The Genetical Relation between Resistance to Insecticides in General and That to Phenylthiourea (PTU) and Phenylurea (PU) in *Drosophila melanogaster*. Zenichi OGITA (Genetical Laboratory, Faculty of Medicine, Osaka University, Osaka, Japan). Received Sep. 26, 1958. *Botyu-Kagaku* **23**, 188, 1958.

35. キイロショウジョウバエにおける殺虫剤抵抗性とフェニールチオウレア (PTU), フェニールウレア (PU) の抵抗性との遺伝学的関係

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キイロショウジョウバエを用いてPTUおよびその対応酸素化合物であるPUに対する抵抗性の研究中, PTUの殺虫作用がDDTのそれに対して負の連関性を有し, PUの殺虫作用はDDTのそれに対して正の連関関係にあるという興味ある現象を見出した. すなわちDDT抵抗性の系統は必ずPTUに対して非抵抗性であり, PTU抵抗性のものはDDTに對して非抵抗性で硫酸ニコチンに対して抵抗性を示した. そしてPU抵抗性のものはDDT, 硫酸ニコチン両者に対して抵抗性であることを見出した. さらにこれらの殺虫作用の間のより深い関係を知るために, PTUおよびPUに対する抵抗性の遺伝学的研究がなされた. その結果, PTU抵抗性は, 性染色体上の不完全優性の変更因子と, 第Ⅱ染色体上 *vg* (67) 附近の劣性遺伝子と第Ⅲ染色体上 50± 附近の優性遺伝子の二つの主要遺伝子との, 少くとも3つの遺伝子に支配されている polygenic な形質である事を見出した. 第Ⅱ染色体上 *vg* (67) 附近の劣性遺伝子はDDT, BHC, パラチオンに対する抵抗性を支配する優性の遺伝子とその位置が一致する. この事はDDT抵抗性の遺伝子がPTUに対して非抵抗性を支配する優性の遺伝子として働く事を暗示しているように思える. さらに第Ⅲ染色体上 50± 附近の優性遺伝子は硫酸ニコチン抵抗性を支配する遺伝子と全く一致した. PU抵抗性に関しては, 第Ⅱ染色体上の *vg* (67) 附近のDDT抵抗性を支配する優性遺伝子と第Ⅲ染色体上の 50± 附近の硫酸ニコチン抵抗性を支配する優性遺伝子によって支配される polygenic な形質である. これらの結果から「第Ⅱ染色体上のDDT抵抗性遺伝子の存在はPUに対する抵抗性とPTUに対する異常な非抵抗性を与え, 第Ⅲ染色体上の硫酸ニコチン抵抗性遺伝子はPTUおよびPUに対する抵抗性を与える.」という仮説を提出した. そしてDDT, BHCやパラチオンなどにPTUを混合使用する事によって抵抗性を得せしめる事なく, さらに, 種々の殺虫剤抵抗性の幼虫をも効果的に殺滅し得る可能性を暗示した.

For some time the author has been conducting biochemical studies on the taste-blindness to PTU* shown by human beings, as discovered by Fox[2].

Recently in order to study the biochemical effect of PTU and PU+, measurements were made of the percentage emergence of adult *Drosophila melanogaster* from a dry yeast medium containing PTU or PU in various concentrations. It was found[7] that strains characterized by resistance to DDT, BHC, parathion and other insecticides were susceptible to PTU, whereas on the other hand the majority of DDT-susceptible strains were resistant to PTU. PU, which is the oxygen compound corresponding to PTU, behaves like DDT and other insecticides in the resistance of *D. melanogaster*. Genetical studies of the resistance to DDT, BHC, parathion, etc., in *D. melanogaster* have been made by Tsukamoto, Ogaki and Kikkawa[12,13], and Oshima[9].

PTU is well-known not only as an inhibitor of melanin formation, but also as a substance capable of dividing people into tasters and non-tasters. The first mention of PTU as an insecticide was the report of Minaff and Wright[6] that 2 % solutions of thiourea or PTU were effective against the webbing clothes moth. Thiourea was reported as a housefly larvicide by McGovran & Piquett[5], and Brown et al.[1], and as fly larvicides which offered promise for the control of resistant populations[15]. Also, its toxicity to *D. melanogaster* larvae was reported by Goldsmith and Harnly[3] who found a very great

* phenylthiourea, also described as phenylthiocarbamide (PTC) by Fox[2].

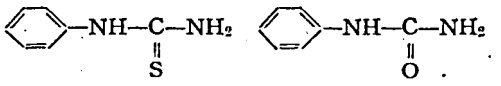
\+ phenylurea

variation between different strains, none of which however were known to be DDT-resistant.

The property of inhibiting the development of DDT-resistant strains but of allowing the survival of DDT-susceptible strains seems to be characteristic of PTU. Knowledge of the genetic mechanism of this interesting phenomenon is important for the settlement of certain problems of insecticide-resistance. Therefore, previous to biochemical study, investigations were made of the genetical relation between resistance to insecticides in general and that to PTU and PU, and these are the subject of the present paper.

Materials and Methods

(1) Materials: The samples of PTU and PU employed were synthesized and purified by the author. PTU and PU have the following chemical constitutions;



Phenylthiourea(PTU) Phenylurea(PU)

The strains of *D. melanogaster* were obtained from the laboratory stocks of Osaka University.

The resistant strains used were as follows;

Hikone-R: resistant not only to DDT, but also to various insecticides such as BHC, parathion, nicotine sulfate, etc.

Hikone-R$_{31}$: derived from the Hikone-*R* strain by single-pair mating; the strain mainly used in this study.

WMB$_{30}$, and *WMD*$_{7-38}$: developed by Tsukamoto in the laboratory from a mixed population of 14 wild strains by rearing them on a medium containing DDT, and resistant to DDT, BHC, parathion and nicotine sulfate.

cn R bw, and *cn R vg bw*: strains with an originally susceptible background but with the 2nd chromosome and the 3rd chromosome respectively derived from the *Hikone-R* strain.

bw; *III* $_{Hikone-R}$, and *bw*; *III* $_{NS-R}$: nicotine-resistant strains with the 3rd chromosome derived from the *Hikone-R* and *NS-R* strains respectively against their originally susceptible background.

bw; *st HR ss*: multichromosomal nicotine-resistant mutant strain in which the 2nd and 3rd chromosome are marked with recessive morphological mutations. The *st* (scarlet) and *ss* (spineless) genes located respectively on the left and right arms of the 3rd chromosome, and the region of the spindle-fibre attachment between them are derived from the *Hikone-R* strain; the remainder derives from an originally susceptible background.

Susceptible strains used were as follows;

Canton-S, Oregon-R(I), Oregon: all wild-type strains.

y-Oregon-R(I), v-Oregon-R(I), y, ywf v, etc.: strains with mutant genes on the X-chromosome.

cn bw, cn vg bw: strains employed mainly as PU-susceptible and PTU-resistant types for the gene analysis on the 2nd chromosome.

bw; st; svn, cn; bar-3; gvl: multichromosomal mutant strains in which the 2nd, 3rd and 4th chromosome are marked with recessive morphological mutations.

bw; st ss: strain employed mainly as the susceptible type for the gene analysis on the 3rd chromosome.

(2) Methods: The method followed in this study is the same as that described as the "adult test" by Tsukamoto and Ogaki[11]. Five male and five female flies, at least 3 days after emergence, were put into a small glass vial containing 15 cc of dry yeast medium (agar 2 g, dry yeast powder 3 g, sugar 4 g, water or the milimolar solution* 100 cc) for 4—5 days to lay their eggs on this medium with or without added insecticide. Then the rate of emergence of the succeeding generation could be calculated by comparing the number of flies from the PTU- or PU-containing media with that from the untreated dry yeast media.

Alternatively, larvae instead of adults were transferred to normal and PTU- or PU-containing media, and the rate of emergence was determined. In this "larval test", 50 larvae were put into each vial of treated or untreated medium. All tests were performed at a temperature approximating 25°.

* 5 mM PTU and 100 mM PU-solutions were prepared as stock solutions, and this solutions were diluted to each milimolar solution with distilled water.

Table 1. Percentage emergence at various concentrations (mM) of PTU and PU by 22 strains of *D. melanogaster* (adult test method).

Strain	PTU				UP					Resistance to DDT	Resistance to nicotine sulfafe p. p. m.
	1.0	2.0	3.0	5.0	10.0	20.0	30.0	50.0	100.0		
y (A_3)	87.2	72.3	33.1	18.5	106.3	82.5	17.3	0	0	S	R to 400
ywf	96.3	79.5	31.7	17.6	64.0	45.3	0	0	0	S	R to 400
w	84.3	82.0	33.9	14.5	71.6	41.1	8.2	0	0	S	R to 400
cn; bar-3; gvl	53.1	39.4	36.1	12.1	82.5	69.1	12.7	0	0	S	R to 400
Canton-S (A_3)	60.8	35.5	14.7	2.0	111.1	70.3	23.6	0	0	S	R to 400
Oregon	88.0	33.3	0	0	96.0	93.1	50.0	0	0	S	S to 400
Oregon-R (*I*)	84.6	7.0	0	0	88.6	53.4	31.3	0	0	S	S to 400
y-Oregon-R(*I*)	17.6	3.4	0	0	98.2	43.2	21.0	0	0	S	S to 400
v-Oregon-R(*I*)	15.8	0	0	0	101.5	37.8	16.8	0	0	S	S to 400
bw; st; sv^n	5.9	0	0	0	127.3	47.5	11.8	0	0	S	S to 400
Hikone-R	23.0	2.9	0	0	155.1	133.6	119.6	102.8	46.7	R	R to 1000
Hikone-R$_{31}$	18.0	4.2	0	0	149.1	128.5	112.4	66.5	24.9	R	R to 1000
WMB_{30}	54.2	3.4	0	0	176.2	113.7	98.5	58.3	7.8	R	R to 400
WMD_{7-38}	32.0	4.4	0	0	180.0	131.7	102.1	31.2	6.3	R	R to 400
cn R bw	11.9	0	0	0	116.0	109.0	74.0	55.7	7.6	R	R to 800
cn R vg bw	4.6	0	0	0	108.2	98.3	80.3	62.1	10.2	R	R to 800
cn bw	69.3	54.9	16.8	6.6	71.6	41.6	8.2	0	0	S	R to 400
cn vg bw	59.1	50.0	17.2	7.3	91.3	74.3	40.6	0	0	S	R to 400
bw; st ss	65.9	0	0	0	98.5	15.0	0	0	0	S	S to 400
bw; st HR ss	—	75.7	59.6	53.2	77.0	50.3	31.3	0	0	S	R to 800
bw; III Hikone-R	—	85.0	70.2	64.0	100.7	59.0	40.6	0	0	S	R to 1000
bw; III NS-R	—	97.0	83.5	74.8	134.0	73.3	44.3	0	0	S	R to 1000

R : resistant, S : susceptible

Table 2. Percentage emergence at various concentrations of PTU, PU, and DDT by 11 strains of *D. melanogaster* (larval test method).

	Strain	*y* (A_3)	*ywf*	*cn; bar-3; gvl*	*cn bw*	*Canton-S* (A_3)	*Oregon-R* (*I*)	*y-Oregon-R* (*I*)	*Hikone-R*$_{31}$	*Hikone-R*	WMB_{30}	WMD_{7-38}
PTU (mM)	1.0	96	72	80	92	87	76	54	79	86	66	42
	2.0	86	70	78	88	74	17	11	54	10	1	6
	2.5	—	68	74	72	46	—	—	—	0	0	0
	3.0	98	42	50	80	36	1	0	0	0	0	0
	5.0	90	38	40	44	19	0	0	0	0	0	0
PU (mM)	10.0	98	92	90	88	92	94	83	100	100	96	98
	20.0	89	84	70	72	68	61	58	82	100	100	96
	25.0	60	76	50	56	56	48	32	86	100	99	98
	50.0	0	0	0	0	0	0	0	89	92	96	91
DDT (p.p.m.)	50.0	84	79	—	—	2	—	—	—	—	—	—
	100.0	48	41	48	38	0	65	74	—	—	—	—
	200.0	24	18	32	28	0	40	48	—	—	—	—
	250.0	10	4	2	0	0	21	28	—	—	94	—
	500.0	0	0	0	0	0	5	4	93	100	93	78
	1000.0	0	0	0	0	0	0	0	84	96	90	70
	2000.0	0	0	0	0	0	0	0	69	84	89	78

Experimental Results

(1) Levels of resistance to PTU and PU

Insecticide-resistant and susceptible strains were tested for their resistance to PTU and PU by means of the "adult test" and "larval test". Some of the results are shown in Tables 1 and 2.

It is seen that the emergence of DDT-susceptible strains (such as *y*, *ywf*, *cn bw*, *cn vg bw*, *cn*; *bar-3*; *gvl* and *Canton-S*) could occur even after rearing in 5 mM PTU, while DDT-resistant strains (such as *Hikone-R*, *Hikone-R*$_{31}$, *WMB*$_{30}$, *WMD*$_{7-38}$ and *cn R bw*), and DDT-susceptible strains (such as *Oregon*, *Oregon-R(I)*, *y-Oreg n-R(I)*, *v-Oregon-R (I)* and *bw*; *st*; *sv*^{n}) were markedly affected by concentrations of 2.0—3.0 mM PTU in the dry yeast medium.

Thus whereas two types of PTU-susceptible strains were present, one DDT-resistant and the other DDT-susceptible, all PTU-resistant strains were suceptible to DDT.

In the tests with PU, the only flies to emerge from media containing 100 mM PU were DDT-resistant, while the emergence of DDT-susceptible strains was inhibited remarkably by 50 mM PU in the dry yeast medium.

The DDT-susceptible strains which had been reared on media containing 3 mM PTU for more than 3 generations were used as the PTU-resistant strain in the present study. Likewise DDT-resistant strains which had been reared on media containing 100 mM PU for more than 2 generations were used as the PU-resistant strains.

(2) Genetical analysis of PTU-resistance

The emergence rate of F_1 flies obtained from the reciprocal crosses between a PTU-resistant and the two types of PTU-susceptible strain was tested by the "adult test" method to ascertain the relation between the resistance and cytoplasmic or sex-linked factors. The results are given in Tables 3, 4, 5, and 6.

a) Reciprocal cross between PTU-resistant strain and DDT-resistant PTU-susceptible strain: The emergence rates of the F_1 generation from the reciprocal cross between PTU-resistant strains

Table 3. Numbers of F_1 flies from reciprocal crosses between PTU-resistant strain and DDT-resistant PTU-susceptible strain which emerged from untreated and PTU-treated media.

Cross	Control			2 mM PTU			3 mM PTU	
	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂
Hikone-R$_{31}$♀ × *y*(*A*$_3$)♂	577	506	1.14	98	88	1.11	0	0
y(*A*$_3$)♀ × *Hikone-R*$_{31}$♂	498	488	1.02	102	99	1.03	0	0
Hikone-R$_{31}$♀ × *ywf*♂	142	128	1.10	30	27	1.11	0	0
ywf♀ × *Hikone-R*$_{31}$♂	130	121	1.07	31	36	0.86	0	0
Hikone-R$_{31}$♀ × *cn*; *bar-3*; *gvl*♂	347	336	1.03	61	58	1.05	0	0
cn; *bar-3*; *gvl*♀ × *Hikone-R*$_{31}$♂	139	129	1.07	29	24	1.21	0	0
Hikone-R$_{31}$♀ × *cn vg bw*♂	179	164	1.09	21	17	1.24	0	0
cn vg bw♀ × *Hikone-R*$_{31}$♂	123	124	0.99	23	28	0.82	0	0
WMB$_{30}$♀ × *y*(*A*$_3$)♂	188	154	1.22	20	17	1.17	0	0
y(*A*$_3$)♀ × *WMB*$_{30}$♂	156	148	1.05	19	23	0.83	0	0
cn R bw♀ × *y*(*A*$_3$)♂	164	135	1.21	0	0	—	0	0
y(*A*$_3$)♀ × *cn R bw*♂	104	124	0.82	0	0	—	0	0
cn R bw♀ × *Canton-S*(*A*$_3$)♂	128	141	0.91	0	0	—	0	0
Canton-S(*A*$_3$)♀ × *cn R bw*♂	104	98	1.06	0	0	—	0	0
cn bw♀ × *Hikone-R*$_{31}$♂	114	110	1.04	64	0	∞	0	0
Hikone-R$_{31}$♀ × *cn bw*♂	229	221	1.03	3	25	0.12	0	0

(such as *cn; bar-3; gvl, cn vg bw, y,* and *Canton-S*) and PTU-susceptible strains (such as *Hikone-R, Hikone-R*$_{31}$, *WMB*$_{30}$, *WMD*$_{7-33}$ and *cn R bw* which have the DDT-resistant gene on the 2nd chromosome) were greatly decreased at the 2.0 and 3.0 mM PTU levels as compared with the PTU-resistant parent (Table 3). These F_1 progeny did not emerge at all 3 mM PTU-concentrations. Accordingly, media containing 3 mM PTU were employed in the genetical analysis for PTU-resistance.

This demonstrates that PTU-resistance is almost completely recessive to the PTU-susceptibility which DDT-resistant strains have, and that except the case of reciprocal cross *cn bw* × *Hikone-R*$_{31}$ maternal or cytoplasmic effects seem to be almost negligible. In these crosses the sex ratio in the F_1 flies emerging from PTU-containing media was normal. Furthermore, the sex ratio in the F_2 generation from the mutual cross between F_1 males and females from the cross of *y* × *Hikone-R*$_{31}$ was also normal under the PTU pressure (Table 4).

In the case of the reciprocal cross *cn bw* × *Hikone-R*$_{31}$, results obtained with media containing 1.5—2.5 mM PTU seem to show that an incompletely dominant factor is present on the sex-chromosome in *Hikone-R*$_{31}$. These results indicate that the resistance shows sex-linkage (Table 5).

b) Reciprocal cross between PTU-resistant strain and DDT-susceptible PTU-susceptible strain. The emergence rates of the F_1 generation from the reciprocal cross between PTU-resistant and PTU-susceptible strains (such as *bw; st; sv*^{n} *Oregon, Oregon-R(I), y-Oregon-R(I)* and *v-Oregon-R(I)* which have the DDT-susceptible gene on the 2nd chromosome) were identical with each other and with the PTU-resistant parent (Table 6). The only exception was the F_1 generation from *bw; st; sv*^{n} ♀ × *y* ♂ on media containing 3 mM PTU.

This demonstrates that the PTU-resistance is almost completely dominant over PTU-susceptibility when the PTU-susceptible parent is also DDT-susceptible. Moreover the maternal or cytoplasmic effects are slight or almost negligible. In these crosses the sex ratio in the F_1 flies emerging from PTU-containing media was normal.

c) Reciprocal cross between DDT-resistant PTU-susceptible strain and DDT-susceptible PTU-susceptible strain: The emergence rate of the F_1 generation from the reciprocal cross between the DDT-resistant PTU-susceptible strain and the DDT-susceptible PTU-susceptible strain was identical with that of the DDT-susceptible PTU-susceptible parent and less than that of the DDT-resistant PTU-susceptible parent (Table 6).

Table 4. Sex ratio of F_2 flies from the mutual backcross between F_1 males and females from the cross *y* × *Hikone-R*$_{31}$, which emerged from untreated and PTU-treated media.

Cross	Control					2 mM PTU					3 mM PTU				
	y		+			*y*		+			*y*		+		
	♀	♂	♀	♂	♀/♂	♀	♂	♀	♂	♀/♂	♀	♂	♀	♂	♀/♂
y(A_3) ♀ × F_1(*y*(A_3) ♀ × *Hikone-R*$_{31}$ ♂) ♂	110	100	0	0	1.10	88	99	0	0	0.89	44	62	0	0	0.71
y(A_3) ♀ × F_1(*Hikone-R*$_{31}$ ♀ × *y*(A_3) ♂) ♂	0	139	116	0	0.83	—	—	—	—	—	0	28	23	0	0.82
F_1(*y*(A_3) ♀ × *Hikone-R*$_{31}$ ♂) ♀ × *y*(A_3) ♂	102	111	116	94	1.11	59	47	40	65	0.84	26	41	34	32	0.82
F_1(*y*(A_3) ♀ × *Hikone-R*$_{31}$ ♂) ♀ × *Hikone-R*$_{31}$ ♂	0	56	110	54	1.0	0	0	6	2	3.0	0	0	0	0	—

Table 5. Sex ratio of F_1 flies from reciprocal crosses between *cn bw* and *Hikone-R*$_{31}$, which emerged from untreated and PTU-treated media.

Cross	Control			1.0mM PTU			1.5mM PTU			2.0mM PTU			2.5mM PTU			3.0mM PTU			5.0mM PTU		
	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂	♂	♂	♂/♂	♀	♂	♀/♂
cn bw ♀ × *Hikone-R*$_{31}$ ♂	175	133	1.31	95	68	1.40	42	3	14.0	22	1	22.0	13	0	∞	0	0	—	0	0	—
Hikone-R$_{31}$ ♀ × *cn bw* ♂	140	125	1.12	99	112	0.88	52	71	0.73	17	47	0.36	0	12	0	0	0	—	0	0	—

Table 6. Numbers of F_1 flies from reciprocal crosses between PTU-resistant strains and DDT-susceptible PTU-susceptible strains (1) and DDT-resistant PTU-susceptible strains and DDT-susceptible PTU-susceptible strains (2) which emerged from untreated and PTU-treated media.

	Cross	Control			2.0 mM PTU			3.0 mM PTU		
		♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂
1	*bw; st; sv*^{n} ♀ × *y*(A_3) ♂	556	533	1.04	293	173	1.69	259	161	1.61
	y(A_3) ♀ × *bw; st; sv*^{n} ♂	640	644	0.99	225	198	1.14	60	72	0.83
	bw; st; sv^n ♀ × *Canton-S*(A_3) ♂	187	190	0.98	98	95	1.03	37	23	1.61
	Canton-S(A_3) ♀ × *bw; st; sv*^{n} ♂	169	156	1.08	74	72	1.02	18	16	1.33
	v-Oregon-R(*I*) ♀ × *y*(A_3) ♂	114	111	1.02	86	73	1.17	18	26	0.69
	y(A_3) ♀ × *v-Oregon-R*(*I*) ♂	160	140	1.14	94	95	0.99	35	38	0.92
2	*v-Oregon-R*(*I*) ♀ × *Hikone-R*$_{31}$ ♂	163	142	1.15	3	5	0.60	0	0	—
	Hikone-R$_{31}$ ♀ × *v-Oregon-R*(*I*) ♂	127	116	1.09	2	3	0.67	0	0	—
	Oregon-R(*I*) ♀ × *WMD*$_{7-38}$ ♂	113	116	0.97	0	0	—	0	0	—
	WMD$_{7-38}$ ♀ × *Oregon-R*(*I*) ♂	153	162	0.94	0	0	—	0	0	—
	Oregon-R(*I*) ♀ × *Hikone-R*$_{31}$ ♂	126	131	0.96	0	0	—	0	0	—
	Hikone-R$_{31}$ ♀ × *Oregon-R*(*I*) ♂	130	126	1.03	0	0	—	0	0	—
	bw; st; sv^n ♀ × *Hikone-R*$_{31}$ ♂	121	132	0.92	0	0	—	0	0	—
	Hikone-R$_{31}$ ♀ × *bw; st; sv*^{n} ♂	128	129	0.99	0	0	—	0	0	—

The results shown in Table 6 indicate that the PTU-susceptible parent (such as *Oregon-R*(*I*), *v-Oregon-R*(*I*) and *bw; st; sv*^{n}) has a factor which decreases the resistant levels in F_1 progeny.

d) Chromosomal location of PTU-resistance: In order to determine which chromosome is responsible for the PTU-resistance, the following backcrosses were carried out using DDT-resistant PTU-susceptible strains (such as *Hikone-R*$_{31}$, *WMB*$_{30}$ and *WMD*$_{7-38}$), the DDT-susceptible PTU-susceptible strain *bw; st; sv*^{n}, and the DDT-susceptible PTU-resistant strains *cn; bar-3; gvl*, *y* and *Canton-S* A_3. Thus *bw; st; sv*^{n}, *cn; bar-3; gvl* are multichromosomal mutant strains in which each 2nd, 3rd and 4th chromosome is marked with a known recessive mutant.

Backcross

(1) *cn; bar-3; gvl* ♀ × F_1 (*Hikone-R*$_{31}$ ♀ × *cn; bar-3; gvl* ♂) ♂

(2) *cn; bar-3; gvl* ♀ × F_1 (*cn; bar-3; gvl* ♀ × *Hikone-R*$_{31}$ ♂) ♂

(3) *bw; st; sv*^{n} ♀ × F_1 (*y* ♀ × *bw; st; sv*^{n} ♂) ♂

(4) *bw; st; sv*^{n} ♀ × F_1 (*bw; st; sv*^{n} ♀ × *y* ♂) ♂

(5) *bw; st; sv*^{n} ♀ × F_1 (*Canton-S* A_3 ♀ × *bw; st; sv*^{n} ♂) ♂

The relation between the phenotypes and the number of surviving flies emerging from PTU-containing or PU-containing media was investigated by the "adult test" method in which 20 F_1 male and 20 female flies of the PTU-resistant multichromosomal strain were put into glass vials of either 90 cc or 180 cc capacity containing 20 or 40 cc of treated or untreated dry yeast medium.

From these backcrosses, the expected proportion of the 8 phenotypes in the F_2 generation emerged from the untreated media; the relation between the phenotype and the number of surviving flies emerging from treated media is shown in Tables 7 and 8.

Since it is known that F_1 hybrid flies from the reciprocal crosses between *Hikone-R*$_{31}$ and *cn; bar-3; gvl* cannot emerge from media containing 3 mM PTU, it is informative to ascertain the percentage survival at this concentration shown by F_2 offspring of the backcrosses of this F_1 hybrid with *cn; bar-3; gvl* females. If they are

Table 7. Numbers of phenotypes of surviving progeny in backcross 1, *cn*; *bar-3*; *gvl* ♀ × F_1 (*Hikone-R*$_{31}$ ♀ × *cn*; *bar-3*; *gvl* ♂) ♂ and backcross 2, *cn*; *bar-3*; *gvl* ♀ × F_1 (*cn*; *bar-3*; *gvl* ♀ × *Hikone-R*$_{31}$ ♂) ♂, which emerged from untreated, and PTU- and PU-treated media.

Backcross		1				2					
No. of experiment		2				3				2	
Sort of medium		Control		3 mM PTU		Control		3 mM PTU		50 mM PU	
Sex		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Phenotype	+ ; + ; +	28	33	0	0	85	72	0	0	68	58
	+ ; *bar-3*; +	43	27	0	0	79	80	0	0	10	9
	+ ; + ; *gvl*	32	25	0	0	89	55	0	0	39	33
	+ ; *bar-3*; *gvl*	19	22	0	0	47	31	0	0	1	1
	cn; + ; +	34	29	24	35	71	106	120	125	8	7
	cn; *bar-3*; +	29	33	19	11	58	66	55	60	1	1
	cn; + ; *gvl*	22	19	18	16	72	75	97	82	2	0
	cn; *bar-3*; *gvl*	19	16	12	15	55	41	36	39	0	0
Total no. of F_2 flies		226	204	73	77	556	526	308	306	129	109
Percentage of total *cn*		46.7		100.0		50.3		100.0		8.0	
Percentage of total *bar-3*		48.4		38.0		42.2		30.9		9.7	
Percentage of total *gvl*		40.5		40.6		43.0		41.4		31.9	
Sex ratio ♀/♂		1.11		0.95		1.06		1.01		1.18	

Table 8. Numbers of phenotypes of surviving progeny in backcross 3, *bw*; *st*; *sv*^{n} ♀ × F_1 (*y* ♀ × *bw*; *st*; *sv*^{n} ♂) ♂, backcross 4, *bw*; *st*; *sv*^{n} ♀ × F_1 (*bw*; *st*; *sv*^{n} ♀ × *y* ♂) ♂, and backcross 5, *bw*; *st*; *sv*^{n} ♀ × F_1 (*Canton-S* A_3 ♀ × *bw*; *st*; *sv*^{n} ♂) ♂, which emerged from untreated and PTU- and PU-treated media.

Backcross		3				4						5			
No. of experiment		3				1						1			
Sort of medium		Control		3mM PTU		Control		3mM PTU		50mM PU		Control		2mM PTU	
Sex		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Phenotype	+ ; + ; +	81	78	67	48	42	38	32	14	0	0	17	17	7	4
	+ ; + ; *sv*^{n}	70	62	47	43	25	26	21	10	0	0	15	12	4	0
	bw ; + ; +	47	43	60	69	29	41	20	24	0	0	22	17	8	3
	bw ; + ; *sv*^{n}	59	32	30	36	22	10	7	5	0	0	8	13	5	6
	+ ; *st* ; +	49	49	0	0	19	34	0	0	0	0	17	14	0	0
	+ ; *st* ; *sv*^{n}	61	57	0	0	18	12	0	0	0	0	20	7	0	0
	bw ; *st* ; +	58	52	0	0	23	28	0	0	0	0	5	9	0	0
	bw ; *st* ; *sv*^{n}	40	29	0	0	26	27	0	0	0	0	9	2	0	0
Total no. of F_2 flies		465	402	204	196	204	216	80	53	0	0	113	91	24	13
Percentage of total *bw*		41.5		48.8		49.0		40.1		0		41.7		59.5	
Percentage of total *st*		45.6		0		44.5		0		0		40.7		0	
Percentage of total *sv*^{n}		47.3		54.8		40.5		32.3		0		42.1		40.5	
Sex ratio ♀/♂		1.13		1.04		0.94		1.50		—		1.24		1.83	

homo zygous for the particular chromosome responsible for the PTU-resistance of the *cn; bar-3; gvl* strain, they would be expected to survive and emerge from the PTU-containing media. However, if they carry the susceptible chromosome from *Hikone-R*$_{31}$ strain in heterozygous condition, they would not be able to emerge.

As shown in Table 7, F_2 larvae of the strains *cn; bar-3; gvl*, *cn; bar-3*, *cn; gvl*, and *cn*, in which the 2nd chromsomes are both derived from the PTU-resistant mutant strain, were able to survive and emerge from media containing 3 mM PTU. Moreover, F_2 progenies in which the 3rd or 4th chromosomes are derived from the DDT-resistant PTU-susceptible strain were scarcely selected by the media containing 3 mM PTU. Besides, there are no significant difference in F_2 flies between the two crosses.

These data evidently indicated that the dominant genetic factor for PTU-susceptibility in the *Hikone-R*$_{31}$ strain is linked with the 2nd chromosome. In other words, it may be concluded that the factor for PTU-resistance on the 2nd chromosome is almost completely recessive to the factor for PTU-susceptibility which characterizes the DDT-resistant strains.

In backcrosses 3, 4, and 5, PTU-susceptible *bw; st; sv*^{n} flies were crossed with PTU-resistant flies such as *y* and *Canton-S*, and the resulting F_1 males were backcrossed to *bw; st; sv*^{n} females. Eight phenotypes might be expected in the F_2 generation. If certain of them are eliminated by PTU, it would suggest that the particular chromosome was concerned with the PTU-susceptibility.

Table 8 gives the number of surviving F_2 flies of each phenotype obtained from the backcrosses 3, 4, and 5. These data apparently suggest that these flies, whose 3rd chromosomes are not derived from the resistant parent (such as *bw; st; sv*^{n}, *bw; st*, *st; sv*^{n}, and *st*), are severely selected by PTU, while most of the flies belonging to other phenotypes can emerge even on media containing 3 mM PTU.

Consequently, it may be concluded that the PTU-resistance is connected with the 3rd chromosome in the PTU-resistant strains such as *y* and *Canton-S*. As shown above, these data evidently indicate that PTU-resistance is a polygenic character mainly controlled not only by the recessive factor on the 2nd chromosome, but also by the dominant factor on the 3rd chromosome.

e) Reciprocal cross between DDT-resistant PU-resistant strain and DDT-susceptible PU-susceptible strain: Although the toxicity of PU is far less than that of PTU, the emergence of DDT-susceptible strains was inhibited markedly in media containing 50 mM PU (Tables 1 and 2). DDT-resistant strains on the other hand could emerge from media containing 100 mM PU.

The emergence rate of F_1 flies from reciprocal crosses between PU-resistant and PU-susceptible strains is shown in Table 9. The reciprocal crosses between the resistant strains such as *Hikone-R*$_{31}$ and *WMD*$_{7-38}$, and the susceptible strains such as *cn; bar-3; gvl*, *cn vg bw*, and *y*,

Table 9. Numbers of F_1 flies from reciprocal crosses between DDT-resistant PU-resistant strains and DDT-susceptible PU-susceptible strains which emerged from untreated and PU-treated media.

Sort of medium	Control			20 mM PU			30 mM PU			50 mM PU			100 mM PU		
Sex	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂
Hikone-R$_{31}$ ♀ × *cn vg bw* ♂	112	106	1.05	96	95	1.01	103	78	1.32	58	81	0.72	22	16	1.39
cn vg bw ♀ × *Hikone-R*$_{31}$ ♂	112	97	1.15	91	89	1.02	73	53	1.37	50	44	1.13	26	29	0.89
WMD$_{7-38}$ ♀ × *cn vg bw* ♂	69	76	0.91	83	76	0.92	61	54	1.13	55	59	0.93	9	4	2.25
cn vg bw ♀ × *WMD*$_{7-38}$ ♂	85	81	1.05	51	50	1.02	46	41	1.12	28	47	0.60	12	15	0.80
Hikone-R$_{31}$ ♀ × *cn; bar-3; gvl* ♂	99	114	0.87	—	—	—	—	—	—	56	53	1.05	35	38	0.92
cn; bar-3; gvl ♀ × *Hikone-R*$_{31}$ ♂	118	103	1.45	—	—	—	—	—	—	62	58	1.06	28	36	1.77
y(A_3) ♀ × *Hikone-R*$_{31}$ ♂	130	108	1.20	82	92	0.89	40	45	0.88	47	36	1.30	9	17	0.53
Hikone-R$_{31}$ ♀ × *y*(A_3) ♂	116	98	1.18	66	83	0.79	72	54	1.33	59	52	1.13	35	38	0.92

were made in order to discover the genetic nature of the PU-resistance of DDT-resistant strains. The hybrids of these crosses were found to be as resistant as the parental PU-resistant strains used. The results make it clear that PU-resistance is almost completely dominant over PU-susceptibility and maternal or cytoplasmic effects are negligible.

f) Chromosomal location of PU-resistance : Backcrosses 2 of section d were carried out with PU-susceptible multichromosomal mutant strain *cn; bar-3; gvl.* The relation between the phenotype and the number of flies emerging from media containing 50 mM PU is shown in Table 7.

If the offspring are heterozygous for the particular chromosome from the *Hikone-R*$_{31}$ strain responsible for PU-resistance, they would be expected to survive and emerge from media containing 50mM PU. If they carry the susceptible chromosome with the mutant marker in homozygous condition, they would not be able to emerge.

As shown in Table 7, evidently the F_2 flies having the recessive mutant marker, and in which both the 2nd chromosomes were derived from the susceptible strain, are strongly selected by PU. The *cn*; +; + individuals, which are homozygous for the 2nd chromosome which has the PU-suceptible gene and are heterozygous for the 3rd and 4th chromosome from the PU-resistant strain, show a slight ability to survive and emerge from media containing 50 mM PU. On the other hand, the +; +; +, +; +; *gvl* individuals which are heterozygous for the 2nd chromosome and 3rd chromosome from the PU-resistant strain, are scarcely selected at all. In contrast, +; *bar-3*; *gvl*, +; *bar-3*; + individuals, which carry the 2nd chromosome in heterozygous condition and are homozygous for the 3rd chromosome with the PU-susceptible factor, are able to show only slight survival on the treated media.

The effect of the 4th chromosomes on the resistance should not be entirely neglected; it was found that the percentage survival of phenotypes of the 4th chromosomal mutant is rather lower on the PU-treated media than in the controls. However, this may not be of major importance in resistance, even if it shows some effect as a modifying factor. The most important effect on resistance may be due to the 2nd and 3rd chromosomes. The data evidently indicate that the mainly dominant genes for resistance to PU are located on the 2nd and 3rd chromosomes.

(3) Analysis of the locus for PTU-resistant gene and PU-resistant gene on the second chromosome

It is confirmed in the above-mentioned experiments that the PTU-resistance of *D. melanogaster* is due to the polygenic system which are manifested by the recessive factor on the 2nd chromosome and the dominant factor on the 3rd chromosome. On the contrary, PU-resistance of *D. melanogaster* is due to the polygenic system which are manifested by the dominant factor on the 2nd chromosome and the dominant factor on the 3rd chromosome, as well as the PTU-resistant factor on the 3rd chromosome.

In order to determine the precise locus for the PTU-resistant factor and the PU-resistant factor on the 2nd chromosome, several crossing experiments were made in order to ascertain the crossover rate between the resistant factors and certain known marker genes.

For this purpose, two mutant PTU-resistant PU-susceptible strains, namely *cn bw*, and *cn vg bw*, were crossed to certain PU-resistant PTU-susceptible strains, such as *Hikone-R*, *Hikone-R*$_{31}$ and *WMD*$_{7-38}$; the resulting F_1 females were backcrossed with mutant males of one of the PTU-resistant PU-susceptible strains.

The crossing procedures were as follows;

Backcross

(8) F_1 (*cn bw* ♀ × *Hikone-R*$_{31}$ ♂) ♀ × *cn bw* ♂

(9) F_1 (*Hikone-R*$_{31}$ ♀ × *cn bw* ♂) ♀ × *cn bw* ♂

(10) F_1 (*cn vg bw* ♀ × *Hikone-R*$_{31}$ ♂) ♀ × *cn vg bw* ♂

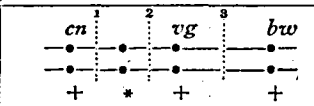
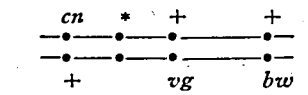
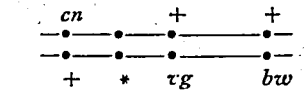
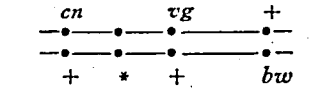
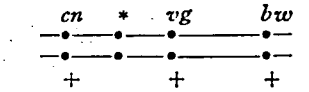
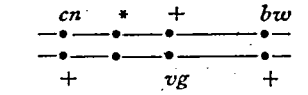
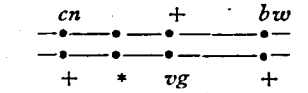
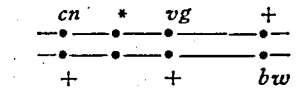
(11) F_1 (*Hikone-R*$_{31}$ ♀ × *cn vg bw* ♂) ♀ × *cn vg bw* ♂

(12) F_1 (*cn vg bw* ♀ × *WMD*$_{7-38}$ ♂) ♀ × *cn vg bw* ♂

In these crossing procedures, the relation between the phenotypes and the number of surviving flies was determined by the "adult test" in which 20 F_1 female and 20 male flies of the mutant strain were put into glass vials containing treated or untreated dry yeast media.

The crossing over in F_1 females in backcross 8

Fig. 1. The constitution of phenotypes on the second chromosome of F_2 flies emerged from treated media.

	Cross-over region		Phenotypes of F_2 flies emerged from PTU-treated media	Phenotypes of F_2 flies emerged from PU-treated media
Non-crossover	0		*cn vg bw*	+ * + +
Single-crossover	1		+ *vg bw*	*cn* * + +
	2		*cn* + +	+ * *vg bw*
	3		*cn vg* +	+ * + *bw*
Double-crossover	1, 2		+ + +	*cn* * *vg bw*
	1, 3		+ *vg* +	*cn* * + *bw*
	2, 3		*cn* + *bw*	+ * *vg* +
Triple-crossover	1, 2, 3		+ + *bw*	*cn* * *vg* +

* : DDT-resistant gene

Table 10. Numbers of F_2 flies of each phenotype obtained from the backcross 8, F_1 (*cn bw* ♀ × *Hikone-R*$_{31}$ ♂) ♀ × *cn bw* ♂ and backcross 9, F_1 (*Hikone-R*$_{31}$ ♀ × *cn bw* ♂) ♀ × *cn bw* ♂ which emerged from untreated, and PTU- and PU-treated media.

Backcross		8						9					
Sort of medium		Control		2 mM PTU		3 mM PTU		Control		3 mM PTU		50 mM PU	
		C. R.		C. R.		C. R.		C. R.		C. R.		C. R.	
Phenotype	*cn bw*	0	187	0	146	0	100	0	68	0	31	1,2	5
	+		183	1,2	12	1,2	4		72	1,2	0	0	34
	cn	1	147	2	106	2	91	1	49	2	26	1	10
	bw		114	1	32	1	14		67	1	10	2	23
Total no. of F_2 flies		631		296		209		256		67		72	
Percentage of total *cn*		52.9		85.1		91.4		45.7		85.1		20.8	
Percentage of total *bw*		47.7		60.1		54.5		52.7		61.2		38.9	

+ ; wild allele from PTU-susceptible PU-resistant strain of DDT-resistance, C. R. : crossover region

and 9 should produce 4 phenotypes in the F_2 generation, i. e., wild-type and *cn bw* (non-crossover class), and *cn* and *bw* (crossover class). Although the *cn bw*, *cn* and *bw* mutant-type flies, as well as the wild-type, can emerge from the untreated media, only wild-type flies are selected on media containing 3 mM PTU. This means that the non-crossover *cn bw* flies, and the crossover *cn* and *bw* flies homozygous for the particular PTU-resistant chromosome from the PTU-resistant strain, would be able to survive and emerge from media containing 3 mM PTU. On the other hand the non-crossover wild-type flies, and the crossover *cn* and *bw* flies heterozygous for the particular PTU-susceptible chromosome from the DDT-resistant strain (vide results in Table 3), would not be able to survive and emerge from media containing 3 mM PTU.

In the case of PU-containing media, many wild-type flies and a few mutant flies can survive at 50 mM concentration. This means that the non-crossover *cn bw* flies, and some of the crossover *cn* and *bw* flies, were selected by PU.

The constitution of genotypes with respect to the 2nd chromosome is schematically represented in Fig. 1.

a) Position of PTU-resistant gene on the 2nd chromosome : As shown in Table 10, non-crossover *cn bw* flies, and crossover *cn* and *bw* flies homozygous for the chromosome with the PTU-resistant factor, can emerge from PTU-containing media, while only wild-type flies heterozygous for the chromosome with the DDT-resistant factor cannot survive from media containing 3 mM PTU.

These crossover data suggest that the dominant PTU-susceptible factor of the *Hikone-R_{31}* strain is located on the right arm of the 2nd chromosome.

Table 11. Numbers of F_2 flies of each phenotype obtained from the backcross 10, F_1 (*cn vg bw* ♀ × *Hikone-R_{31}* ♂) ♀ × *cn vg bw* ♂, backcross 11, F_1 (*Hikone-R_{31}* ♀ × *cn vg bw* ♂) ♀ × *cn vg bw* ♂ and backcross 12, F_1 (*cn vg bw* ♀ × *WMD$_{7-38}$* ♂) ♀ × *cn vg bw* ♂ which emerged from untreated, and PTU- and PU-treated media.

Backcross			10						11						12			
No. of experiment			3						2						2			
Sort of medium			Control		3 mM PTU		50 mM PU		Control		3 mM PTU		50 mM PU		Control		3 mM PTU	
			C.R.		C.R.		C.R.		C.R.		C.R.		C.R.		C.R.		C.R.	
Phenotype	*cn*	*vg*	*bw*	0	227	0	286	1,2	6	0	190	0	167	1,2	8	0	116	0	123
	+	+	+		299	1,2	0	0	240		250	1,2	0	0	115		153	1,2	0
	cn	+	+	1	64	2	13	1	28	1	51	2	10	1	8	1	34	2	12
	+	*vg*	*bw*		44	1	39	2	13		40	1	21	2	6		22	1	31
	cn	*vg*	+	2	136	3	193	1,2,3	13	2	113	3	123	1,2,3	4	2	78	3	96
	+	+	*bw*		223	1,2,3	0	3	167		176	1,2,3	0	3	71		119	1,2,3	0
	cn	+	*bw*	1,2	12	2,3	2	1,3	11	1,2	11	2,3	1	1,3	1	1,2	3	2,3	0
	+	*vg*	+		17	1,3	18	2,3	5		15	1,3	12	2,3	1		7	1,3	14
Total no. of F_2 flies			1022		551		483		846		334		214		532		276	
Percentage of total *cn*			43.0		89.7		12.0		43.1		90.1		9.8		43.3		83.7	
Percentage of total *vg*			41.5		97.3		7.7		42.3		96.7		8.9		42.1		95.7	
Percentage of total *bw*			49.5		59.3		40.8		49.3		56.6		40.2		49.1		55.8	

Table 12. Percent frequency of the 3 mutant marker types in the F_2 flies which emerged from PTU-treated and PU-treated media.

Sort of medium	3 mM PTU			50 mM PU		
Mutant marker	*cn*	*vg*	*bw*	*cn*	*vg*	*bw*
Backcross 10	89.7	97.3	59.3	12.0	7.7	40.8
Backcross 11	90.1	96.7	56.6	9.8	8.9	40.2
Average	89.9	97.0	57.9	10.9	8.3	40.5
Map distance from mutant marker to PTU- and PU-resistant genes	10.1	3.0	42.1	10.9	8.3	40.5

Therefore, it appears that a gene for PTU-resistance is involved, which is located slightly to the left of the centre of the region between *cn* and *bw*; that is the PTU-resistant gene seems to be nearer to *cn* than to *bw*.

In order to determine the precise locus for the PTU-resistant gene, the *cn vg bw* strain was employed. In this strain the positions of the mutant markers are suitable for the precise determination of such a gene on the 2nd chromosome. The crossing experiment was performed by the same procedure as above, and results are set out in Table 11.

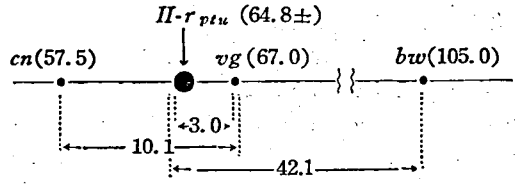
It is seen that those F_2 flies which carry the mutant marker *vg* emerged from PTU-containing media in greater numbers than those with the other markers (see crosses 10, 11, 12). These results indicated that the PTU-resistant gene is located on the right arm of the 2nd chromosome near the *vg* (II-67) gene, since the linkage is very close. The crossover rates between the PTU-resistant gene and the mutant marker may be calculated from Table 12, in which the percentages of total flies with the mutant markers *cn*, *vg* or *bw* surviving PTU treatment are presented.

The locus for the PTU-resistant gene is calculated from the average percentages as follows:

Map distance from mutant marker to PTU-resistant gene

cn ⟶ *II-r*$_{ptu}$: 100—89.9=10.1(%)
II-r$_{ptu}$ ⟶ *vg* : 100—97.0=3.0 (%)
II-r$_{ptu}$ ⟶ *bw* : 100—57.9=42.1(%)

The locus for the PTU-resistant gene may be graphically represented thus;



Here the PTU-resistant gene on the 2nd chromosome is designated as *II-r*$_{ptu}$.

Consequently, the estimated true position of this resistant gene lies between 64 and 67 on the 2nd chromosome near *vg* (II 67); i. e. the locus for the recessive gene for PTU-resistance is slightly to the left of the *vg* locus. In other words the locus for the dominant gene for PTU-susceptibility in the DDT-resistant strain is slightly to the left of the *vg* locus.

It is very interesting that this locus closely agrees with the locus for resistance to DDT, BHC and parathion determined by Tsukamoto & Ogaki, and by Kikkawa[12,13], and especially with the locus (II-64.5) for resistance to parathion determined with the same strain *cn vg bw* by Kikkawa[4].

As shown in Table 11, when the strain *WMD*$_{7-38}$ is used instead of the *Hikone-R*$_{31}$ as the DDT-resistant PTU-susceptible strain, the results indicated that the dominant gene for PTU-susceptibility had the same locus, despite its different origin.

b) Position of PU-resistant gene on the 2nd chromosome: The locus for the PU-resistant gene on the 2nd chromosome was determined by a similar procedure. As shown in Table 10, the *cn*, *bw*, and *cn bw*, flies which could emerge from PU-containing media seem to be carrying, in heterozygous condition, the resistant gene on their chromosomes acquired by crossing over. These data provide evidence that the resistant

gene is closer to *cn* than to *bw*.

The details of these experimental results are set out in Table 11. The frequencies of the 3 mutant types in the F_2 generation reared on PU-containing media are expressed as percentages in Table 12. As shown in Table 11, F_2 flies having the mutant marker *vg* were more heavily selected by PU than flies with the other markers. These results indicate that the PU-resistant factor is located on the right arm of the 2nd chromosome near the *vg* (II-67) gene.

The rate of emergence of single-crossover F_2 flies from PU-containing media is somewhat higher than that of the value expected from the triple-crossover class (morphologically single-crossover class *cn vg*+). Thus it appears that the non-resistant flies have not sufficiently been selected.

The locus for PU-resistant gene is calculated from the average percentage as follows :

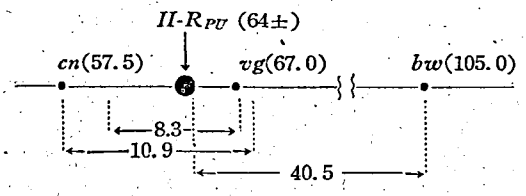
Map distance from mutant marker to PU-resistant gene.

cn ⟶ *II-R*$_{PU}$: 10.9 (%)

II-R$_{PU}$ ⟶ *vg* : 8.3 (%)

II-R$_{PU}$ ⟶ *bw* : 40.5 (%)

The locus for the PU-resistant gene may be graphically represented thus :



Here the PU-resistant gene on the 2nd chromosome is designated as *II-R*$_{PU}$.

Consequently, the estimated true position of this resistant gene seems to lie between 64 and 67 on the 2nd chromosome near the gene *vg* (II-67). This locus appears to agree closely with the locus of the gene for resistance to DDT, BHC and parathion.

However, the locus as calculated from the *vg* flies is slightly different from the locus as calculated from the *cn* and *bw* flies. This difference may derive from the fact that the susceptible flies were not completey selected at a concentration of 50mM PU. Further experiments should use higher concentrations.

Also, if this difference is due to pleiotropic effects of other genes contributive to PU-resistance, the precise locus for the resistant gene itself may be determined only by eliminating the effect of other genes. For this purpose, special strains are being now synthesized.

(4) Analysis of the locus for the PTU-resistant and PU-resistant gene on the 3rd chromosome.

a) Position of PTU-resistant gene on the 3rd chromosome: Since PTU-susceptibility and PU-resistance may be mutually pleiotropic expression of DDT-resistant gene on the 2nd chromosome, as mentioned above, the precise determination of the locus for the factor on the 3rd chromosome for resistance to PTU on the one hand and to PU on the other was determined by a procedure in which the pleiotropic effect between these genes was eliminated.

In order to determine the precise locus for the dominant PTU- and PU-resistant factor on the 3rd chromosome, certain PTU-resistant strains such as *bw*; *III*$_{Hikone\text{-}R}$, and *bw*; *III*$_{NS\text{-}R}$ were used. These two strains have the 3rd chromosome derived from the *Hikone-R* and *NS-R* strains respectively in their originally DDT-susceptible background. As the typical PTU- and PU-susceptible strain *bw*; *st ss* was mainly used. By the one of these special strains in crossing experiments with the susceptible strain, the differences may be restricted to the 3rd chromosome without interference by factors on the other chromosome.

In order to determine the precise locus for the PTU-resistant factor on the 3rd chromosome, the following backcrosses were carried out.

Backcross

(13) F_1 (*bw*; *st ss* ♀ × *bw*; *III*$_{Hikone\text{-}R}$ ♂) ♀ × *bw*; *st ss* ♂

(14) F_1 (*bw*; *st ss* ♀ × *bw*; *III*$_{NS\text{-}R}$ ♂) ♀ × *bw*; *st ss* ♂

From the crossover data in Table 13, the locus for the PTU-resistant gene on the 3rd chromosome could be determined.

It was calculated by assuming that the gene was located between *st* (III-44.0) and *ss* (III-58.5). At what point along this map distance of 14.5 the resistant gene was located could be

Table 13. Numbers of F_2 flies of each phenotype obtained from the backcross 13, F_1 (*bw*; *st ss* ♀ × *bw*; *III* $_{Hikone-R}$ ♂) ♀ × *bw*; *st ss* ♂, and backcross 14, F_1 (*bw*; *st ss* ♀ × *bw*; *III*$_{NS-R}$ ♂) ♀ × *bw*; *st ss* ♂, which emerged from untreated, and PTU-and PU-treated media.

Backcross		13			14	
No. of experiment		5		2	2	
Sort of medium		Control	3 mM PTU	30 mM PU	Control	3 mM PTU
Phenotype	*bw*; *st* *ss*	986	8	5	455	2
	bw; *st* +	209	107	4	58	17
	bw; + *ss*	230	174	7	69	31
	bw; + +	1164	1701	269	444	332
Total no. of F_2 flies		2589	1990	285	1024	382

determined from the rate of the *st* and *ss* F_2 flies which emerged from the media containing PTU.

In the case of backcross 13, the locus for the resistant gene on the 3rd chromosome was calculated from the crossover data (Table 13) as follows:

$$st \longrightarrow III\text{-}R_{PTU} \quad 44.0 + \frac{8+107}{1990} \times 100 = 49.8$$

$$III\text{-}R_{PTU} \longrightarrow ss \quad 58.5 - \frac{8+174}{1990} \times 100 = 49.4$$

With a similar procedure, the locus for the resistant gene was calculated from the crossover data of backcross 14 (Table 13) as follows:

$$st \longrightarrow III\text{-}R_{PTU} \quad 44.0 + \frac{2+17}{382} \times 100 = 49.0$$

$$III\text{-}R_{PTU} \longrightarrow ss \quad 58.5 - \frac{2+31}{382} \times 100 = 49.9$$

Thus the results of both crosses indicate that the PTU-resistant gene on the 3rd chromosome derived from the *Hikone-R* and *NS-R* strains is located approximately at III-50± on the linkage map, i. e. on the right arm of the 3rd chromosome slightly to the right of the spindle-fibre attachment.

It is very interesting that the locus for the dominant PTU-resistant gene on the 3rd chromosome closely agrees with that for the nicotine-resistant gene reported by Tsukamoto and Hiroyoshi [10,14].

Here, the PTU-resistant gene on the 3rd chromosome is designated as *III-R*$_{PTU}$.

b) Position of PU-resistant gene on the 3rd chromosome: In order to determine the locus for the PU-resistant gene on the 3rd chromosome, the offspring of backcross 13 were reared on media containing 30 mM PU.

This backcross was suitable for determining the locus of the PTU-resistant gene because both the strains involved had the PTU-resistant gene on the 2nd chromosome. However, these backcrosses were not suitable for determining the locus of the PU-resistant gene on the 2nd chromosome, because these strains lacked the PU-resistant gene on the 2nd chromosome. Consequently, a concentration of 30 mM PU was employed, since 50 mM PU exerted too strong a selective effect for the separation of phenotypes.

But backcross 13 proved unsuitable for determining the locus of the PU-resistant gene on the 3rd chromosome because the emergence rate of the non-crossover F_2 flies from PU-treated media was somewhat higher than the amount expected from the double-crossover class (*bw*; *st ss*). This may be due to the insufficient selective effect of 30 mM PU for the non-resistant flies. Therefore preliminary estimations were based on only the numbers of the mutant F_2 phenotypes in the single-crossover class and the map distance between the marker genes was divided in proportion to the emergence rate of these phenotypes from the PU-treated media. The locus for the PU-resistant gene on the 3rd chromosome may be calculated by dividing the map distance 14.5 between *st* (III-44.0) and *ss* (III-58.5) on the basis of the emergence rate of the *st* and *ss* flies as follows:

$$st \longrightarrow III\text{-}R_{PU} \quad 44.0 + 14.5 \times \frac{4}{4+7} = 49.3$$

$III\text{-}R_{PU} \longrightarrow ss \quad 58.5 - 14.5 \times \frac{7}{4+7} = 49.3$

Thus it may be assumed that the PU-resistant gene on the 3rd chromosome is located at approximately the same position as the gene for PTU-resistance on this chromosome.

Discussion and Conclusion

It is noteworthy that the results obtained by the "adult test" agreed so closely with these obtained by the "larval test". This would indicate that the presence of the chemical (PTU or PU) in the medium had little effect on the oviposition rate in the concentrations used. Therefore in this investigation the data were obtained almost exclusively by means of the "adult test".

The genetic relation between resistance to insecticides in general and that to PTU and PU. Both the DDT-resistant nicotine-resistant strains (e. g. *Hikone-R*, *Hikone-R*$_{31}$, *WMB*$_{30}$ and *WMD*$_{7\text{-}38}$) and the DDT-susceptible nicotine-susceptible strains (e. g. *bw; st; sv*^{n} and *bw; st ss*) were alike in their susceptibility to PTU. On the other hand, strong resistance to PTU is shown by strains which are DDT-susceptible but resistant to nicotine (e. g. *bw*; $III_{Hikone\text{-}R}$, *bw*; $III_{NS\text{-}R}$, *bw; st HR ss*). Moderate tolerance of PTU is also shown by strains such as *y*, *ywf*, *Canton-S*, *cn*; *bar-3*; *gvl*, and *cn bw* which are DDT-susceptible but can tolerate 400 p. p. m. nicotine sulfate. whereas the *cn R bw* strain had acquired DDT-resistance while acquiring susceptible to PTU, by introduction of the 2nd chromosome of *Hikone-R* into the originally DDT-susceptible PTU-resistant *cn bw* background, by contrast the *bw; st HR ss* strain acquired PTU-resistance alone with the nicotine resistance introduced on the 3rd chromosome of *Hikone-R*.

PTU-resistance was shown in high degree by strains that were simultaneously DDT-susceptible and nicotine-resistant, but not shown at all by strains that were susceptible both to DDT and nicotine nor by strains that were resistant both to DDT and nicotine. Hence it may by concluded that all PTU-resistant strains are susceptible to DDT and resistant to nicotine sulfate, while the PTU-susceptible strains are divided two types, one resistant and the other susceptible both to DDT and to nicotine (Table 1).

The results of reciprocal crosses shown in Tables 3, 4, 5, and 6 suggest that PTU-resistance may be controlled by three genes at least; i. e. two main genes and one modifier. Of the main genes, one is recessive and the other is dominant, while the modifying gene is incompletely dominant and located on the sex chromosome.

Resistance to PU, a compound far less insecticidal than PTU, is shown by the DDT-resistant *Hikone-R*$_{31}$, *WMB*$_{30}$ and *WMD*$_{7\text{-}38}$ strains. Complete susceptibility to PU at half the dosage that fail to kill the resistants is shown by the DDT-susceptible strains.

The relationship between PTU- and PU-resistance and the chromosomes. As mentioned above, there is some evidence (Table 5) that PTU-tolerance may be partially enhanced by a factor on the sex chromosome which is incompletely dominant over susceptibility. Also, as shown in the reciprocal crosses between PTU-resistant and DDT-susceptible PTU-susceptible strains (Table 6), cytoplasmic or maternal effects may have a slight effect on the resistance of F_1 progeny. More detailed experiments are required before any conclusion may be drawn on these points, which are of secondary importance in controlling PTU-resistance.

For this reason, an autosome analysis for resistance was carried out. This consisted of backcrosses between the DDT-susceptible PTU-resistant *cn; bar-3; gvl* strain and the DDT-resistant PTU-susceptible *Hikone-R*$_{31}$ strain (backcrosses 1 & 2), and between the DDT-susceptible PTU-susceptible *bw; st; sv*^{n} strain and the DDT-susceptible PTU-resistant *y* and *Canton-S* strains (backcrosses 3, 4 & 5).

The results of backcrosses 1 and 2 (Table 7) indicate that the recessive PTU-resistant gene possessed by the PTU-resistant strains is located on the 2nd chromosome. The results of backcrosses 3, 4 and 5 (Table, 8) indicate that the dominant PTU-resistant gene possessed by the PTU-resistant strains is located on the 3rd chromosome. These results therefore indicate that PTU-resistance is controlled not only by the recessive gene on the 2nd chromo-

some but also by the dominant gene on the 3rd chromosome.

PU-resistance, as reciprocal crosses show (Table 9), is almost completely dominant over PU-susceptibility. Moreover here cytoplasmic or maternal effects seem to be almost negligible, the sex ratio in the F_1 flies being normal under PU pressure. These results indicate that PU-resistance is controlled by a dominant factor. The results of backcrosses between PU-susceptible marker strains with the DDT-susceptible gene, and a PU-resistant strain with the DDT-resistant gene (Table 7) indicate that this factor derives mainly from dominant genes for PU-resistance located on the 2nd and 3rd chromosomes.

The locus for the PTU-and PU-resistant genes on the 2nd chromosome. This was estimated from the results of the backcrosses reported in Tables 10 and 11. They indicate that the recessive PTU-resistant gene is located on the right arm of the 2nd chromosome near the *vg* (II-67) gene, and that the dominant PU-resistant gene is also located near the *vg* gene on the 2nd chromosome. This locus for the PTU- and PU- resistant genes closely agrees with the locus determined for DDT- and BHC-resistance by Tsukamoto and Ogaki[12], and also with that reported for parathion-resistance by Kikkawa[4].

Therefore, it may be assumed that the PU-resistant gene on the 2nd chromosome occupies the same locus as the gene controlling a general resistance to DDT, BHC and parathion. There is the possibility, but no direct evidence that these genes are pseudoallele each other. Moreover the DDT-resistant gene might act phenotypically as the PU-resistant gene in the presence of PU, at least in the DDT-resistant strains such as *Hikone-R*$_{31}$, *WMB*$_{30}$, *WMD*$_{7-35}$, and *cn R bw*.

Similarly, the PTU-resistant gene evidently occupies the same locus on the 2nd chromosome as the gene controlling general resistance to insecticides.

But whereas the PU-resistant gene is dominant over the susceptible allele, the PTU-resistant gene is recessive to the susceptible allele. Hence it is very interesting that the presence of PTU-resistant gene on the 2nd chromosome apparently confers a susceptibility to DDT, BHC, parathion and PU. Vice versa, the presence of the PTU-susceptible gene on the 2nd chromosome means to confer a resistance to DDT, BHC, parathion and PU.

It is therefore possible that the gene for resistance to DDT (and to PU, BHC and parathion) and the PTU-susceptible gene are either pseudoalleles or closely linked genes, but is preferable to conclude that PTU-susceptibility results from the pleiotropic expression of the DDT-resistant gene. If this is so, the investigation of PTU-susceptibility may be useful for the study of cross-resistant mechanism, and *Drosophila* which are generally resistant to DDT, BHC and parathion may be controlled by selection pressure from PTU.

The locus for PTU-and PU-resistant genes on the 3rd chromosome. This was estimated from the results of the backcrosses shown in Table 13. They indicate that the dominant PTU-resistant gene is located slightly to the right of the spindle-fibre attachment (III-47) on the 3rd chromosome; i. e. the locus III-50 ± calculated for the PTU-resistant gene of the *bw*; *III*$_{Hikone-R}$ strain from the data of backcross 13 closely coincides with the value III-49.0 calculated for the *bw*; *III*$_{NS-R}$ strain.

An assumed locus of III-50± for this PTU-resistant gene closely agrees with the locus of the gene for nicotine-resistance reported by Tsukamoto and Hiroyoshi[14]. Thus it may be assumed that the PU-resistant gene on the 3rd chromosome is located approximately between the *st* and *ss*, and that the dominant gene for PTU-and PU-resistance occupies the same locus on the 3rd chromosome as the dominant nicotine-resistant gene.

The following hypothesis may be drawn from the results mentioned above. The dominant gene at II-64~66 which confers resistance to DDT, BHC and parathion also confers resistance to PU (phenylurea), and abnormal susceptibility to PTU (phenylthiourea). The dominant gene at III-50 ± which confers resistance to nicotine, also confers resistance to PTU as well as to PU.

A logical outcome of this reasoning is to consider that PTU pressure could restore DDT-

susceptibility to the surviving strain, but in so doing it could assemble the nicotine-resistant gene on the 3rd chromosome. Therefore, if nicotine-resistant gene on the 3rd chromosome does not appear the high level as DDT-tolerance, this assumption suggests the possibility that the mixture of DDT, BHC or parathion with a substance correlated negatively to these compounds, such as PTU, might have an effective insecticidal action and might not produce flies resistant to these mixtures even after the continuous use. On the other hand, PU pressure could restore DDT-resistance and nicotine-resistance in a few generations. Several experiments are in progress on the effects of selection pressure with PTU and PU, and will be reported in a subsequent paper.

The author is greatly indebted to Prof. H. Kikkawa and Mr. M. Tsukamoto for their comments during the course of the study and for supplying the insecticide-resistant strains, and to Dr. A.W.A. Brown of W.H.O. and Dr. J.F. Crow of the University of Wisconsin for their kind assistance in the preparation of the English manuscript.

Summary

(1) The levels of resistance to PTU (phenylthiourea) and PU (phenylurea) were determined for strains of *D. melanogaster* some of which were resistant to DDT, BHC, parathion and others of which were susceptible to these insecticides. It was found that the resistant *Hikone-R*, WMB_{30}, WMD_{7-38} and *cn R bw* strains were less resistant to PTU than the susceptible *Canton-S*, *cn bw*, etc. strains. In contrast, the resistant strains were more resistant to PU than the susceptible strains.

(2) All the PTU-resistant strains were susceptible to DDT, but the PTU-susceptible strains were of two types, one DDT-resistant and the other DDT-suceptible.

(3) Genetical analysis indicated that the PTU-resistance was mainly controlled by a recessive gene on the 2nd chromosome and a dominant gene on the 3rd chromosome with a contribution from an incompletely dominant factor on the sex chromosome. On the other hand, the PU-resistance is mainly controlled by two dominant genes respectively on the 2nd and 3rd chromosomes.

(4) The 2nd chromosomal recessive gene for PTU-resistance was located on the right arm of the 2nd chromosome near the *vg* (II-67) gene, while the dominant gene for PU-resistance was also trace to the same locus on the 2nd chromosome. This locus (i. e. II-64~66) for PTU- and PU-resistance closely agrees with the locus already established for resistance to DDT, BHC and parathion. It may therefore be assumed that PTU-susceptibility and PU-resistance result from the pleiotropic expression of the dominant gene for resistance to these other insecticides.

(5) The 3rd chromosomal dominant gene for PTU-resistance was traced to the same locus (III-50±) as the dominant gene already described for nicotine-resistance. The 3rd chromosomal dominant gene for PU-resistance also seems to occupy the same locus as the nicotine-resistant dominant gene, although a precise determination could not be made.

(6) From these results, the following hypothesis was introduced; that is, "the dominant gene (II-64~66) on the 2nd chromosome which confers resistance to DDT, BHC and parathion also confers resistance to PU and abnormal susceptibility to PTU. The dominant gene (III-50±) on the 3rd chromosome which confers resistance to nicotine sulfate, also confers resistance to PTU as well as PU.

(7) It was suggested that there was the possibility of new type of insecticides which might have an effective insecticidal action to insecticide-resistant strains and might not produce flies resistant to the new type of insecticides even after the continuous use.

Literature

1) Brown, A.W.A. et al.: Canad. J. Res. D. **26**, 117 (1948).
2) Fox, A. L.: Proc. Nat. Acad. Sci. U. S. **18**, 115 (1932).
3) Goldsmith, E. D. and M. Harnly: Science **103**, 649 (1946).
4) Kikkawa, H.: Japan. J. Gen., **28**, 171(1953).
5) McGovran, E. R. and P. G. Piquett: J. Econ. Entomol. **36**, 936 (1943).
6) Minaeff, M.G. and T.H. Wright: Ind. Eng. Chem. **21**, 1187 (1929).
7) Ogita, *Z.*: Drosophila Information Service

31, 146 (1957).
8) Ogita, Z.: Inform. Cir. Resistance Problem, W.H.O. 12, 4 (1958).
9) Oshima, C. and T. Hiroyoshi: Botyu-Kagaku 21, 65 (1956).
10) Tsukamoto, M.: Botyu-Kagaku 20, 73 (1955).
11) Tsukamoto, M. and M. Ogaki: Botyu-Kagaku 18, 39 (1953).
12) Tsukamoto, M. and M. Ogaki: Botyu-Kagaku 19, 25 (1954).
13) Tsukamoto, M., M. Ogaki and H. Kikkawa: Proc. Intern. Genet. Symposia 442 (1956).
14) Tsukamoto, M. and T. Hiroyoshi: Botyu-Kagaku 21, 71 (1956).
15) Wichmand, H.: Working paper 8 Add. 1, Expert committee on Insecticide, W. H. O. (1956).

The Effects of Rotenone and Its Derivatives on the Respiration of Brain in Guinea Pig.

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36. モルモット脳の呼吸に及ぼすロテノーンおよびその誘導体の影響 深見順一（東京大学農学部 害虫学研究室）・富沢長次郎（農林省農業技術研究所） 33. 9. 26 受理

ロテノーンは昆虫体内で神経および筋肉の細胞呼吸を抑制し，死に至らしめるが，その細胞呼吸抑制の一部はグルタミン酸脱水素酵素の抑制によるものである[5]．しかし昆虫におけるグルタミン酸脱水素酵素の細胞呼吸における役割は不明である．そこで筆者らはグルタミン酸脱水素酵素の役割が細胞呼吸においてかなり理解されているモルモットの脳を使用してロテノーンの影響を検討した．モルモット脳ホモジエネートおよびミトコンドリアに基質無添加，コハク酸およびグルタミン酸を添加した場合の酸素消費に対するロテノーンおよびその誘導体の影響を調べたところ，その阻害の強度は昆虫の場合と同じ傾向を示した．

The authors have concluded in the previous reports that rotenone inhibits the respiration of nerve and muscle in insects, and the inhibition of the respiratory metabolism is partly due to the inhibition of the glutamic dehydrogenase activity[4,5]. It was also found in rotenone and its derivatives that a close correlation exists between the *in vivo* toxicity against insects and the degree of the inhibition of glutamic dehydrogenase in insect muscle[6].

Although the role of glutamic dehydrogenase in cellular respiration is not known in insects, it is more fully demonstrated in the brain of mammals. The present study was therefore undertaken with the purpose of determining whether rotenone and its derivatives inhibited the homogenate respiration with or without succinate and the glutamic dehydrogenase activity from the brain of the guinea pig.

The authors wish to thank Assist. Prof. T. Yamasaki and Mr. T. Narahashi of the Faculty of Agriculture, University of Tokyo for their guidance during the course of this work.

Materials and Methods

All animals used were male guinea pigs weighing approximately 250 g.

Measurement of oxygen consumption of brain homogenate: Experiments were carried out using the Warburg manometers at 37°C. The animals were killed by decapitation. A brain (1.5g) which contained cerebrum, cerebellum and medulla oblongata was homogenized with five times as much as volume of phosphate buffer (1/15 M, pH 7.4) as the brain and filtered through gauze. These procedures were carried out at 10°C. Flask contents were as follows: 1/15 M phosphate buffer (pH 7.4) 1.2 ml, substrate 0.4 ml (final concentration, 0.033 M), homogenate 1.3 ml, distilled water 0.7 ml or distilled water 0.3 ml plus inhibitor 0.4 ml.

Measurement of oxygen consumption by the

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