Summary

Several acyl phosphorates were prepared and their biological activities were tested. These acyl phosphorates showed a little higher activity towards insect than Dipterex and the toxicities of these acyl phosphorates towards mice were also higher than that of Dipterex.

Acknowledgement

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An Attempt to Reduce and Increase Insecticide-Resistance in *D. melanogaster* by Selection Pressure. Genetical and Biochemical Studies on Negatively Correlated Cross-Resistance in *Drosophila melanogaster* I. Zenichi Ogita (Department of Genetics, Faculty of Medicine, Osaka University, Osaka, Japan). Received Nov. 22, 1960. *Botyu-Kagaku*, **26**, 7, 1961. (in English)

3. Selection pressure によって殺虫剤抵抗性を減少させ増加させる試み キイロショウジョウバエにおける negatively correlated cross-resistance の遺伝生化学的研究 I.

荻田善一 (大阪大学医学部 遺伝学教室) 35. 11. 22 受理

キイロショウジョウバエの第Ⅱ染色体上65±附近にある優性の DDT 抵抗性遺伝子の存在は，BHC, parathion および phenylurea (PU) に対して交叉抵抗性 (cross-resistance) を与えるが，phenylthiourea (PTU) に対しては逆に異常な非抵抗性 (逆相関交叉抵抗性 negatively correlated cross-resistance) をもたらす．また第Ⅲ染色体上 50 ±附近にある硫酸ニコチン抵抗性の遺伝子の存在は，これらの薬剤に対して交叉抵抗性をもたらすことをすでに報告した．したがって，PTU-selection pressure によっては DDT, BHC, parathion に対して非抵抗性でニコチンに対して抵抗性の系統をもたらし，PU-selection pressure はこれらの薬剤に対して抵抗性の系統をもたらすことを暗示した．実際，DDT 抵抗性と非抵抗性の系統からなる混合 population に PTU-selection pressure を働かせることによって DDT 抵抗性遺伝子を追い出し，生き残った系統は DDT, BHC, parathion 非抵抗性で硫酸ニコチン抵抗性であった．また PU-selection pressure より生き残った系統には，DDT, BHC, parathion 抵抗性と硫酸ニコチン抵抗性を同時にもたらすことを証明した．

The cross-resistance pattern of *Drosophila melanogaster* to DDT, BHC, and parathion is negatively correlated with its resistance to phenylthiourea(PTU), but is positively correlated with phenylurea(PU)-resistance. As a result of the genetical analyses in previous papers, the following working hypothesis was introduced by the author: "The dominant PTU-susceptibility and dominant PU-resistance may be a pleiotropic expression of the dominant gene for resistance to DDT, BHC and parathion at locus *65*± on the 2nd chromosome; and the dominant factor for PTU- and PU-resistance on the 3rd chromosome is a pleiotropic expression of the dominant gene for resistance to nicotine sulfate at locus *50*±.
The present investigation has aimed to test the hypothesis by means of applying selection pressure with PTU and PU to synthetic populations consisting of several strains resistant to DDT, BHC and parathion, and strains susceptible to those insecticides.

Introduction

In previous papers[11,12], the author reported that the resistance to DDT, BHC, parathion and PU (phenylurea) in *D. melanogaster* was negatively correlated with resistance to PTU (phenylthiourea).

Examples of "negatively-correlated substances" are found in DDT-resistant houseflies for a crude preparation of diisopropyl tetrachloroethylphosphate by Mitlin et al.[10], and for potassium bromide and cetyl bromoacetate by Ascher[1,2,3,4], although these workers have not carried out genetical analyses.

A genetical analysis of this interesting phenomenon would seem to be important in working towards some solution of the insecticide-resistance problem. As a result of the genetical analyses in previous papers, the following hypothesis was introduced by the author: A dominant gene at *II-65±* which confers resistance to DDT, BHC and parathion, also confers resistance to PU and abnormal susceptibility to PTU, while another dominant gene at *III-50±* which confers resistance to nicotine sulfate, also confers resistance to PTU as well as to PU. Thus, resistance to PTU and PU may be due to a polygenic system which simultaneously requires two main genes on the 2nd and 3rd chromosomes respectively.

Accordingly, it is assumed that the dominant PTU-susceptibility and PU-resistance associated with locus *II-65±* is a result of the pleiotropic expression of the dominant gene for resistance to DDT, BHC and parathion; while the PTU, PU-resistance associated with the 3rd chromosome results from the pleiotropic expression of the dominant gene for resistance to nicotine sulfate at locus *III-50±*.

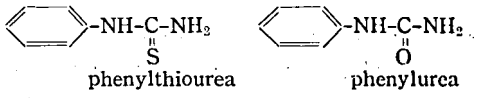
If this hypothesis is correct, selection pressure with PTU should eliminate the dominant allele for resistance to DDT, BHC and parathion on the 2nd chromosome, and thus reduce insecticide resistance in the surviving flies; on the other hand, selection pressure with PU should increase resistance in the surviving flies. Meanwhile selection pressure from PTU or PU should produce a strain of flies resistant to nicotine sulfate.

The present investigation has aimed to test this hypothesis by means of applying selection pressure with PTU and PU to *D. melanogaster*.

Materials and Methods

I. Chemicals

The samples of PTU and PU employed in the present study were synthesized and purified by the author. PTU and PU have the following chemical constitutions:



phenylthiourea　　phenylurea

Stock solutions of 10 mM PTU-, and 100 mM PU-solutions were prepared and were diluted to the appropriate millimolar solutions with distilled water.

II. Media

The constituents of the medium for culturing flies were as follows:

Agar	2g
Dry yeast powder	3g
Sugar	4g
Distilled water	100 ml

Lots of 15 ml of the media were poured into small glass vials of 60 ml capacity. PTU and PU-media were made with the appropriate millimolar solutions substituted for water.

III. Biological materials

The strains of *D. melanogaster* kept in the laboratory of Osaka University were used.

1. Wild strains

Hikone-R$_{31}$: derived by a single pair mating from the *Hikone-R* strain which is resistant not only to DDT, but also to various insecticides such as BHC, parathion, nicotine sulfate etc.

IIL2-Q: a DDT-resistant strain which was obtained from Dr. J. Bennett in the U. S. A.

WMB$_{30}$ and *WMD*$_{7-28}$: developed by Tsukamoto in the laboratory from a mixed population by rearing them on a medium containing DDT. The strain is resistant not only to DDT, but also to BHC, parathion and nicotine sulfate.

KSL: a highly parathion-resistant strain which was selected by Dr. B. Rasmuson in Sweden. This strain is also highly resistant to DDT and BHC, but moderately resistant to nicotine sulfate.

Canton-S: an unusually DDT-susceptible strain,

but moderately resistant to nicotine sulfate.

+;+; HR_3 : a DDT-susceptible, but nicotine sulfate-resistant strain with the 3rd chromosome of the *Hikone-R* strain, and with the 1st and the 2nd chromosomes of the *Canton-S* strain.

2. Mutant strains

cn bw : a DDT-susceptible mutant strain in which the 2nd chromosome is marked with the visible mutants *cn* (cinnabar) and *bw* (brown) respectively. This strain has a moderate tolerance to nicotine sulfate.

cn R bw ; HR_3 : strain with an originally susceptible background but with a part of the 2nd chromosome and the entire 3rd chromosome derived from the *Hikone-R* strain. This strain is resistant to DDT, BHC, parathion and nicotine-sulfate.

bw ; *st ss* : a multichromosomal strain, susceptible to DDT and nicotine sulfate, in which the 2nd and 3rd chromosomes are marked with some recessive visible mutants. The *st* (scarlet) and *ss* (spineless) genes are located respectively on the left and right arms of the 3rd chromosome.

bw ; *st* HR_3 *ss* : a multichromosomal nicotine sulfate-resistant mutant strain in which the region of the spindle-fibre attachment between *st* and *ss* genes on the 3rd chromosome was derived from the *Hikone-R* strain. The strain is resistant to nicotine sulfate and susceptible to DDT, BHC and parathion.

IV. Methods

The methods of determining resistance and of genetical analysis are the same as those described in the previous papers as the "larval test" method ; that is, first-instar larvae were picked up by the tip of a needle from agar plates on which eggs were laid and were transferred to normal and insecticide-containing media ; then the number of flies that emerged from these media was counted.

Adult flies with wings extended normally were counted to establish the rate of emergence, but flies which could not extend their wings and died immediately after emergence were not counted as survivors. The numbers of flies that emerged from these media were then compared with each other. In this "larval test", 50 or 100 larvae were put into each vial of treated or of untreated medium.

The effective concentration of the medium is given in millimoles per liter. All tests were performed at a temperature approximating 25°C.

Experimental Results

I. Tests for resistance to various chemicals

Levels of resistance to DDT, BHC, parathion, nicotine sulfate, PU and PTU were estimated by means of the "larval test" method in several insecticide-resistant and susceptible strains of *D. melanogaster*. Some of the results are shown in Fig. 1.

Tsukamoto and Ogaki[16,17], and Kikkawa[7], and Tsukamoto, Ogaki and Kikkawa[18] have concluded that DDT-, BHC-, and parathion-resistance is mainly controlled by a single dominant gene (*II-65*±) located on the 2nd chromosome, and Tsukamoto[15], and Tsukamoto and Hiroyoshi[19] have concluded that nicotinesulfate resistance is mainly controlled by a single dominant gene (*III-50*±) located on the 3rd chromosome in *D. melanogaster*. Thus the level of DDT-resistance refers mainly to the 2nd chromosome and the level of nicotine sulfate-resistance refers mainly to the 3rd chromosome ; this point will be discussed later.

The Canton-S strain for example is unusually susceptible to DDT, BHC and parathion and lacks the DDT-resistant allele on the 2nd chromosome ; it is however somewhat resistant to nicotine sulfate and moderately resistant to PTU.

The +; +; HR_3 strain, which contains the 3rd chromosome of the *Hikone-R* strain and the background of the *Canton-S* strain, is almost as DDT-susceptible as the *Canton-S*, although the resistant level of the +; +; HR_3 is somewhat higher than that of the *Canton-S*. The resistance to nicotine sulfate in this strain is somewhat lower than that of the *Hikone-R* strain. Thus there is no resistance to PU, although the *II-65*± allele has high resistance to PTU.

The *bw*; *st* HR_3 *ss* strain, which contains the 3rd chromosome derived from the *Hikone-R* strain on the DDT- and nicotine sulfate-susceptible background of the *bw*; *st ss* strain, is resistant to PTU, but not resistant to PU.

PTU-resistance is not found in such strains as *Hikone-*R_{31}, *cn R bw* ; HR_3, *HL2-Q*, WMB_{30},

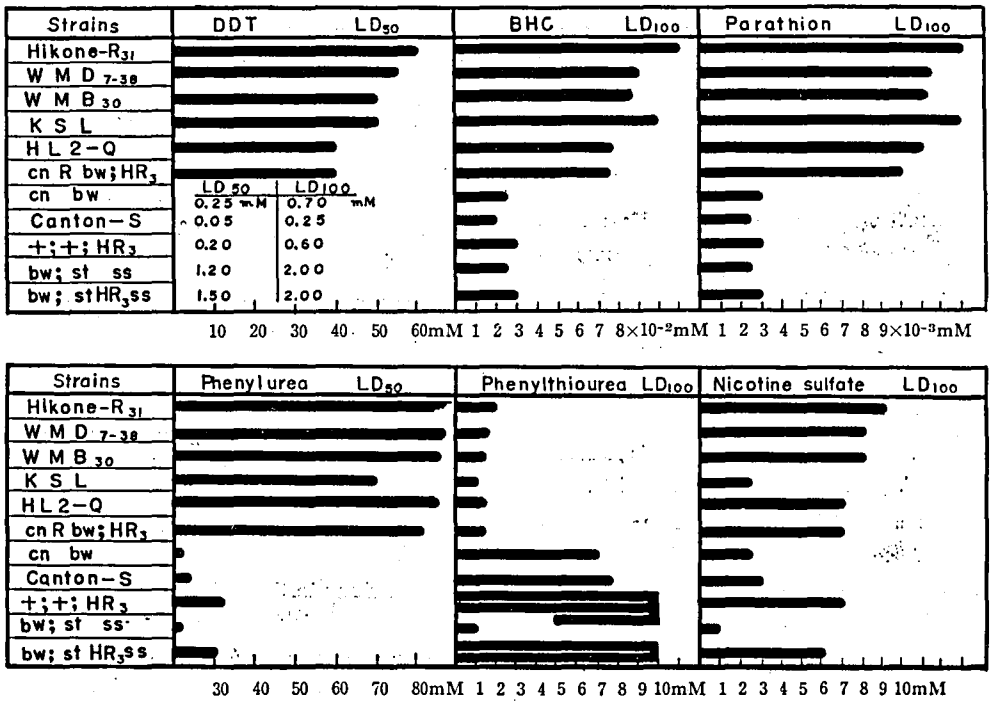


Fig. 1. Level of resistance to DDT, BHC, parathion, nicotine sulfate, phenylurea and phenylthiourea in the strains of *D. melanogaster*.

mM concentrations of various insecticides show dose which brought LD_{50} or LD_{100} to strains of *D. melanogaster*. 500 larvae were used by the "larval test" method.

WMD$_{7-38}$ and *KSL* which have the highly DDT-resistant allele (*II-65*±) on the 2nd chromosome.

On the other hand, PU-resistance is found in such strains as *Hikone-R*$_{31}$, *cn R bw*; *HR*$_3$, *WMB*$_{30}$ and *WMD*$_{7-38}$ which have both the DDT-resistant gene and the nicotine sulfate-resistant gene.

The *KSL* strain, which lacks the highly nicotine sulfate-resistant gene on the 3rd chromosome but possesses the DDT-resistant gene on the 2nd chromosome, shows a level of PU-resistance which is somewhat lower than the *Hikone-R*$_{31}$ strain.

The results reported in Fig. 1 accordingly support the hypothesis, advanced in previous papers, that PTU-resistance is found in strains which have no DDT-resistant gene on the 2nd chromosome but possess a nicotine sulfate-resistant gene on the 3rd chromosome, but that PTU-resistance is not found at all in strains which possess both these resistant gene alleles. Meanwhile on the other hand, PU-resistance is highly developed in strains which have both the DDT-resistant and the nicotine sulfate-resistant genes, as in the *Hikone-R* strains, but is entirely absent in strains which have only one of these two resistant genes.

These results indicate that all PTU-resistant strains are susceptible to DDT, BHC, parathion, and resistant to nicotine sulfate, while the PTU-susceptible strains are divided into three types, one being resistant to DDT, the second resistant to both DDT and nicotine sulfate, and the third susceptible to both DDT and nicotine-sulfate.

All of PU-resistant strains are resistant to both DDT and nicotine sulfate, while PU-susceptible strains are divided into three types, the first susceptible to both DDT and nicotine sulfate, and the other two susceptible to one or other of the insecticide groups.

Thus all the strains used in the present study belong to one of four types with respect to cross-resistance characteristics, as shown in Table 1.

Table 1. Types of insecticide resistance in *D. melanogaster*.

Type	gene combination	BHC, parathion and DDT	nicotine sulfate	PTU	PU	example
A	*II-s_{DDT} ; III-s_{NS}*	S	S	S	S	*bw ; st ss* *bw ; st ; sv^n*
B	*II-s_{DDT} ; III-R_{NS}*	S	R	R	S	*+ ; + ; HR_3* *bw ; st HR_3 ss* *bw ; HR_3* *bw ;III NSR*
C	*II-R_{DDT} ; III-s_{NS}*	R	S	S	S	*HR_2 ; bw ; st ss* *HR_2 ; st ss*
D	*II-R_{DDT} ; III-R_{NS}*	R	R	S	R	*Hikone-R_{31}* *WMB_{30}, WMD_{7-38}* *cn R bw ; HR_3* *cn R vg bw ; HR_3*

The following abbreviations are used ;

II-R_{DDT} : DDT-resistant gene on the 2nd chromosome
III-R_{NS} : nicotine sulfate-resistant gene on the 3rd chromosome
II-s_{DDT} : DDT-susceptible gene
III-s_{NS} : nicotine sulfate susceptible gene
S : susceptible
R : resistant
PTU : phenylthiourea
PU : phenylurea

Each one was submitted to selection pressure with either PTU or PU.

II. Change of the resistance level to various insecticides by selection pressure with PTU or PU

In order to determine whether selection pressure with PTU or PU brought about any change of the resistance level to DDT, BHC, parathion or nicotine sulfate, the following backcrosses were carried out with the PTU-susceptible strains (D type) such as *Hikone-R_{31}*, *HL2-Q*, *WMB_{30}*, *WMD_{7-38}* and *KSL* (DDT- and nicotine sulfate-resistant strains), the PTU-resistant strain (B type) such as *bw;st HR_3 ss* (DDT-susceptible, nicotine sulfate-resistant strain) and the PTU-susceptible strain (A type) such as *bw; st ss* (DDT- and nicotine sulfate-susceptible strain).

Backcross

(1) *bw ; st ss* ♀ × F_1(*bw ; st ss* ♀ × *Hikone-R_{31}*♂)♂
(2) *bw ; st ss* ♀ × F_1(*bw ; st ss* ♀ × *KSL*♂)♂
(3) *bw ; st ss* ♀ × F_1(*bw ; st ss* ♀ × *HL2-Q*♂)♂
(4) *bw ; st ss* ♀ × F_1(*bw ; st ss* ♀ × *WMB_{30}*♂)♂
(5) *bw ; st ss* ♀ × F_1(*bw ; st ss* ♀ × *WMD_{7-38}*♂)♂
(6) *bw ; st HR_3 ss* ♀ × F_1(*bw ; st HR_3 ss* ♀ × *Hikone-R_{31}*♂)♂
(7) *bw ; st HR_3 ss* ♀ × F_1(*bw ; st HR_3 ss* ♀ × *WMD_{7-38}*♂)♂

One hundred first-instar F_2 larvae resulting from each of these backcrosses were put into a 60 cc glass vial containing 15 cc of dry yeast medium with or without chemicals.

The results are shown in Tables 2—6.

The effects of the selection pressure of these chemicals may be determined by comparing the phenotypes of surviving flies that emerged from treated media with the expected proportion of 4 phenotypes that emerged from untreated media.

The *bw ; ++* flies resulting from backcrosses 1 to 5 and, the *bw ; ++* and *bw ; st ss* flies obtained from backcrosses 6, 7 (i. e. the *bw ; st ss* flies are genotypically *bw ; st HR_3 ss*, the original strain of B type), are homozygous for the 2nd chromosome lacking the DDT-resistant allele, and are either heterozygous or homozygous for the 3rd chromosome with the nicotine sulfate-resistant gene. These flies were able to emerge from the media containing more than 3 mM PTU, but the flies of other phenotypes could not. Thus, the 2nd chromosome which carries the DDT-resistant gene is completely eliminated by selection pressure with PTU.

On the other hand, the + ; ++, and + ; *st ss*, (i. e. $\frac{+ \quad bw}{R_{DDT} \quad +}$; $\frac{st\ HR_3\ ss}{st\ HR_3\ ss}$ in crosses 6, 7) flies which carry the 2nd chromosome with the

Table 2. The percentage of phenotypes of surviving progeny in backcross (1). *bw* ; *st ss*♀×F_1 (*bw* ; *st ss*♀×*Hikone-R_{31}*♂)♂ in treated media.
(500 F_2-larvae were used for test by the larval test method)

	Phenotype		*bw* ; *st ss*	*bw* ; + +	+ ; *st ss*	+ ; + +
Chemical	DDT	10mM	0	0	83.9	99.5
		20mM	0	0	39.9	71.6
		30mM	0	0	29.6	68.5
	BHC	3×10^{-2}mM	0	0	55.0	79.5
	Parathion	1×10^{-2}mM	0	0	17.8	39.5
	Nicotine sulfate	1.5mM	0	96.0	55.0	99.2
		2.0mM	0	85.4	0	95.5
		3.0mM	0	27.8	0	33.0
	PTU	3mM	0	90.5	0	0
		5mM	0	86.5	0	0
		10mM	0	53.9	0	0
		20mM	0	18.3	0	0
		30mM	0	3.9	0	0
	PU	50mM	0	14.2	2.5	85.0
		70mM	0	0	0	85.8

Table 3. The percentage of phenotypes of surviving progeny in backcross (2). *bw* ; *st ss*♀×F_1 (*bw* ; *st ss*♀×*KSL*♂)♂ in treated media.
(500 F_2-larvae were used for test by the larval test method)

	Phenotype		*bw* ; *st ss*	*bw* ; + +	+ ; *st ss*	+ ; + +
Chemical	DDT	10mM	0	0	85.0	92.9
		20mM	0	0	37.2	74.2
		30mM	0	0	25.6	58.6
	BHC	3×10^{-2}mM	0	0	56.2	84.3
	Parathion	1×10^{-2}mM	0	0	24.0	40.6
	Nicotine sulfate	1.5mM	0	43.1	25.6	94.5
		2.0mM	0	16.4	1.6	45.3
		3.0mM	0	0	0	0
	PTU	3mM	0	25.0	0	0
		5mM	0	4.3	0	0
		10mM	0	0	0	0
	PU	50mM	0	0	0	74.2
		70mM	0	0	0	60.9

Table 4. The percentage of phenotypes of surviving progeny in backcross (3). *bw* ; *st ss*♀×F_1 (*bw* ; *st ss*♀×*HL2-Q*♂)♂ in treated media.
(500 F_2-larvae were used for test by the larval test method)

	Phenotype		*bw* ; *st ss*	*bw* ; + +	+ ; *st ss*	+ ; + +
Chemical	DDT	10mM	0	0	91.4	97.0
		20mM	0	0	52.3	76.9
		30mM	0	0	42.9	60.0
	BHC	3×10^{-2}mM	0	0	52.4	50.4
	Parathion	1×10^{-2}mM	0	0	12.3	31.1
	Nicotine sulfate	1.5mM	0	82.4	32.3	80.7
		2.0mM	0	63.8	0	72.2
		3.0mM	0	10.0	0	15.6
	PTU	3mM	0	48.7	0	0
		5mM	0	24.4	0	0
		10mM	0	6.7	0	0
		20mM	0	0	0	0
	PU	50mM	0	5.0	0	72.6
		70mM	0	0	0	51.1

Table 5. The percentage of phenotypes of surviving progeny in backcross (4) *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*WMB*$_{30}$♂)♂, and backcross (5) *bw* ; *st ss*♀ ×F_1 (*bw* ; *st ss*♀ ×*WMD*$_{7-38}$♂)♂, which emerged from PTU- and PU-treated media. (500 F_2-larvae were used for test by the larval test method)

Backcross		4		5	
Sort of medium		3mM PTU	70mM PU	3mM PTU	70mM PU
Phenotype	*bw* ; *st ss*	0	0	0	0
	bw ; + +	48.1	0	46.8	0
	+ ; *st ss*	0	0	0	0
	+ ; + +	0	74.4	0	74.8

Table 6. The percentage of phenotypes of surviving progeny in backcross (6) *bw* ; *st* HR_3 *ss*♀ ×F_1(*bw* ; *st* HR_3 *ss*♀ ×*Hikone-R*$_{31}$♂)♂, backcross (7) *bw* ; *st* HR_3 *ss*♀ ×F_1(*bw* ; *st* HR_3 *ss*♀ ×*WMD*$_{7-38}$♂)♂, which emerged from PTU-and PU-treated media.
(500 F_2-larvae were used for test by the larval test method)

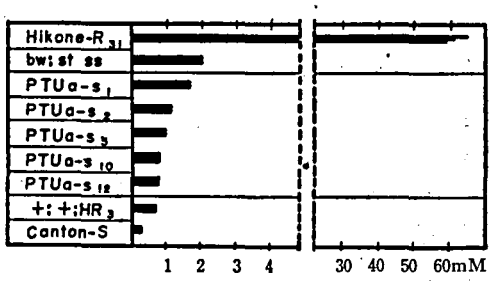
Backcross		6		7	
Sort of medium		3mM PTU	70mM PU	3mM PTU	70mM PU
Genotype	$\frac{+\ bw}{+\ bw}$; $\frac{st\ HR_3\ ss}{st\ HR_3\ ss}$	96.0	0	102.6	0
	$\frac{+\ bw}{+\ bw}$; $\frac{st\ HR_3\ ss}{+\ R_{NS}\ +}$	101.6	0	96.9	0
	$\frac{+\ bw}{R_{DDT}\ +}$; $\frac{st\ HR_3\ ss}{st\ HR_3\ ss}$	0	81.7	0	74.0
	$\frac{+\ bw}{R_{DDT}\ +}$; $\frac{st\ HR_3\ ss}{+\ R_{NS}\ +}$	0	81.4	0	77.6

DDT-resistant gene in a heterozygous condition, and the 3rd chromosome with the nicotine sulfate-resistant gene in a heterozygous or a homozygous condition, could not emerge from media containing more than 3 mM PTU, whereas they could emerge from media containing 70 mM PU.

Therefore, the effect of selection pressure with PU is the same as selection pressure with the mixture of DDT, BHC or parathion with nicotine sulfate and it can therefore assemble the DDT-resistant gene on the 2nd chromosome together with the nicotine-resistant gene on the 3rd chromosome.

The surviving flies from the selection pressure with PTU and PU were also tested for their resistance to DDT, BHC, parathion, nicotine-sulfate, PU and PTU. The results are shown in Figs. 2 and 3.

With regard to PTU selection pressure, the



mM concentration of DDT which brought mortality of 100 per cent to larvae

Fig. 2. The change of the resistant level to DDT in the surviving flies from backcross (1), *bw* ; *st ss* ♀ ×F_1 (*bw*;*st ss*♀ ×*Hikone-R*$_{31}$♂)♂ by PTU-selection pressure.

PTU a-S$_1$; Surviving flies from one time of 10mM PTU-selection pressure of flies which emerged from media containing 5mM PTU. Numbers show times of 10mM PTU-selection pressure.
500-larvae were used by the "larval test" method.

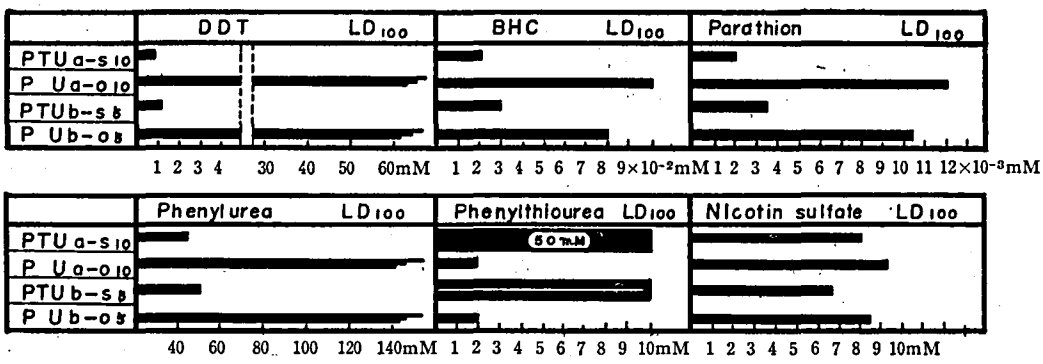


Fig. 3. The resistance level to various insecticides of surviving flies from media containing PTU and PU. mM concentrations of various insecticides show dose which brought LD_{100} to larvae.

PTU a-S_{10} ; Surviving flies from *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*Hikone-*R_{31}♂)♂ by 10mM PTU-selection pressure of 10 times.
PTU b-S_5 ; Surviving flies from F_1(*cn R bw* ; HR_3♀ ×+ ; + ; HR_3♂)♀ ×*cn R bw* ; HR_3♂ by 5mM PTU-selection pressure of 5 times.
PU a-O_{10} ; Surviving flies from *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*Hikone-*R_{31}♂)♂ by 100mM PU-selection pressure of 10 times.
PU b-O_5 ; Surviving flies from F_1(*cn bw*♀ ×*Hikone-*R_{31}♂)♀ ×*cn bw*♂ by 100mM PU-selection pressure of 5 times.
500-larvae were used by the "larval test" method.

bw; ++flies which emerged from the media containing 5 mM PTU in backcross (1) were selected with 10 mM PTU once more. The surviving flies from this additional PTU pressure were tested for their resistance to DDT, BHC, parathion, nicotine sulfate, PU and PTU. It may be seen from Fig. 2 that the DDT-susceptibility of the *bw*; ++ flies was increased more than that of the *bw* ; *st ss* strain by the selection pressure with PTU.

However, even with 10 mM PTU, the DDT-susceptibility of the *bw* ; ++ flies was not as great as that of the very susceptible *Canton-S* strain, but approximated the level of the + ; + ; HR_3 strain. The resistance to nicotine sulfate of these surviving flies was also as high as that of the + ; + ; HR_3 strains.

In a second test, repeated selection pressure with PU was applied ; + ; ++ flies that had emerged from the media containing 70 mM PU in cross (1) were selected with 100 mM PU once more. The surviving flies from this additional PU pressure proved to approach the *Hikone-R* strains in their resistance to DDT, BHC, parathion and nicotine sulfate, and their susceptibility to PTU.

From these results, as shown in Fig. 3, it may be concluded that PTU-pressure could restore DDT-, BHC, parathion- and PU-susceptibility in the surviving flies, while PU-pressure could restore DDT-, BHC- and parathion-resistance in the surviving flies ; pressures from both PTU and PU brought about nicotine sulfate-resistant flies.

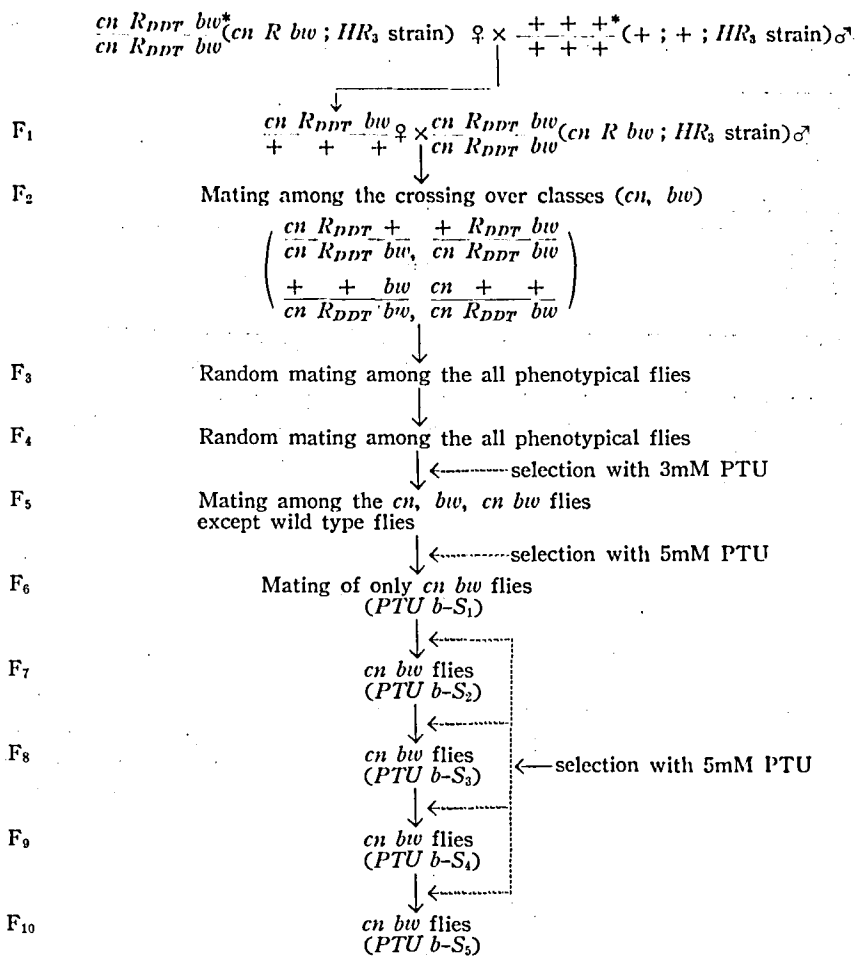
III. Elimination of the DDT-resistant allele on the 2nd chromosome

The 2nd chromosome having a DDT-resistant gene was eliminated by the PTU-selection pressure, and this restored DDT-susceptibility in the surviving flies.

Besides, elimination of the DDT-resistant allele on the 2nd chromosome was tried by using selection pressure with PTU and recombination simultaneously.

For this purpose, backcrosses as shown in Fig. 4 were made with the DDT-resistant *cn R bw*; HR_3 strain, and the DDT-susceptible and nicotine sulfate-resistant + ; + ; HR_3 strain. These strains have the 3rd chromosome from the *Hikone-R* strain, with the nicotine sulfate-resistant gene on it. Therefore, PTU-selection pressure operates only on the 2nd chromosome.

In these crossing procedures, 20 F_1 wild females and 20 *cn R bw* ; HR_3 male flies were put into glass vials with untreated dry yeast media. Owing to



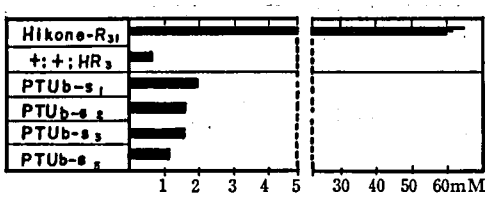
* shows gene combination on the 2nd chromosome.

Fig. 4. Elimination of DDT-resistant gene (*II 65±* locus)

the crossing-over which would occur in the F_1 females, 4 F_2 phenotypes would be produced ; that is, wild type and *cn bw* (non-crossover classes) and *cn* and *bw* (crossover classes containing 4 genotypes, i. e. $\frac{cn\ +\ +}{cn\ R_{DDT}\ bw}$, $\frac{cn\ R_{DDT}\ +}{cn\ R_{DDT}\ bw}$, $\frac{+\ +\ bw}{cn\ R_{DDT}\ bw}$, $\frac{+\ R_{DDT}\ bw}{cn\ R_{DDT}\ bw}$). The *cn* and *bw* flies in the cross over were crossed freely in the media containing 3 mM PTU. *cn*, *bw* and wild flies in a homozygous condition for the particular PTU-resistant gene, which originated from the PTU-resistant strain + ; + ; HR_3, would be able to emerge from the media containing 3 mM PTU. *cn* and *bw* flies selected with PTU-pressure were used for continuous selection in the medium containing 5 mM PTU. *cn bw* flies that emerged from this medium were tested for their resistance to DDT, nicotine sulfate, PU and PTU. The results are shown in Fig. 3, and Fig. 5.

It may be seen in Fig. 5 that the highly DDT-resistant gene was eliminated from the 2nd chromosome by this treatment, and thus it may be concluded that PTU-susceptibility may be a result of the pleiotropic expression of the dominant gene for resistance to DDT on the 2nd chromosome.

It may be due to the effect of the nicotine sulfate-resistant gene on the 3rd chromosome that the lower DDT-resistant factor on the 2nd chromosome could not be eliminated by several selections with 5mM PTU.



mM concentration of DDT brought mortality of 100 per cent to larvae.

Fig. 5. The change of resistant level to DDT in the surviving flies from backcross F_1(*cn R bw* ; HR_3♀ ×+ ; + ; HR_3♂)♀ ×*cn R bw* ; HR_3♂ by PTU-selection pressure.

PTU b-S_1 ; Surviving flies from one time of 5mM PTU-selection pressure of flies which emerged from media containing 3mM PTU. Numbers show times of 5mM PTU selection pressure.

500-larvae were used by the "larval test" method.

With regard to PU-selection pressure, backcrosses as shown in Fig. 4 were carried out with the DDT-susceptible, nicotine sulfate-resistant flies, the *cn bw* strain obtained from PTU-selection pressure, and the DDT- and nicotine sulfate-resistant *Hikone-*R_{31} strain.

It is seen in Fig. 3 that the DDT-resistant gene has been recovered in the 2nd chromosome of the *cn bw* flies and thus it may be concluded that the PU-resistance may be a result of the pleiotropic expression of the dominant gene for resistance to DDT on the 2nd chromosome.

Discussion and Conclusions

In previous papers the author has advanced the hypothesis that the dominant gene (*II-65*±) for resistance to DDT, BHC, parathion on the 2nd chromosome can be eliminated by PTU-selection pressure, but that the nicotine-resistant gene on the 3rd chromosome cannot be eliminated. Therefore, if the nicotine sulfate-resistant gene or other genes which cannot be eliminated by PTU-selection pressure have no connection with DDT-, BHC, and parathion-tolerance, an insecticide-susceptibility as low as that of the unusually susceptible *Canton-S* strain should be attainable by means of selection pressure with PTU.

However, the level of DDT-susceptibility restored by one or several selections with 10 mM PTU was not as great as that of the *Canton-S* strain, but approximated that of the + ; + ; HR_3 strain.

The conclusions of other workers on the genetics of DDT-, BHC- and parathion-resistance in *Drosophila* are not in agreement. Tsukamoto and Ogaki[16] have concluded that DDT-resistance in *D. melanogaster* is controlled by a single dominant gene (*II-66*±) located on the 2nd chromosome, while Crow[5,6] and King[8,9] have maintained that many dominant and recessive factors located on almost all chromosomes control DDT-resistance. Oshima[13,14] has also concluded that DDT-resistance is due to a polygenic system and that the effect of the dominant resistant gene on the 2nd chromosome of the highly resistant *Hikone-R* strain is markedly higher than the effect of the gene on the 3rd chromosome.

The author's genetical analyses, shown in Table 2, 3, and 4, agree with Oshima's conclusion ; that is, DDT-resistance and nicotine sulfate-resistance are due to a polygenic system involving two factors on the 2nd and 3rd chromosomes at least.

Thus, the factor on the 2nd chromosome may be regarded as the main gene for DDT-resistance and to act as a modifier for nicotine sulfate-resistance. The factor on the 3rd chromosome may be regarded as the main gene for nicotine sulfate-resistance and to act as a modifier for the DDT-resistance.

Hence, flies having no DDT-resistance factor on the 2nd chromosome cannot show resistance to DDT in high degree, even if they have the DDT-tolerance gene on the 3rd chromosome. This is exemplified in the level of DDT-resistance of the + ; + ; HR_3 strain, which has the 3rd chromosome derived from the *Hikone-R* strain against the background of the unusually DDT-susceptible *Canton-S* strain ; the DDT-resistance level of this + ; + ; HR_3 strain is not very much higher than that of the very susceptible *Canton-S* strain.

This implies that the presence of the dominant allele on the 3rd chromosome is almost powerless in itself to cause the expression of a higher resistance to DDT. Nevertheless, it is able to raise slightly the DDT-tolerance of flies that were homozygous for the wild type DDT-susceptible allele on chromosome II. It is persistence of the 3rd chromosome allele which prevents PTU selection

pressure from decreasing the DDT-resistance to a level as low as that of the *Canton-S* strain.

As shown in Fig. 2, 3 and 5, the flies which survived the PTU selection pressure had become susceptible to PU, BHC and parathion, as well as to DDT, while the flies which survived the PU selection pressure had developed an increased resistance to DDT, BHC, and parathion, but a reduced resistance to PTU.

The flies which survived a few selections with PU had the resistant gene to DDT and PU in heterozygous condition, the PU-resistant gene being dominant. Therefore their offspring would be expected to contain both DDT-resistant and PTU-resistant (DDT-susceptible) flies.

Flies homozygous for PTU-susceptibility can be obtained by continuous selection pressure with PU. It has also been demonstrated that flies homozygous for the DDT-resistant gene on the 2nd chromosome are developed by PU selection pressure. The proportion of flies in the population that are homozygous for the DDT-susceptible gene would be determined from the survival percentage to PTU.

The flies which survived selection pressure from either PTU or PU showed nicotine sulfate-resistance. However, the level was higher with PU selection pressure than with PTU-selection pressure. Whereas PU selection pressure accumulates alleles for DDT-resistance as well, PTU-selection pressure eliminates the DDT-resistance alleles on the 2nd chromosome. Therefore it is possible that the DDT-resistant allele has effect of enhancing nicotine-resistance, and it is also possible that nicotine sulfate-resistance is a polygenic character controlled by a main resistant factor on the 3rd chromosome and a modifier on the 2nd chromosome which may be the same as the DDT-resistant gene. From the results noted above, it may be assumed that PTU-susceptibility is a pleiotropic expression of the dominant gene for resistance to DDT, BHC and parathion on the 2nd chromosome.

This assumption suggests that the admixture with DDT, BHC, or parathion of a substance negatively correlated to these compounds, such as PTU, might avoid the development of resistance to these insecticides even after their prolonged usage.

The possibility of a new type of insecticide formulation composed of such an admixture has already been described in previous papers. Further experiments on dosage levels suitable from the stand point of population genetics are now in progress.

Summary

Selection pressure with PTU were applied to synthetic populations consisting of several strains resistant to DDT, BHC and parathion, and strains susceptible to these insecticides. The highly DDT-resistant gene on the 2nd chromosome which showed cross-resistance to BHC and parathion was eliminated in only one generation, but the DDT-susceptibility of surviving flies was not so low as that of the *Canton-S* strain (which had extraordinary susceptibility). Continuous selection pressure with PTU progressively eliminated the lower DDT-resistant gene allele on the 2nd chromosome and thus restored DDT-susceptibility to the surviving flies, although this susceptibility did not approach to that of the *Canton-S* strain.

Selection pressure with PU restored DDT-resistance in these strains. Selection pressure with either PTU or PU increased the resistance of the strains to nicotine sulfate.

These results support the hypothesis advanced in previous papers that the dominant PTU-susceptibility and the dominant PU-resistance may be a pleiotropic expression of the dominant gene for resistance to DDT, BHC and parathion at locus *65*± on the 2nd chromosome ; and that the dominant factor for PTU- and PU-resistance on the 3rd chromosome is a pleiotropic expression of the dominant gene for resistance to nicotine sulfate at locus *50*±.

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Relationship between the Structure of Compounds and Negatively Correlated Activity. Genetical and Biochemical Studies on Negatively Correlated Cross-Resistance in *Drosophila melanogaster*. II.

Zenichi Ogita (Department of Genetics, Faculty of Medicine, Osaka University, Osaka). Received Jan. 20, 1961. *Botyu-Kagaku*, **26**, 18, 1961.

4. 化学構造と negatively correlated activity との関係 キイロショウジョウバエにおける negatively correlated cross-resistance の遺伝生化学的研究. 荻田善一（大阪大学医学部 遺伝学教室）36. 1. 20 受理

DDT, BHC, parathion に対して逆相関交叉抵抗性 (negatively correlated cross-resistance) を示す phenylthiourea (PTU) と，その対応酸素化合物である phenylurea (PU) の化学構造を種々の置換基でかえることによって PTU のもっている negatively correlated activity (N. C. activity) や PU のもっている positively correlated activity (P. C. activity) がどのように変化するかを遺伝学的解析によってしらべた．その結果，化学構造とこれらの activity との間の関係を示すことができた．

すなわち，alkylthiourea や thiourea は，いずれも毒性は arylthiourea 誘導体よりもはるかに高いけれども，N. C. activity を示さない．また，urea は毒性が殆んどなく P. C. activity も示さなかった．そして arylthiourea 誘導体の中で PTU に含まれるベンゼン核のパラ位置の水素のハロゲン置換体のみが PTU よりも N. C. activity 及び毒性もともに高い．これと同様に arylurea 誘導体の中で PU に含まれるベンゼン核のパラ位置の水素のハロゲン置換体のみが PU よりも P. C. activity 及び毒性もともに高い．しかし，オルソ位置の水素の塩素による置換体は PTU や PU の毒性を高めなかった．その外，他の基による置換体は毒性が低下し N. C. activity や P. C. activity を失なう．更に PTU や *p*-chlorophenylthiourea に含まれる thioureido 基のメチル基による N-置換体や S-置換体は毒性の低下とともに N. C. activity をも減少させる．したがって，PTU が N. C. activity を示すためには構造中に含まれるベンゼン核，そのパラ位置の水素の置換基および thioureido 基が大きな役割をはたしていると結論された．

The cross-resistance pattern to DDT, BHC and parathion observed in *Drosophila melanogaster* is negatively correlated with the resistance to phenylthiourea (PTU), but is positively correlated with phenylurea (PU)-resistance.

In order to understand the biochemical mechanism of the negatively correlated cross-resistance, and to find substances having toxicities stronger than PTU, the author has investigated the relationship between molecular structure and negatively correlated activity in a number of compounds, using genetical analyses.