Genetical Studies on Actions of Mixed Insecticides with Negatively Correlated Substances. Genetical and Biochemical Studies on Negatively Correlated Cross-Resistance in *Drosophila melanogaster*. III. Zenichi Ogita (Department of Genetics, Faculty of Medicine, Osaka University, Osaka, Japan). Received July 26, 1961. *Botyu-Kagaku*, 26, 88, 1961. (in English).

15. 逆相関交叉抵抗性物質を混合した殺虫剤の殺虫作用に関する遺伝学的研究

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 III. 荻田善一（大阪大学医学部遺伝学教室）36. 7. 26 受理

キイロショウジョウバエにおいて phenylthiourea やその *p*-halogen 置換体の殺虫作用が DDT, BHC や parathion の殺虫作用と逆相関関係にあることは，すでに報告した．殺虫作用が相互に逆相関関係にある薬剤の混合物のもつ殺虫作用を遺伝学的解析によつて研究した．これらの混合物の殺虫作用は長期間の連用によつても，薬剤抵抗性をその集団内に生ぜしめないし，又，薬剤抵抗性と非抵抗性からなる昆虫の混合集団に対しては，見かけ上，顕著な "Joint toxic action" をもたらす．しかし，これは今までいわれて来た "Joint toxic action" とは本質的に異なつている．

The author already reported that the insecticidal action of phenylthiourea and of *p*-halogenophenylthioureas had relation with negatively correlated to that of DDT, BHC or parathion. The insecticidal actions of the mixed insecticides consisted of those which had mutual relations of negatively correlated cross-resistance pattern were studied by using genetical analyses.

Those results led to the conclusion that these mixed insecticides had an effective insecticidal action for the mixed population consisted of insecticide-resistant and susceptible flies, and it appeared to be same with the so-called "Joint toxic action" outwardly, though it was essentially different from the usual "Joint toxic action". And also, those might not produce any resistant flies to the mixtures of these insecticides even after continuous use.

Introduction

In previous papers[1,2,3,4], on the basis of a result of genetical analyses, the author has deduced the following conclusion: "The dominant susceptibility to phenylthiourea and *p*-halogenophenylthioureas in *D. melanogaster* resulted from the pleiotropic expression of the dominant gene for resistance to DDT, BHC and parathion on the 2nd chromosome. Therefore, mixed insecticides consisted of those which had mutual relations of negatively correlated cross-resistance pattern, might have an effective insecticidal action for the mixed population and might not produce any resistant flies to these mixtures even after continuous use". In this paper, such a new combined insecticidal composition has actually employed, and it has been concluded that these mixtures are essentially different from ordinary ones.

Materials and Methods

General insecticide-resistant strains such as *Hikone-R*$_{31}$, *HL2-Q* and *KSL*, and a susceptible multichromosomal mutant strain *bw*; *st ss*, were used for the genetical analyses.

The "Larval test" method described in the previous papers, was carried out by using 100 first-instar larvae in each glass vial.

Phenylthiourea and its *p*-halogeno-substituents were synthesized and purified in this laboratory. Other insecticides were obtained from the Japan Agricultural Chemicals and Insecticides Co.

The concentrations of chemicals are expressed in milimols per liter of yeast medium. All these tests were performed at approximately 25°C.

Experimental Results and Discussion

To investigate the relationship between single insecticidal action of chemicals such as phenylthiourea and its *p*-halogenosubstituents, or of various insecticides and that of mixed insecticides, the first-instar F_2-larvae resulting from the following backcrosses were used:

Backcross

(1) *bw*; *st ss*♀ × F_1(*bw*; *st ss*♀ × *Hikone-R*$_{31}$♂)♂

Table 1. Numbers of each phenotype in F_2 progeny from backcross (1) *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *Hikone-R*$_{31}$♂)♂ emerging from media containing phenylthioureas or insecticides expressed as a percentage of those emerging from untreated media.

(Based on 500 F_2 larvae by larval test method.)

Chemical		*bw* ; *st ss*	*bw* ; ++	+ ; *st ss*	+ ; ++
DDT	0.5 mM	15.0	7.0	90.9	91.3
	1.0	1.5	1.2	81.0	90.5
	1.5	0	0	74.4	77.1
	2.0	0	0	80.2	85.2
	10.0	0	0	82.5	86.5
	20.0	0	0	36.2	72.4
	30.0	0	0	28.6	65.5
BHC	1×10^{-2} mM	85.8	84.2	101.7	90.6
	3×10^{-2}	0	0	52.8	65.3
	5×10^{-2}	0	0	4.1	12.1
parathion	5×10^{-4} mM	69.9	66.6	103.3	101.5
	1×10^{-3}	24.3	24.6	102.4	98.4
	2.5×10^{-3}	12.2	27.8	90.2	100.8
	1×10^{-2}	0	0	17.3	37.4
nicotine sulfate	1.5 mM	0	96.0	55.0	99.2
	2.0	0	85.4	0	95.5
	3.0	0	27.8	0	33.0
phenylthiourea (PTU)	2.0 mM	0	102.4	0	9.6
	3.0	0	94.8	0	0
	5.0	0	87.2	0	0
	10.0	0	54.4	0	0
	30.0	0	10.9	0	0
p-chlorophenylthiourea (*p*-Cl-PTU)	0.5 mM	96.8	99.2	69.6	72.0
	1.0	64.0	97.6	8.0	4.0
	1.5	0	88.0	0	0
	2.0	0	95.2	0	0
	3.0	0	102.4	0	0
p-bromophenylthiourea (*p*-Br-PTU)	0.5 mM	32.0	98.4	4.0	20.0
	1.0	8.0	96.8	0	0
	2.0	0	93.6	0	0
	3.0	0	95.2	0	0

(2) *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *HL2-Q*♂)♂

(3) *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *KSL*♂)♂

One hundred first-instar F_2-larvae were put into a 60 ml glass vial containing 15 ml of yeast medium with various insecticides, chemicals or their mixtures, and the number of each phenotypical fly emerging from these media was examined. Only the results obtained from the backcross (1) were shown in Tables 1~5, because the results obtained from others (2 and 3) were almost the same to those of (1).

As shown in Table 1, + ; *st ss* and + ; ++ (wild type) could emerge from media containing high concentration of DDT, BHC and parathion. These flies had the 2nd chromosome carrying the DDT-resistant gene derived from the resistant strain (*Hikone-R*$_{31}$) in the heterozygous condition. No flies of other phenotypes could emerge. Moreover, wild type flies which had the 3rd chromosome carrying nicotine sulfate-resistant gene in the heterozygous condition, emerged more frequently than + ; *st ss* flies. Flies which could emerge from media containing phenylthiourea, *p*-chlorophenylthiourea, or *p*-bromophenylthiourea were only *bw* ; ++ in phenotype, which were DDT-, BHC-, parathion-susceptible and nicotine sulfate-resistant.

The *bw* ; ++ and + ; ++ flies could emerge from media containing nicotine sulfate. Such flies had nicotine sulfate-resistant gene derived from

resistant strain in the heterozygous condition. No flies of other phenotypes could emerge.

These results indicated, as reported in the previous papers, that the insecticidal action of *p*-halogeno-phenylthioureas as well as phenylthiourea was negatively correlated to the insecticidal action of DDT, BHC and parathion, and that the insecticidal action of nicotine sulfate was different from that of DDT, BHC and parathion, as reported by M. Tsukamoto and T. Hiroyoshi[5].

The flies which obtained resistance to DDT showed cross-resistance to BHC and parathion as reported by M. Tsukamoto, M. Ogaki and H. Kikkawa[6]. Therefore, the insecticides which are similar in insecticidal action, or the mixtures of these insecticides would be ineffective for flies resistant to one of these. As shown in Table 2, the + ; ++ progenies which had the gene responsible for cross-resistance to DDT, BHC and parathion on the 2nd chromosome, and the gene responsible for resistance to nicotine sulfate on the 3rd chromosome simultaneously, could emerge from media containing mixtures consisted of 2 mM nicotine sulfate and 2 mM DDT which are sufficient to kill susceptible flies for these insecticides. Accordingly, to kill the resistant flies a large amount of an insecticide or of the mixture of these insecticides should be used. However, even in such cases, when time goes on, there will appear the flies which had accumulated resistant genes against these insecticides.

The method of applying the insecticide with its synergist to the insecticide-resistant flies may also be expected to be effective at the beginning. However, if the insecticidal action of the insecticide was simply strengthened by the synergistic action, the application would bring higher resistance to the mixture in coming generations by selection. At any rate, even by the mixtures of insecticides which have mutually different insecticidal actions or by the mixtures of the insecticides with the synergists, the resistance to these mixtures will be brought in the population by continuous use.

On the contrary, as shown in Tables 3, 4 and 5, mixed insecticides consisted of those which had mutual relation of negatively correlated cross-resistance pattern, had an effective insecticidal action to these F_2-larvae, and it is suggested that the mixtures might not produce any resistant flies to these mixtures even after continuous use. That is, in these experimental populations, the mixtures of phenylthioureas of the minimum amount which is sufficient to kill DDT-, BHC- and parathion-highly resistant flies (*i. e.*, phenylthiourea susceptible flies), and of these insecticides of the lowest amount which is enough to kill phenylthiourea-resistant flies (these insecticide-susceptible and nicotine sulfate-resistant flies), were effective to exterminate all populations. And also, even if resistant flies to an insecticide which was one of components of the mixture would appear in the population by gene mutation, they must necessarily be susceptible to the insecti-

Table 2. Numbers of each phenotype in F_2 progeny from backcross (1) *bw* ; *st ss* ♀ × F_1(*bw* ; *st ss* ♀ × *Hikone-R*$_{31}$♂)♂ emerging from media containing mixed DDT with nicotine sulfate expressed as a percentage of those emerging from untreated media.

(Based on 500 F_2 larvae by larval test method.)

Chemical			*bw* ; *st ss*	*bw* ; ++	+ ; *st ss*	+ ; ++	Average
nicotine sulfate	+	DDT					
2.0 mM	+	0 mM	0	92.4	20.0	106.9	54.8
1.6	+	0.4	0	28.3	31.1	95.3	38.7
1.2	+	0.8	0	0	8.9	102.3	27.8
0.8	+	1.2	0	0	33.3	88.3	30.4
0.4	+	1.6	0	0	37.7	95.3	33.3
0	+	2.0	0	0	97.8	102.9	50.2
2.0 mM	+	2.0 mM	0	0	8.3	70.3	19.7
2.0	+	4.0	0	0	0	41.0	10.3

Table 3. Numbers of each phenotype in F_2 progeny from backcross (1) *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*Hikone-R*$_{31}$♂)♂ emerging from media containing mixed DDT with phenylthiourea expressed as a percentage of those emerging from untreated media.

(Based on 500 F_2 larvae by larval test method.)

Chemical				*bw* ; *st ss*	*bw* ; ++	+ ; *st ss*	+ ; ++
PTU	3.0mM	+	DDT 0.5mM	0	79.8	0	0
		+	DDT 1.0	0	25.7	0	0.4
		+	DDT 1.5	0	0	0	0
		+	DDT 2.0	0	0	0	0
		+	DDT 5.0	0	0	0	0
PTU	2.0mM	+	DDT 1.0mM	0	15.5	27.8	30.0
		+	DDT 2.0	0	0.4	0	17.4
		+	DDT 5.0	0	0	0	4.9
PTU	1.0mM	+	DDT 1.0mM	17.0	21.1	44.3	59.5
		+	DDT 1.5	5.4	8.4	55.7	56.2
		+	DDT 2.0	0	0	27.8	31.4
p-Cl-PTU	2.0mM	+	DDT 1.0mM	0	20.3	0	0
		+	DDT 1.5	0	2.4	0	0
		+	DDT 2.0	0	0	0	0
p-Cl-PTU	1.0mM	+	DDT 1.0mM	4.8	37.4	0	31.7
		+	DDT 1.5	9.7	5.0	0	14.2
		+	DDT 2.0	0	0	0	7.9
p-Br-PTU	2.0mM	+	DDT 1.0mM	0	22.9	0	0
		+	DDT 1.5	0	11.8	0	0
		+	DDT 2.0	0	0	0	0
p-Br-PTU	1.0mM	+	DDT 1.0mM	2.0	9.8	0	2.4
		+	DDT 1.5	8.7	5.7	0	6.3
		+	DDT 2.0	0	0	0	3.2

Table 4. Numbers of each phenotype in F_2 progeny from backcross (1) *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*Hikone-R*$_{31}$♂)♂ emerging from media containing mixed BHC with phenylthioureas expressed as a percentage of those emerging from untreated media.

(Based on 500 F_2 larvae by larval test method.)

Chemical				*bw* ; *st ss*	*bw* ; ++	+ ; *st ss*	+ ; ++
PTU	3.0mM	+	BHC 1×10^{-2}mM	0	56.9	0	0
		+	BHC 2×10^{-2}	0	18.2	0	0
		+	BHC 3×10^{-2}	0	0	0	0
p-Cl-PTU	2.0mM	+	BHC 1×10^{-2}mM	0	72.9	0	0
		+	BHC 2×10^{-2}	0	19.8	0	0
		+	BHC 3×10^{-2}	0	0	0	0
p-Cl-PTU	1.0mM	+	BHC 1×10^{-2}mM	4.0	67.2	0	34.8
		+	BHC 2×10^{-2}	0	47.4	0	16.5
		+	BHC 3×10^{-2}	0	3.9	0	14.2
p-Br-PTU	2.0mM	+	BHC 1×10^{-2}mM	0	54.5	0	0
		+	BHC 2×10^{-2}	0	22.9	0	0
		+	BHC 3×10^{-2}	0	0	0	0
p-Br-PTU	1.0mM	+	BHC 1×10^{-2}mM	1.6	36.3	0	9.5
		+	BHC 2×10^{-2}	0	18.1	0	7.1
		+	BHC 3×10^{-2}	0	5.5	0	8.7

Table 5. Numbers of each phenotype in F_2 progeny from backcross (1) *bw* ; *st ss*♀ ×F_1(*bw* ; *st ss*♀ ×*Hikone*-R_{31}♂)♂ emerging from media containing mixed parathion with phenylthioureas expressed as a percentage of those emerging from untreated media.

(Based on 500 F_2 larvae by larval test method.)

Chemical				*bw* ; *st ss*	*bw* ; ++	+ ; *st ss*	+ ; ++
PTU	3.0mM	+	parathion 2.5×10^{-3}mM	0	14.2	0	0
		+	parathion 1×10^{-2}	0	0	0	0
p-Cl-PTU	2.0mM	+	parathion 2.5×10^{-3}mM	0	18.1	0	0
		+	parathion 1×10^{-2}	0	0	0	0
p-Cl-PTU	1.0mM	+	parathion 2.5×10^{-3}mM	12.9	26.9	6.4	22.4
		+	parathion 1×10^{-2}	0	0	0	24.8
p-Br-PTU	2.0mM	+	parathion 2.5×10^{-3}mM	0	14.3	0	0
		+	parathion 1×10^{-2}	0	0	0	0
p-Br-PTU	1.0mM	+	parathion 2.5×10^{-3}mM	9.7	24.5	0	3.2
		+	parathion 1×10^{-2}	0	0	0	11.0

cide of the other component of the mixture which had mutual relation of negatively correlated cross-resistance pattern. Therefore, the resistance to these insecticides will be not brought in the population by continuous use.

The above mentioned results indicated that these insecticides showed a stronger insecticidal effect when used together than when used separately for the mixed population consisted of insecticide-resistant and susceptible flies. This phenomenon appears to be identical with the so-called "Joint toxic action" as usually seen in the combination among other insecticides which have mutually different insecticidal actions (Table 2), or among the insecticides and the synergists. The "Joint toxic action" which was brought by the mixtures consisted of the negatively correlated substances and the insecticides, however, is essentially different from the forsaid usual "Joint toxic action", though they appear to be same outwardly.

This is clear from the fact that the mixtures of the negatively correlated substances and the insecticides showed an outward "Joint toxic action" only for the mixed population consisted of insecticide-resistant and susceptible flies. The phenomenon does not take place for the pure population consisted of equal resistant levels because the flies are killed by either of components of the mixtures.

If the flies having various resistant levels to DDT, BHC or parathion were present in natural population, they would have also various resistant levels to phenylthioureas which were quite reversed to the pattern of resistant levels of DDT, BHC or parathion. And also, as described in previous papers, the nicotine sulfate resistant gene on the 3rd chromosome acts advantageously to the selection pressure with phenylthioureas as well as to that with DDT, BHC or parathion. Accordingly, the flies which have a nicotine sulfate highly resistant gene and a low resistant gene to DDT, BHC or parathion simultaneously, may survive to an amount of phenylthioureas which is enough to kill flies which have simultaneously the gene responsible for high resistance to DDT, BHC or parathion on the 2nd chromosome, and for high resistance to nicotine sulfate on the 3rd chromosome. These problems are being tested experimentally.

Summary

The mixture insecticides consisted of insecticides which had mutual relation of negatively correlated cross-resistance pattern, had an effective insecticidal action for the mixed population and might not produce any resistant flies to the mixture of these insecticides even after continuous use. This phenomenon appears to be same with the so-called "Joint toxic action" outwardly. However, the "Joint toxic action" which was brought by the

mixtures consisted of the negatively correlated substances and the insecticides, is essentially different from the usual "Joint toxic action", because the procedure described here is applicable only for the mixed population consisted of the insecticide-resistant and susceptible flies.

Acknowledgments

The author wishes to express his sincere gratitude to Prof. H. Kikkawa for his kind guidance and encouragement. This work was supported by a Research Grant from the W. H. O. and by a Grant for Scientific Research from the Ministry of Education, Japan.

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Genetical Relationship between Ali-Esterase Activity and Insecticide-Resistance in *Drosophila melanogaster*. Genetical and Biochemical Studies on Negatively Correlated Cross-Resistance in *Drosophila melanogaster* IV. Zenichi OGITA (Department of Genetics, Faculty of Medicine, Osaka University, Osaka, Japan). Received July 26, 1961. *Botyu-Kagaku*, 26, 93, 1961 (in English).

16. キイロショウジョウバエにおけるアリエステラーゼ活性と薬剤抵抗性との遺伝学的関係.

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 IV. 荻田善一（大阪大学医学部遺伝学教室） 36. 7. 26. 受理.

最近，Van Asperen や Oppenoorth は diazinon や malathion に対して抵抗性の系統のイエバエがもつているアリエステラーゼの活性（methylbutyrate 加水分解活性）が，非抵抗性のイエバエのもつている活性に比して低いことを報告した．この関係がショウジョウバエにおいても存在するかどうかをしらべるために，DDT，BHC，parathion-抵抗性の系統と非抵抗性の系統とを用いてアリエステラーゼとこれらの薬剤抵抗性の関係を遺伝学的に解析した結果，キイロショウジョウバエにおいては DDT, BHC, parathion に対して交叉抵抗性をもたらす第Ⅱ染色体上の gene とは関係なく，アリエステラーゼ活性に関係する遺伝因子は第Ⅲ染色体上に存在することが明らかとなつた．

Recently, Van Asperen and Oppenoorth reported that organophosphorous-resistant strains of housefly which have single gene responsible for the diazinon- and malathion-resistances, exhibited lower ali-esterase activity.

The author found that methylbutyrate was not hydrolyzed by homogenate of two mutants of *D. melanogaster*, while homogenate of wild flies had highly methylbutyrate-splitting capacity. The relation between low ali-esterase activity and insecticide-resistance was studied in the DDT, BHC and parathion-resistant and susceptible strains.

These results obtained led to the conclusion that low ali-esterase activity and DDT, BHC and parathion-resistances are independent on each other, and that the factor responsible for the activity of ali-esterase was involved in the 3rd chromosome.

Introduction

Recently, Van Asperen and Oppenoorth[1,2,3] reported that organophosphorous-resistant strains of housefly exhibited lower ali-esterase activity which seemed to depend upon single gene in both a diazinon-resistant and a malathion-resistant strains.

As it may bring solution on the mechanism of negatively correlated cross-resistance, it appeared to be of some interest to ascertain whether the same phenomenon is recognized in *D. melanogaster* or not. Therefore, genetical analysis of the factors responsible for ali-esterase in this insect has been performed.

Methods and Materials

The following strains of *D. melanogaster* were