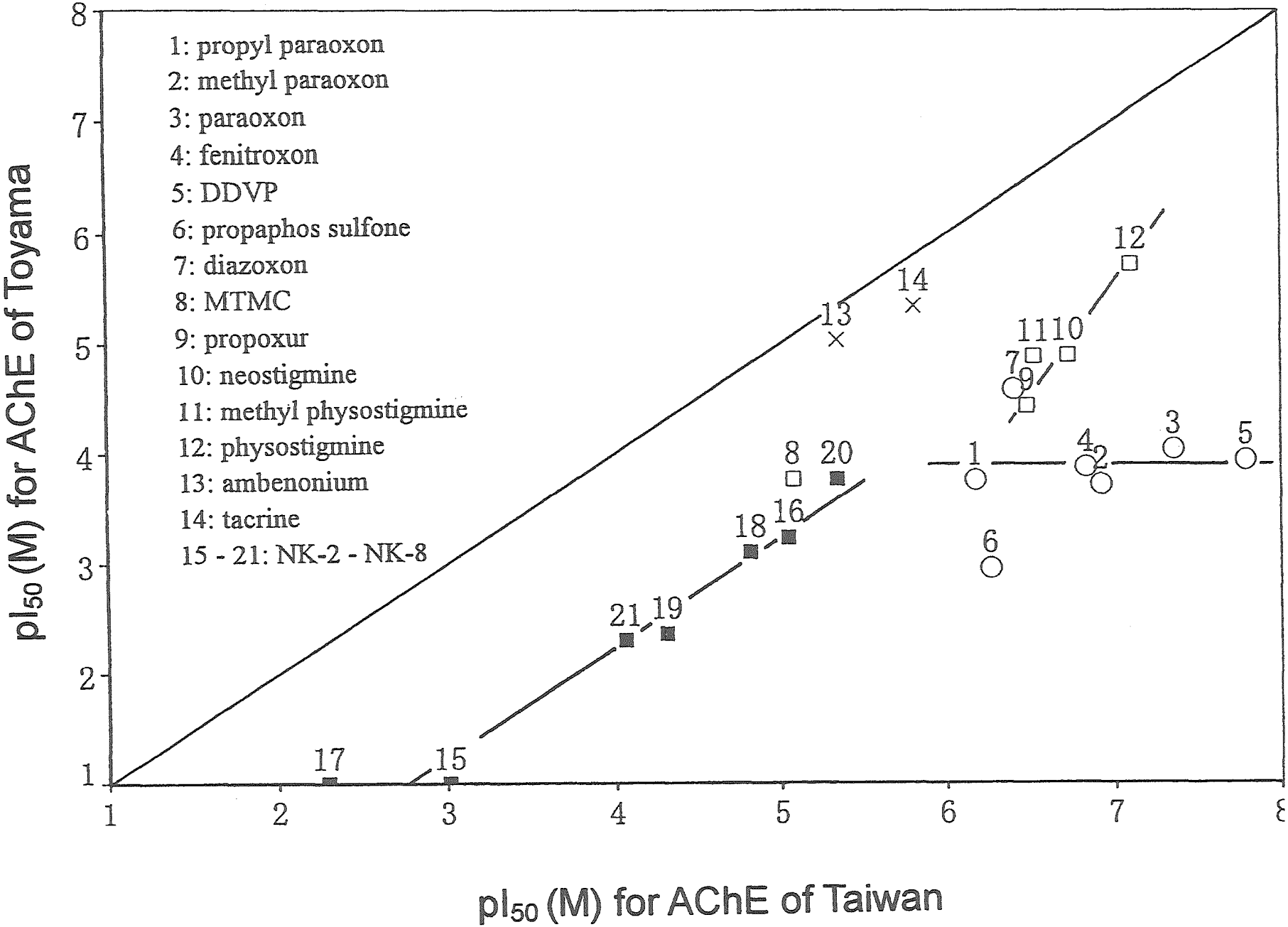


Fig. 3, by Kono and Tomita

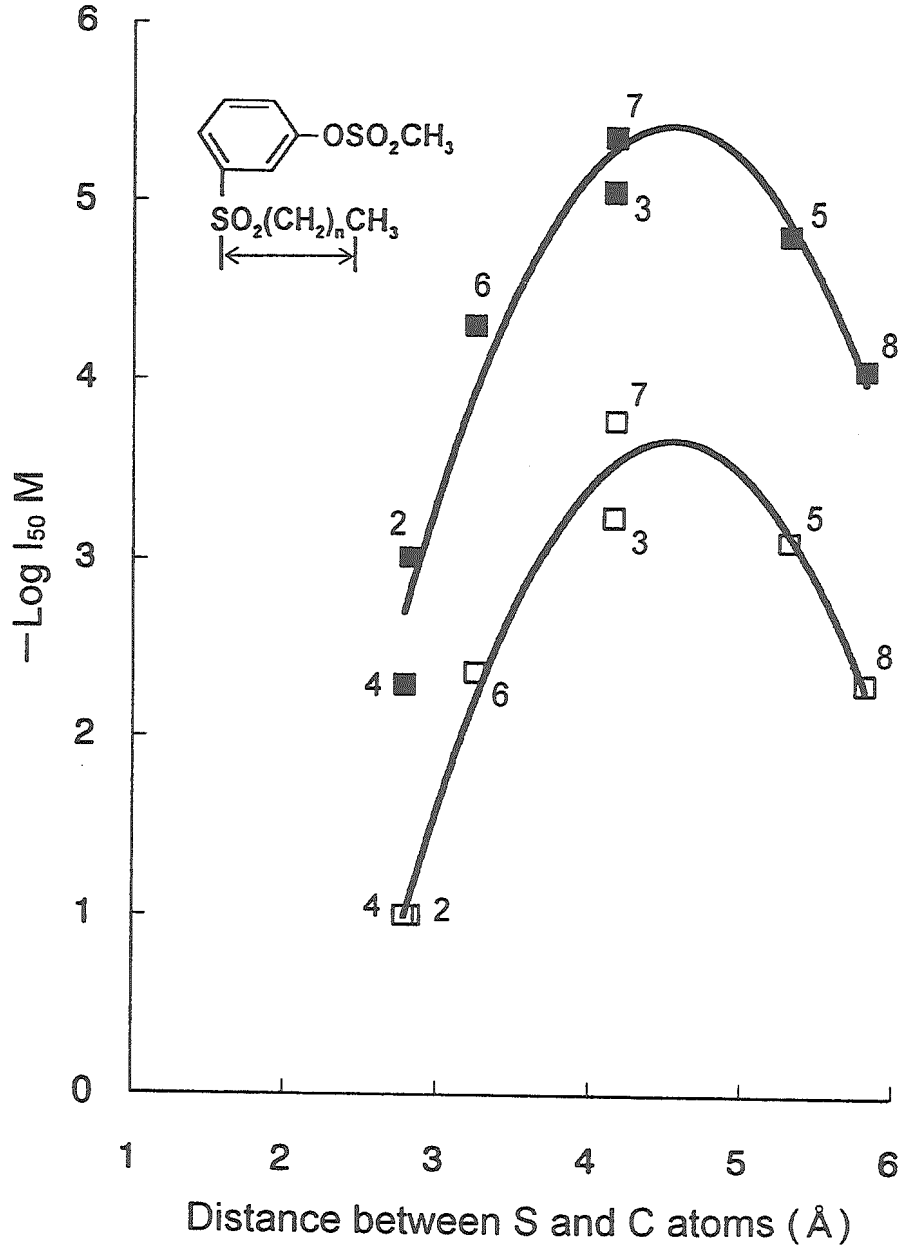


Fig. 4, by Kono and Tomita

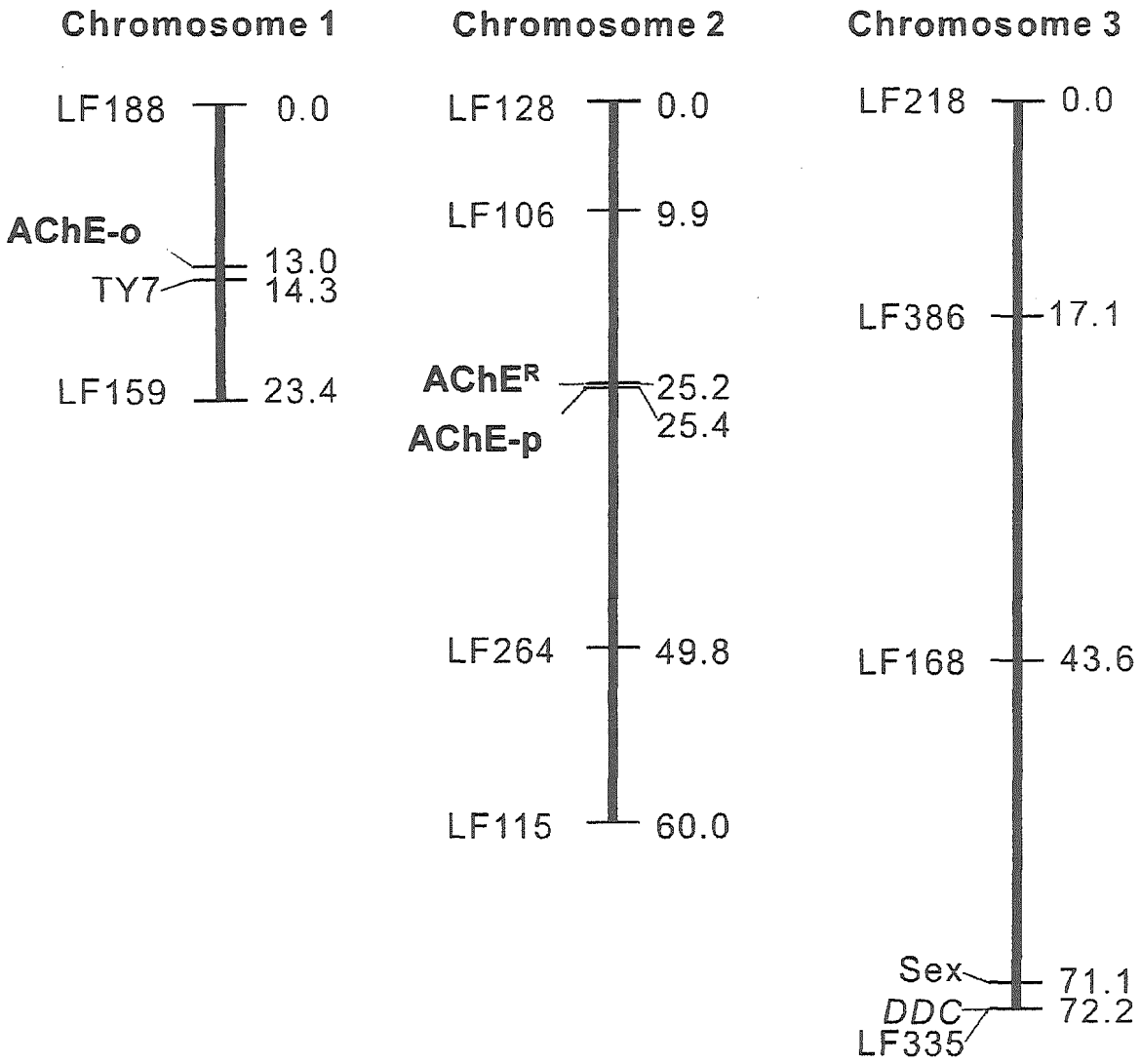


Fig. 5, by Kono and Tomita

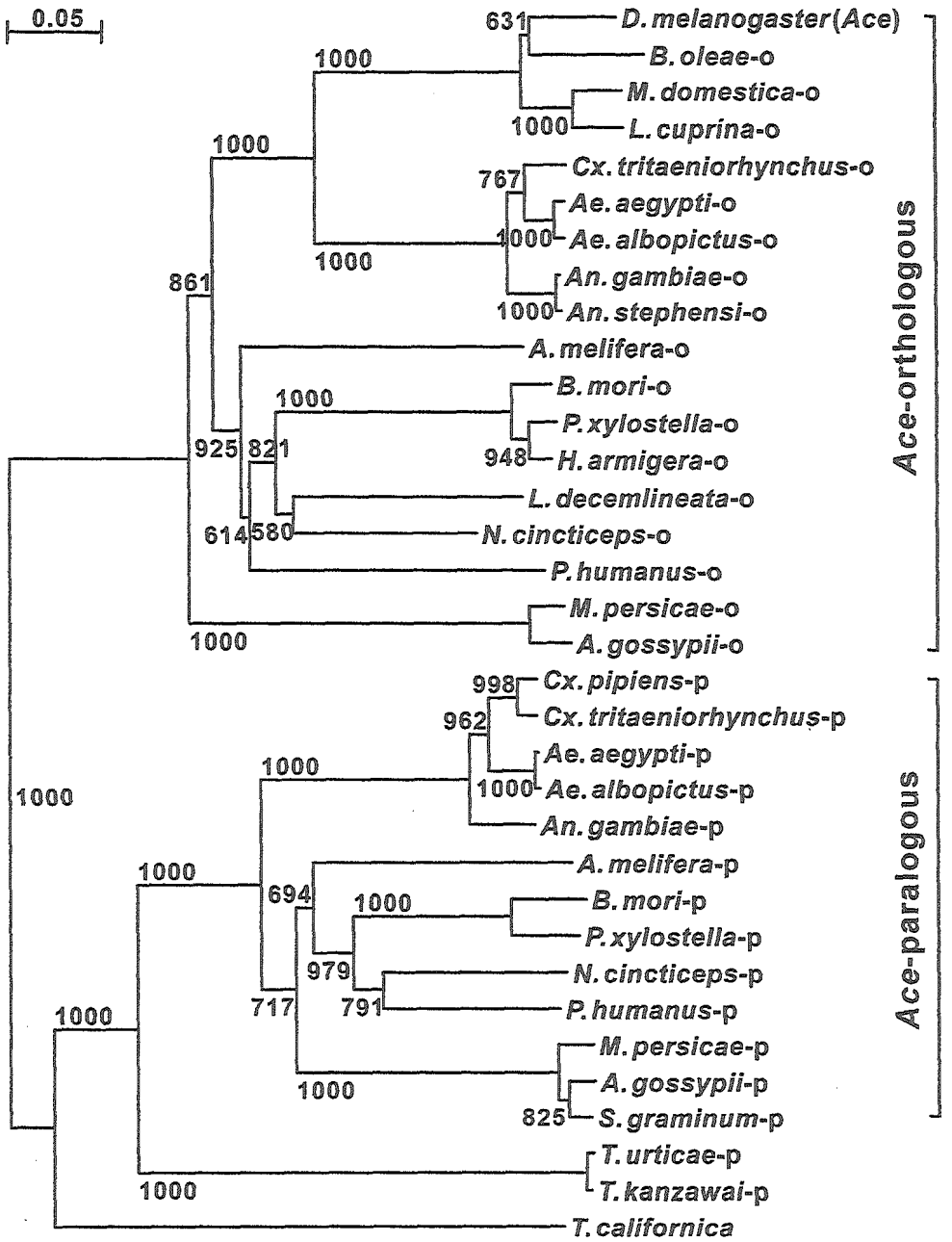


Fig.6 by Kono & Tomita

オオチョウバエの人工汚水を用いた室内飼育

水谷　澄・新庄　五朗・田中　生男
財団法人日本環境衛生センター環境生物部
〒210-0828 川崎市川崎区四谷上町 10-6

A Trial for Laboratory Culturing of a Moth Fly *Clogmia albipunctatus* Williston by Artificial Sewage Water

Kiyoshi MIZUTANI, Goro SHINJO and Ikuo TANAKA
Department of Environmental Biology, Japan Environmental Sanitation Center
10-6, Yotsuyakamicho, Kawasaki-ku, Kawasaki-shi, Kanagawa, 210-0828 Japan

摘要. オオチョウバエの各種飼育法の概要を示した．さらに人工汚水と森谷らが 1969 年に報告したエビオス水溶液による飼育方法を室内観察で比較検討した．

その結果，雌成虫が産卵適期であれば両法共培養液を設置後すぐ産卵した．25℃ の温度下では，設置 52 時間後に一部 1 齢幼虫が孵化したので，卵期間は 2~3 日であった．その後設置 4 日後まで双法の間で幼虫の発育に差は認められなかった．しかしそれ以降は人工汚水区の方がエビオス水溶液区より発育が早く進行した．成虫は両法とも自然個体と同様な大きさの個体が得られた．卵期間は 2~3 日，幼虫期間は人工汚水区が 8~14 日，エビオス水溶液区が 10~15 日，蛹期は双法とも 3~4 日であった．成虫の寿命は比較的短く，通常の湿度環境 (60~80%RH) では 2 週間以内にほとんどが死亡した．

キーワード: オオチョウバエ，室内飼育法，人工汚水

Abstract. 1) The growth of a moth fly *Clogmia albipunctatus* were compared by using two media, an artificial sewage water and a 0.1% dry yeast solution under the conditions of 25℃, 60~80%RH and L12 : D12 in photoperiodism. 2) The artificial sewage water is composed by peptone 0.03w/v%, Ehrligh meat extract 0.02%, $(NH_4)NO_3 \cdot H_2O$ 0.005%, NaCl 0.015%, $Na_2HPO_4 \cdot 12H_2O$ 0.05%, KCl 0.0007%, $CaCl_2$ 0.0007%, $MgSO_4 \cdot 7H_2O$ 0.0005%, laboratory chew 0.05% and H_2O filling up 100 m*l*. 3) Normal and standard sized adults were obtained in both culture solutions. 4) The egg period was 2~3 days, larval stages ranged 8~14 days, pupal period was 3~4 days and adults survived 3 to 18 days by the artificial sewage water but larval period in the dry yeast solution was delayed by 1 to 2 days.

Key words: *Clogmia albipunctatus*, culture solution, artificial sewage water

はじめに

チョウバエは発生場所から想像される汚いイメージによって不快害虫として認識されて久しいが，防除の対象種になっている割には研究報告が少ない．また調査研究のための累代飼育もあまり行われていない．

本研究は厚生労働省科学研究補助金（H15-がん予防-092）による研究の一部として行った．
2004 年 9 月 8 日受付 (Received 8 September 2004)
2004 年 9 月 29 日受理 (Accepted 29 September 2004)

チョウバエの幼虫は浄化槽，汚水だまり，下水溝，排水溝，下水処理場の散水濾床，汚泥など有機物の多い水域に生息する（住環境の害虫獣対策，2002）．この仲間は卵，幼虫，蛹を経て成虫になる完全変態を行う昆虫で60種程が記録されているが，代表種はここに示すオオチョウバエと小型種のホシチョウバエの2種のみである

オオチョウバエは1960年頃から多くなった（森谷ら，1969）害虫で，特に近年ビル環境の地下汚水槽等から発生が目立ち，その対策が要望されている．

著者らは平成13年6月に川崎市内の某事業所からオオチョウバエ幼虫を採取，その後試行錯誤の末現行の方法を確立して累代飼育を行っている．

ここでは飼育法を記述すると共に，エビオス水溶液と人工汚水による飼育を比較検討したので，その結果を報告する．

材料と方法

1．供試昆虫：オオチョウバエ　*Clogmia albipunctatus* Williston

平成13年6月に川崎市内夜光地区の某事業所で採取し，約1年間採取地の汚泥を原培地として水分と粉末飼料で調整しながら累代飼育を行い，その後人工汚水をベースとした飼育に移行，試行錯誤の末，平成15年6月頃より現行の方法を確立して累代飼育している集団でここでは川崎コロニーと呼ぶ．

2．飼育法の概要

これまで報告されている方法と当研究室で行ってきた方法を合わせると，本種の飼育法はおおむね下記に記述した3つに分けられるので，各方法について述べる．なお飼育室は温度25℃±1℃，相対湿度60~80%，明暗12時間の条件であったが，飼育ケージは低湿度になることを避けるため常にビニールシートで覆ったので内部の湿度は90 RH以上となった．

2-1．発生源の汚泥・汚水による方法

幼虫・蛹を含む汚泥・汚水を200 m*l*程度の容器に移し，ケージ内で羽化させる．成虫にはザラメ5%液浸漬綿を与える．時折汚泥に水分と粉末飼料を適量加えおくと，1週間以内に容器内壁の湿った場所に産卵する．3~4日すると若齢幼虫が目視できる．

その後も水分と飼料を調整しておけばケージ内で累代飼育ができる．なお産卵用の培地は汚泥というより汚水でよく，容器は傾斜を付けて設置する方が産卵させるには都合が良い（傾斜角5度）．30×20 cmの飼育バットの例では，深い部分は水深が2 cmになるが浅い部分は底が露出する．

採取汚泥・汚水をポリバットに深さ2 cmほど入れて，布蓋をして20℃以上の室内に傾斜を付けて放置するだけで，少なくとも3~4ヶ月間その中で世代を繰り返し種の維持が可能である．

2-2．エビオス水溶液による方法（森谷ら，1969）

成虫を飼育かごに収容して，容器に入れた標

表1　人工汚水の組成

成　分	構成比 (w/v%)
ペプトン	0.03
肉エキス	0.02
固形飼料	0.05
$(NH_4)NO_3 \cdot H_2O$	0.005
NaCl	0.015
$Na_2HPO_4 \cdot 12H_2O$	0.05
KCl	0.0007
$CaCl_2$	0.0007
$MgSO_4 \cdot 7H_2O$	0.0005
	水を加えて100とする

注）ペプトン・肉エキス・固形飼料の量は適宜調整してよい．

記水溶液に円錐形に折った濾紙を浸漬しておくと湿った内壁に産卵する．これを所定濃度(0.1~0.2%)のエビオス水溶液で飼育する．

2-3. 人工汚水による方法

成虫を飼育かごに収容して，**表1**に示す組成の人工汚水を20 m*l*ほど入れた腰高シャーレ等を傾斜を付けて設置する．産卵と1齢幼虫を確認したら，少量の砕いた固形飼料（幼虫の密度にもよるが通常0.1~0.5 gr程度）を加える．幼虫は脱皮を3回行い2, 3, 4齢幼虫に成長し約10日後に蛹となり，2週後に羽化する．1ケージ当たり5~6個以上の飼育容器（腰高シャーレなど）を使用すれば，定期的に1区20匹，20区（400匹）程度の終令幼虫を確保することができる．

3. オオチョウバエの飼育例—人工汚水とエビオス水溶液による比較

ビニールシートで覆った30 cm角の金網ケージ内で蛹を羽化させる．羽化成虫が約100匹となった時点で蛹を除去し，3日後に人工汚水とエビオス水溶液(0.1%)各20 m*l*宛入れた腰高シャーレを2日間設置して回収，その後の生育状況を観察した．

なお容器により産卵数に著しい差が生じたので，多数卵区と少数卵区は除いて孵化幼虫が近似した数（100匹前後）であった各2区で比較した．

人工汚水区は孵化2~3日後に固形飼料片約10 mgを加えた．これはエビオス水溶液区の栄養素量と一致させるためである．

結果と考察

25℃の温度下で実施した飼育例の結果を**表2**に示した．

人工汚水区ならびにエビオス水区のいずれも，雌成虫が産卵適期であれば設置後すぐ産卵することが確認された．この飼育例でも2日間の設置期間中に産卵が認められた．

人工汚水区とエビオス水区とも設置52時間後に一部の1齢幼虫が孵化したので，卵期間は2~3日であると思われる．人工汚水区とエビオス水区の設置4日後までの幼虫の成長に著しい差は認められなかった．しかし6日後に，人工汚水区は齢がそろって順調な成長が得られたが，エビオス区では幼虫の成長に若干ばらつきが認められた．設置10日後には，人工汚水区で一部蛹が観察されたが，エビオス水区では12日後に蛹化が始まった．羽化開始も人工汚水区の方が2日早く設置12日後であった．なお羽化のピークは人工汚水区は設置後14~15日後であったが，エビオス水区では16日以降にずれ込んだが，いずれの試験区も標準的な成虫が認められた．

成虫の寿命は人工汚水区で主に観察したが，

表2 オオチョウバエの飼育例(25℃)

経過日数	人工汚水	エビオス水溶液(0.1%)
0	設置	設置
0~2	産卵	産卵
2	1部1齢幼虫	同左
3	1齢幼虫	同左
4	1~2齢	同左
6	2~3齢	1~3齢
8	2~4齢	同左
10	3~4齢　1部蛹	2~4齢
12	3~4齢　蛹　羽化開始	2~4齢　蛹
14	4齢　蛹　羽化多数	3~4齢　蛹　羽化
15~18	4齢　蛹多数　羽化多数	3~4齢　蛹　羽化

表3 飼育結果から得た各期所用日数

期間令期	人工汚水区日数	エビオス水溶液区日数
卵	2~3	2~3
幼虫	8~14	10~15
1齢	2~3	3
2齢	2~3	3
3齢	3	3
4齢	3~4	3~4
蛹	3~4	3~4
成虫*	3~18*	観察せず

* 相対湿度60~80%

3日後に死亡虫が見られ，7日後に50%強が，2週間後には大部分が致死した(60~80%RH).

表3は今回行った飼育結果で得られた各期所用日数を示したものである.

人工汚水区とエビオス水区を比較すると，両区の間で産卵嗜好や孵化日数に特記する差は認められなかった．幼虫期の生育速度は，後者より前者の方が優れており，かつ齢のそろった成長を示した．この結果が蛹化や羽化に至る日数に2日ほどの差をつけた.

この差は両飼育溶液区の栄養素量の安定性と質的な違いによるものと思われる.

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