原　著

Genetical Studies on the Resistance to Parathion in *Drosophila melanogaster*. II. Induction of a Resistance Gene from its Susceptible Allele. Hideo KIKKAWA (Department of Genetics, Medical School, Osaka University, Osaka) Received July 16, 1964. *Botyu-Kagaku*, 29, 37 1964

8. キイロショウジョウバエにおけるパラチオン抵抗性の遺伝学的研究. II. 感受性遺伝子より抵抗性遺伝子の誘発

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第1報でのべたようにキイロショウジョウバエにおけるパラチオン抵抗性は第2染色体の 64.5 の位置に存在する優性遺伝子によってひきおこされる．この優性遺伝子をホモまたはヘテロの状態にもつ個体は DDT, BHC, ディルドリン，マラチオン等に対し交差抵抗性を示す．しかしフェニールチオ尿素およびそのハロゲン誘導体に対しては却つて感受性である．

著者はかような特性を示すパラチオン抵抗性遺伝子が果して突然変異によって感受性遺伝子から誘発されるか否かを調べるため，現在最も感受性と思われる Canton-S の系統に X 線を照射してその子孫を調査した．その結果，一つの中程度の抵抗性を示す系統を得ることができた．この系統について遺伝子分析を行ったところ，これまでに見出されたパラチオン抵抗性の系統と同じく第2染色体の 64.5 の位置にある感受性遺伝子が突然変異をおこして抵抗性になったものであることを確めた．

この系統からは自然的にもっと強い抵抗性を示す系統も生じたが，反対にもとの Canton-S と同程度の感受性を示す系統に復帰することもあった．つまり感受性の遺伝子が強い抵抗性遺伝子に変わる場合，一段階ではおこらず非常に不安定な中程度のものをへておこることがわかった．

以上の結果のほか，この実験から推察されることとして前にのべた交差抵抗性を示したり PTU に却って感受性という現象も一つの遺伝子の変化によってひきおこされること，また世界の各地にみられるパラチオン抵抗性の系統は大部分各地方で独立に抵抗性の突然変異がおこって生じたものであろうということが推論された．

Introduction

As reported in my previous paper[1], the resistance to parathion in *D. melanogaster* is mainly controlled by a dominant gene located at 64.5 on the second chromosome. A fly carrying this dominant gene in a homozygous or in a heterozygous state shows cross-resistance to DDT, BHC, Dieldrin, Malathion etc. Of great interest is the fact that such a resistant fly is susceptible to phenylthiourea and its halogen derivatives[2,3].

However, there is still no datum as to the origin of the resistance gene itself. This led me to do the following experiment.

Materials and Methods

Since it was the most susceptible strain to parathion, the Canton-S strain was used as the material. Other appropriate strains were also used as the occasion demanded. The test method was the same as that used in my previous paper[1].

Experimental results

Adult males of the Canton-S strain were treated with X-rays of 2000r and 3000r respectively, then mated to non-treated virgin females of the same strain. The F_1 first instar larvae were raised on a medium containing 0.5 p. p. m. parathion. The result is shown in Table 1.

Table 1. Induction of parathion-resistance gene by X-ray treatment. (Selected with 0.5 p. p. m. parathion).

Source	Larvae tested	Survivors
Control	25,000	0
2000 r	12,500	2(♀1, ♂1)
3000 r	16,000	2(♀1, ♂1)

As shown in this table, four adult flies survived in the experimental series. Among these survivors, one female derived from the 3000r treatment was dead before laying eggs. The other three

flies were mated to *cn* (cinnabar, 2-57.5) *RI*$^{+}$ (parathion-susceptible, 2-64.5) *bw* (brown, 2-104.5). The F_1 males of each mating should have the genotypic constitution of either *cn*+ *bw*/+++; or *cn*+*bw*/+*RI*+, in the case where a reistance gene (*RI*) was actually induced from its susceptible allele of the Canton-S strain. Then each male derived from the three survivors was mated to a virgin *cn* (cinnabar, 2-57.5) *Cy* (Curly, 2)/*Tft* (Tuft, 2) parathion-susceptible female. Among the progeny, only *Cy* phenotypic flies were selected, and they were crossed *inter se*. From the progeny, only wild type flies were selected, and brother-sister matings were performed. Thus eight strains of either *RI*$^{+}$/*RI*$^{+}$ or *RI*/*RI* were established from each survivor, so that twenty-four strains were obtained in all.

It was exepected that half of them would be resistant, while the remaining half susceptible. Contrary to this expectation, the majority of them were susceptible or nearly so, and only one strain derived from the male survivor of the 2000 r treatment showed resistance to parathion. At that time, there was no clear explanation as to this curious phenomenon. The resistant strain was named "RI8", and properties to various insecticides were examined in comparison with those of the Hikone strain (the most resistant strain to parathion) and of the original Canton-S strain. The results are represented in Tables 2 and 3.

Table 2. Degrees of resistance to various insecticides. (Percentage of survivors).

Strain		Hikone	RI8	Canton-S
Parathion	0.0 p. p. m.	96.5	84.0	90.0
	0.25 p. p. m.	93.0	62.5	0.0
	0.5 p. p. m.	91.0	68.5	0.0
	1.0 p. p. m.	85.5	57.0	0.0
	1.5 p. p. m.	87.5	51.5	0.0
BHC	10 p. p. m.	75	41	3
PTU*	3 mM	0	11	54

200 larvae were used for each test of parathion experiments.
100 larave were used for each test of BHC and PTU experiments.
* PTU=phenylthiourea

Table 3. Degrees of resistance to various insecticides. (Percentage of survivors).

Strain		Hikone	RI8	Canton-S
Parathion	0.0 p. p. m.	92	81	83
	1.0 p. p. m.	92	57	0
	2.0 p. p. m.	59	1	0
	3.0 p. p. m.	16	0	0
	4.0 p. p. m.	9	0	0
Nicotine-sulfate	400 p. p. m.	19	2	1
	800 p. p. m.	9	0	0
PTU	2 mM	3	21	42
	3 mM	0	9	38
	4 mM	0	12	30
	5 mM	0	5	33

100 larvae were used for each test.

As shown in these tables, responses to various insecticides were nearly intermediate between those of the Hikone and of the original Canton-S strain.

In order to analyze the gene or genes responsible for parathion-resistance in the RI8 strain, the following experiments were performed. Males of the RI8 strain were mated to the *bw* (brown, 2-104.5); *st* (scarlet, 3-44.0); *sv*^{n} (shaven-naked, 4-0.0±) parathion-susceptible females and the

Table 4. Linkage analysis of *RI8* gene.
bw ; *st* ; *sv*^{n} ♀ × *bw*/+ ; *st*/+ ; *sv*^{n}/+ ♂

Phenotype	Control	Experiment (0.5 p. p. m. parathion)
bw ; + ; +	52	1
bw ; *st* ; +	55	2
bw ; + ; *sv*^{n}	42	2
bw ; *st* ; *sv*^{n}	33	0
+ ; *st* ; +	74	73
+ ; *st* ; *sv*^{n}	44	68
+ ; + ; *sv*^{n}	53	59
+ ; + ; +	53	82
Total	406	287
Larvae tested	500	750
Rate of emergence	81.2%	38.3%
Frequency of appearance of each mutant character (%)		
bw :	44.8	1.7
st :	50.7	49.8
sv^{n} :	42.4	44.9

F_1 males were back-crossed to the *sw* ; *at* ; sv^n females. The first instar larvae from this back-cross were raised on media with or without parathion. In Table 4 is shown the result.

From this table, it is easily concluded that the parathion-resistance gene involved in the RI8 strain is located on the second chromosome.

Next, to find the locus of the resistance gene on the second chromosome, males of the RI8 strain were mated to the *cn RI*$^+$ *vg* (vestigial, 2-67.0) *bw* females, and the F_1 females were back-crossed to the *cn RI*$^+$ *vg bw* males. The first instar larvae produced from this mating were raised on media with or without parathion. Table 5 represents the result.

Table 5. Locus analysis of *RI8* gene.

$\frac{cn\ +\ vg\ bw}{+\ RI8\ +\ +}$ ♀ × *cn* + *vg wb* ♂

Phenotype	Control	Experiment (0.5 p. p. m. parathion)
cn vg bw	114 (0*)	1 (1, 2*)
+ + +	173 (0)	213 (0)
bw + +	13 (1)	16 (1)
+ *vg bw*	5 (1)	3 (2)
cn vg +	53 (2)	0 (1, 2, 3)
+ + *bw*	63 (2)	46 (3)
cn + *bw*	2 (1, 2)	2 (1, 3)
+ *vg* +	0 (1, 2)	0 (2, 3)
Total	423	281
Larvae tested	500	750
Rate of emergence	84.6%	37.5%

Recombination value

cn-vg :	4.7	*cn-RI8* :	6.8
vg-bw :	27.9	*RI8-vg* :	1.4
		vg-bw :	17.1

* Showing crossover region.
Locus of *RI8* : 64.3-65.6

From the result of Table 5, it was ascertained that the resistance gene involved in the RI8 strain was located at the same locus as the RI gene (2-64.5) which was involved in all parathion-resistant strains[1]. This result suggested that a resistance gene, although it was not so strong in resistance, was actually induced from its susceptible allele.

Next, in order to examine whether a more resistant allele could be obtained spontaneously from the RI8 gene, the RI8 strain was mated to the *cn RI*$^+$ *bw* strain, and the F_1 first instar larvae were raised on the medium containing 2.5 p. p. m. parathion. Table 6 shows the result.

Table 6. Attempt to obtain high-resistance genes from RI8 strain.

Cross	Selection with 2.5 p. p. m. parathion Larvae tested	Survivors
A. RI8♀ × *cn*+*bw*♂	4,000	22(♀11, ♂11)
B. *cn*+*bw*♀ × RI8♂	4,000	14(♀ 5, ♂ 9)
Total	8,000	36(♀16, ♂20)

As shown in this table, thirty-six survivors were obtained from the total 8,000 larvae tested. Among these survivors, ten males were chosen arbitrarily, and they were mated to the *cn Cy/Tft* females, respectively. Among the progeny of each vial, only *Cy* flies were selected, and they were crossed *inter se*. Thus, homozygous resistant

Table 7. Degrees of resistance to parathion in some resistant strains derived from the RI8.

Strain	Concentration of parathion (p. p. m.) 0.0	0.5	1.0	2.0	3.0	4.0
RI8-A	67*	41	40	7	1	0
RI8-B	76	73	82	44	1	0
RI8-D	65	65	55	22	0	0
RI8-E	85	63	35	22	0	1
RI8-F	83	33	40	8	0	0
RI8-G	84	0	0	0	0	0
RI8-H	85	78	51	31	0	0
RI8-I	66	43	43	12	0	0
RI8-J	78	25	5	0	0	0

* Number of survivors. 100 larvae were used for each test.

Table 8. Properties of some resistant and susceptible strains.

Strain		Hikone	RI8-B	RI8-H	RI8-O*	RI8-G	Canton-S
Parathion	0.0 p. p. m.	83**	78	79	82	93	89
	0.5 p. p. m.	80	77	79	31	0	0
	1.0 p. p. m.	71	68	53	26	0	0
	2.0 p. p. m.	66	36	31	2	0	0
	3.0 p. p. m.	37	4	2	0	0	0
	4.0 p. p. m.	2	1	0	0	0	0
PTU	3mM	0	2	8	23	42	40
DDT	4000 p. p. m.	69	66	44	17	0	0

* Original RI8

** Number of survivors. 100 larvae were used for each test.

strains were established by the same procedures described before. They were designated as RI8-A~J, respectively. However, since one of the ten RI8 males (RI8-C) used at the start contained a recessive lethal gene on the second chromosome, it was impossible to get a homozygous resistant strain. The remaining nine strains were kept without any further selection, and the degree of resistance of each strain was examined from time to time.

Strange to say, degrees of resistance of these strains were very variable. For instance, the results for larvae of the twenty-first generation were as follows (Table 7).

As seen in this table, some strains such as RI8-B and RI8-H showed high resistance to parathion, while the RI8-G strain was susceptible to this insecticide. Properties of these three strains were compared with those of the original RI8 (RI8-O) and Canton-S, and also of the Hikone strain. In Table 8 is shown the result.

As seen in this table, both RI8-B and RI8-H strains showed high resistance to parathion, although they were still weaker in resistance than the Hikone strain. On one hand, the RI8-G strain showed a complete susceptibility which was nearly equal to the original Canton-S strain. The fact that the degree of resistance in the RI8-O strain was slightly weaker than that shown in Tables 2 and 3, might be due to back-mutation of the resistance gene involved in the RI8-O strain.

Of great interest obtained from these experiments is the fact that the strains which show resistance to parathion are also resistant to DDT, but susceptible to PTU, and *vice versa* (Table 8). This fact indicates clearly that these pleiotropic phenomena are caused by the mutation of a single gene.

Another important point deduced from these experiments is that the intermediate-resistance gene such as RI8-O seems to be unstable, and tends to mutate to either a susceptible or a more resistant direction.

In order to elucidate this point, the RI8-O strain was mated to the *cn RI⁺ bw* strain, and the F_1 first instar larvae were raised on media with or without PTU. Table 9 shows the result.

Table 9. Attempt to obtain back mutants from the RI8-O strain.

Cross	Selection with PTU 0.0 mM	3 mM	5 mM
A. RI8-O ♀ × *cn*+*bw*♂	76.6*	47.0	33.0
B. *cn*+*bw* ♀ × RI8-O♂	77.2	35.6	26.9

* Percentage of survivors.

In either A or B series, 500 larvae were used as the control, and 1000 larvae were used for each experiment of PTU. As shown in Table 9, more surviviors than expected were obtained even in the medium of 5 mM PTU. Among the survivors in the experiment of 5 mM PTU, ten males were chosen arbitrarily, and the homozygous strains were established by the procedure described before. Of them, nine strains showed a weak resistance to parathion, while the remaining one showed a complete susceptibility to

Table 10. Properties of some resistant and susceptible strains.

Strain		Hikone	RI8-B	RI8-G	RI8-RV
Parathion	0.0 p. p. m.	87*	80	90	81
	0.5 p. p. m.	88	82	0	0
	2.0 p. p. m.	86	45	0	0
PTU	3 mM	0	0	43	42
DDT	4000 p. p. m.	63	50	6**	0
Sevin	100 p. p. m.	67	22	0	0

* Number of survivors. 100 larvae were used for each test.
** Flies of dying state.

parathion. This susceptible strain was named "RI8-RV" (revertant). Properties of the RI8-RV strain are shown in Table 10, together with data for other related strains.

As shown in this table, both RI8-G and RI8-RV strains showed a complete susceptibility not only to parathion, but also to DDT and Sevin. However, they were resistant to PTU. These facts confirm again the assumption that the pleiotropic responses to various drugs are caused by the mutation of a single gene.

Discussion

As shown in a series of experiments, the parathion-susceptible gene (*RI*$^+$) which was located at 64.5 on the second chromosome was able to mutate to a resistance gene artificially or spontaneously. However, in this case, the mutation did not take place in a single step, but it occurred through an intermediate state. This intermediate type seems to be unstable, and tends to mutate to either a more resistant or a susceptible direction. The process may be represented as follows:

$$\text{Susceptible} \rightleftarrows \text{Intermediate resistance} \quad \text{Intermediate resistance} \leftarrow ? - \rightarrow \text{High resistance}$$

Such a phenomenon has already been reported by Auerbach[4], and by Demerec[5], with some plausible explanations. I am now performing an experiment to determine whether a high-resistance gene mutates to a susceptible one *via* an intermediate state or not. After this experiment has been done, the problem will be discussed thoroughly.

It is apt to be considered that the establishment of a highly resistant strain *via* intermediate states is the result of accumulation of polygenes for resistance through selection by insecticide pressure. However, it is also plausible that an intermediate and unstable resistance gene which was present in the population mutated to a more resistant state during selection. In this case, the establishment of resistant strain is to be attributed to successive mutations of a single gene.

Another important point derived from the results of these experiments is a demonstration that a resistance gene was actually induced from its susceptible allele. It should also be noted that the resistance gene is the same as the one which is found in other resistant strains of the world. This fact seems to indicate that the same mutation takes place independently in many strains, as in the case of gene-mutation of a visible marker. In other words, the majority of resistant strains found in various regions of the world may be due to polyphyletic origins.

Lastly, it has been shown that the phenomenon of cross-resistance to various insecticides and a negatively correlated effect for PTU, might be due to pleiotropic expressions of a single gene (*RI*, 2-64.5).

Summary

In *D. melanogaster*, a parathion-resistance gene was derived from its susceptible allele both by X-ray treatment and spontaneously. However, such a resistance gene was obtained *via* an intermediate state which was unstable and was liable to mutate to either a more resistant or a

susceptible direction.

The chromosome, the locus and the property of this resistance gene were in accordance with the ones which were found in various resistant strains of the world. This fact suggests strongly that the majority of resistant strains may be due to polyphyletic origins.

Lastly it was pointed out that the phenomenon of cross-resistance to various insecticides and a negatively correlated effect for PTU, might be due to pleiotropic expressions of a single gene.

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The Genetic Study on the Resistance to Sevin in *Drosophila melanogaster*. Hideo KIKKAWA (Department of Genetics, Medical School, Osaka University, Osaka) Received July 16, 1964 *Botyu-Kagaku*, **29**, 42, 1964

9. キイロショウジョウバエにおけるセビン抵抗性に関する遺伝学的研究

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最近セビン，ピロラン等のカーバメート系殺虫剤がカやシラミの駆除に広く用いられるようになった．興味のあることはカやシラミではカーバメート系殺虫剤と DDT 等の間に逆相関の効果，すなわち DDT に強いものは却ってカーバメート系殺虫剤に弱いという現象があるらしいといわれている．

キイロショウジョウバエではまだカーバメート系殺虫剤の抵抗性についての研究がないと思われるので，上記の逆相関の現象と結びつけて実験を行った．カーバメート系殺虫剤としてはセビンを用いたが，実験の結果キイロショウジョウバエのセビン抵抗性は DDT やパラチオン抵抗性と同じく第2染色体の 64.5 の位置にある一つの優性遺伝子によってひきおこされることがわかった．つまりショウジョウバエでは一つの遺伝子の変化に伴って塩素系，有機燐系およびカーバメート系のどの殺虫剤に対しても交差抵抗性のおこることが見出された．従ってカやシラミに見出されたような DDT とカーバメート系殺虫剤との逆相関の関係は存在しないものと思われる．

Introduction

Within the past few years, carbamate insecticides have become widely used for the control of vectors such as mosquitoes and body lice. Recent work suggests the possibility of a negatively correlated effect between carbamate insecticides and chlorinated hydrocarbons[1,2]. Such a negatively correlated effect was found by Ascher and his coworkers[3,4] in *Musca domestica*, viz., some drugs such a cetylbromoacetate were more effective to DDT-resistant flies than to susceptible ones. Subsequently, Ogita[5,6] found in *Drosophila melanogaster* that phenylthiourea and its halogen derivatives were more effective to DDT-resistant larvae than to susceptible ones. A review concerning this phenomenon has been reported by Brown[7]. Since there seems no available datum as to the resistance to carbamate insecticides in *D. melanogaster*, a genetic study was undertaken in connection with the problem of a negatively correlated effect mentioned above.

Materials and Methods

As a carbamate insecticide, Sevin (1-naphthyl-