the maximum synergistic action is obtained could be determined theoretically. The minimum of z calculated is 2.297. It is concluded that the maximum synergistic toxicity for the house fly would be obtained when lindane and Hercules 5727 were mixed in the ratio of 3.7:2.3.

Summary

Joint toxic action between lindane and Hercules 5727 for the common house fly was synergistic. The maximum mortality would be obtained when lindane and Hercules 5727 were mixed in the ratio of 3.7:2.3.

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Genetic Analyses of DDT-Resistance in Two Strains of The Housefly, *Musca domestica* L.*

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16. イエバエにおける DDT 抵抗性の遺伝学的分析 塚本増久・鈴木玲子 (大阪大学 医学部遺伝学教室) 39. 11. 5 受理

従来イエバエの殺虫剤抵抗性の遺伝に関する論文は数多く報告されているが，そのほとんどは簡単な交配実験の域を出ないものであって，その結論についても遺伝学的には不適切不充分なものが多かった．そこで筆者らはイエバエのいくつかの可視突然変異を用いて起原の異なる DDT 抵抗性の2系統の DDT 抵抗性および DDT+DMC 抵抗性について遺伝学的分析をおこなった．その結果，イエバエの DDT 抵抗性には，すくなくとも第2染色体の不完全劣性遺伝子および第5染色体の優性遺伝子の2つの主要遺伝子が含まれていること，そのうち第5染色体の抵抗性遺伝子は *cm* ミュータントの近くに存在していることなどが明らかとなった．また，この第5染色体の DDT 抵抗性遺伝子は DDT の協力剤 DMC によってその働きが抑制されるが，第2染色体の遺伝子ではそのような影響は認められなかった．DMC は DDT 脱塩酸酵素の阻害剤でもあるので，第5染色体の抵抗性遺伝子は脱塩酸酵素による DDT の代謝を支配していること，第2染色体の抵抗性遺伝子は DMC では阻害されない別の抵抗性メカニズムに関与していることなどが推論された．

Inheritance of knockdown-resistance to DDT in an Italian strain of the housefly was first found by Harrison[7] in 1951 to be due to a single recessive genepair. Since then, a number of studies on the inheritance of resistance to insecticides, such as crossing experiments between genetically unmarked strains, were reported in various insects. These results on the mode of inheritance were reviewed by Brown[2], Crow[4], Davidson and Mason[6], Milani[19,21], etc. Especially on DDT-resistance of the housefly, some investigators indicated that a simple recessive factor was concerned to the knockdown-resistance (Barbesgaard and Keiding[1], Keiding[11], and Milani[18]) or that a dominant monofactorial one was concerned to the inheritance of kill-resistance (Lichtwardt[13] and Maelzer and Kirk[16]). However, the majority of early investigators concluded that multifactorial systems were responsible for the kill-resistance to DDT because they could not find any evidence of a typical Mendelian segregation in filial generations of crosses between resistant and susceptible strains (Bruce and Decker[3], D'Alessandro and Mariani[5], La Face[12], March[17], Norton[24], etc.). Maelzer and Kirk[16] also reported that, although the inheritance of high DDT-resistance was monofactorial, the inheritance of intermediate (or "weak") resistance to DDT seemed to be multifactorial. In addition,

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Harrison[8,9] studying kill-resistance stated that the F_1 flies had an intermediate resistance level but the F_2 data were indicative of multifactorial inheritance in contrast to the monofactorial character of knockdown-resistance.

Furthermore, Johnston, Bogart and Lindquist[10] concluded that the factor responsible for DDT (kill)-resistance was carried in the cytoplasm. However, their data were insufficient to draw such a conclusion.

Using an Italian strain (Latina), Milani[18] first gave the symbol *kdr* for knockdown resistance gene which was inherited as an incompletely recessive autosomal character in agreement with the findings of Harrison[7]. Later he[20] demonstrated that the *kdr* and two visible markers, *bwb* (brown-body) and *dv* (divergent), belonged to the 2nd linkage group with the following recombination values: *bwb-dv*: 40.90%; *bwb-kdr*: 48.61%; *dv-kdr*: 45.83%. Furthermore, the researches reported by Milani and his co-workers[22,23] suggested that the resistance character in an American resistant strain (Orlando-R) was recessive and was assorted to the 2nd chromosome, but the *kdr* and the resistance factor of the Orlando-R strain were not allelic to each other, and hence another symbol *kdr-o* was given by them for the latter gene.

Lovell and Kearns[15] demonstrated the parallelism in the inheritance between high DDT-dehydrochlorinase activity and DDT-resistance in an American strain resistant to a mixture of DDT+DMC. It is, however, not yet sure whether the resistance factor is identical to one of the 2nd chromosomal resistance genes mentioned above, or whether the higher enzyme activity is truely due to the action of resistance gene itself or merely due to other gene located on the same chromosome by chance.

Although preliminary results of genetic analyses of insecticide-resistance in the housefly were already reported by us at the annual meeting of the Japanese Society of Sanitary Zoology in 1962[25] and the annual meeting of the Genetic Society of Japan in 1963[31], almost all the results were not yet published. The purpose of the present paper is, therefore, to describe details of results on the linkage group analysis of DDT-resistance factors and on the map position of the 5th chromosomal major gene in two different resistant strains of the housefly.

Materials and Methods

Housefly Strain: JIR: Highly resistant both to DDT and to DDT+DMC. Visually wild phenotype. Selected on bait for DDT-resistance for more than 3 years and further for DDT+DMC-resistance for 2 years in this laboratory from a mixed population of various Japanese strains collected from fields in 1958. At present almost all the adults can survive at a topical dose of 100μg DDT+100μg DMC/fly.

DMC-R: Highly resistant to DDT and to DDT+DMC. Originally derived from the University of California, Riverside, U. S. A., then the University of Illinois, Urbana, U. S. A. A subcolony of this strain was reared as "Strain L" in the Laboratory for Research on Insecticides, Utrecht, Netherlands, from where we received the strain in 1962. Identical with the DMC-R strain used by Lovell and Kearns[15]. At that time, this strain consisted of about 23% susceptible and 77% DDT-resistant individuals. Therefore, further selections for DDT+DMC-resistance were continued on bait for successive generations in this laboratory. As the results of selections, the susceptible portion was practically eliminated from the strain: i. e., at present practically no mortality is observed at a dose range of 10–200μg DDT or DDT+DMC/fly. Visually wild phenotype.

R (*bwb*; *ocra*; *ar*; *acv*): A DDT-resistant strain marked with *bwb* (brown-body, 2nd chromosome), *ocra* (ocra eyes, 3rd chromosome), *ar* (aristapedia, 5th chromosome) and *ac* (ali curve, 6th chromosome). Synthesized by hybridizingthe JIR strain and a multichromosomal mutant strain. Resistance level to DDT is similar to that of the JIR strain.

Lab: A highly susceptible laboratory strain originated from the NAIDM 1948 strain. Obtained from Mrs. E. T. Lichtwardt, Kansas, U. S. A., as a subcolony of the IS-1 strain[13] in 1960. LD_{50}: 0.03-0.04 μg/fly.

ro; *ext*; *cm*; *acv*: A highly susceptible multichromosomal mutant strain marked with *ro* (rough

eyes, 2nd (chromosome), *ext* (extended wings*, 4th chromosome), *cm* (carmine eyes, 5th chromosome) and *acv* (anterior-cross-veinless, 6th chromosome**). LD_{50}: 0.03–0.04μg/fly.

ro;cm;acv: A highly susceptible strain marked with the mutant *ro*, *cm* and *acv* (2;5;6). LD_{50}: 0.03～0.04μg/fly.

ar cm: A susceptible strain. Marked with the 5th chromosomal mutants, *ar* and *cm*. LD_{50}: 0.15 μg/fly.

Susceptibility test to DDT: Treatments were performed by topical application of *p,p'*-DDT (or *p,p'*-DDT+DMC) in one μl acetone onto the dorsum of the thorax of one-day-old flies. Extremely higher doses of DDT, such as 200μg/fly or more, were based on the application of two drops (2μl/fly). This volume of acetone alone did not give any appreciable mortality in preliminary experiments. Mortality counts were done at 24-hours after the treatment with DDT. Moribund flies were counted as dead.

For the susceptibility test or resistance test of strains used or their progeny to obtain the ld-p line, usually 50 females and 50 males were used as a group for each dose. For genetic analyses of resistance, however, mixed sex one-day-old fies were randomly treated with a given dose of the insecticide in question.

Crossing procedure: Usually at least 100 virgin females were collected within 10 hours after emergence of adults and were used in mass-matings. Determination of linkage groups of both dominant and recessive resistance factors was based on the F_1 male backcross and subsequent factorial analysis of data (Tsukamoto[26]) and determination of locus of a major resistance gene on the chromosome was based on the F_1 female backcross and subsequent calculating method described in a previous paper[27]. Actual crossing experiments carried out are listed in Table 1.

Breedings of flies and tests with insecticides were carried out at a constant temperature of 25°C.

* A new mutant isolated from an experimental cross. Wings extended horizontally from the body axis. Assorted to the 4th linkage group.

** The former 4th linkage group to which the mutant *acv* was assorted[29] has recently been revised to be the 6th linkage group[30].

Determination of Linkage Group

Experiments with DDT Cross 1: As shown in Figure 1, the F_1 progeny of this cross were highly resistant to DDT, indicating dominance of DDT-resistance character: i.e. almost all the hybrid flies could survive at a dose range of 0.5μg/fly and even at higher doses of 50 or 100μg/fly mortality did not exceed an average of 10%. F_1 males were backcrossed to virgin females of the susceptible mutant strain, *ro;ext;cm;acv* (or briefly "reca" in figures), without any application of the insecticide. This backcross was designed to detect dominant effect of resistance factor, if any, on the 2nd, 4th, 5th and 6th chromosomes. The resultant backcross progeny were treated with various doses of DDT at the next day of emergence of adults.

Examination of the shape of the ld-p line for the backcross progeny shown in Figure 1 strongly suggests the existance of at least one major dominant resistance factor. Namely, the 1:1 segregation ratio of the R and S genotypes seems to have resulted in the plateau at a dose range of 0.5–15μg/fly.

For factorial analysis to detect each chromo-

Table 1. Crossing schemes employed in analyses of DDT-resistance

Cross	Procedure	For determining
1	*ro;ext;cm;acv*♀♀ × F_1(JIR♀♀ × *ro;ext;cm;acv*♂♂)♂♂	Linkage group (2;4;5;6)
2	JIR♀♀ × *ro;ext;cm;acv*♂♂⟶F_1♀♂⟶F_2	Linkage group (2;4;5;6)
3	R(*bwb;ocra;ar;ac*)♀♀ × F_1{R(*bwb;ocra;ar;ac*)♀♀ × Lab♂♂}♂♂	Linkage group (2;3;5;6)
4	*ro;cm;acv*♀♀ × F_1(DMC-R♀♀ × *ro;cm;acv*♂♂)♂♂	Linkage group (2;5;6)
5	F_1(JIR♀♀ × *ar cm*♂♂)♀♀ × *ar cm*♂♂	Gene locus
6	F_1(DMC-R♀♀ × *ar cm*♂♂)♀♀ × *ar cm*♂♂	Gene locus

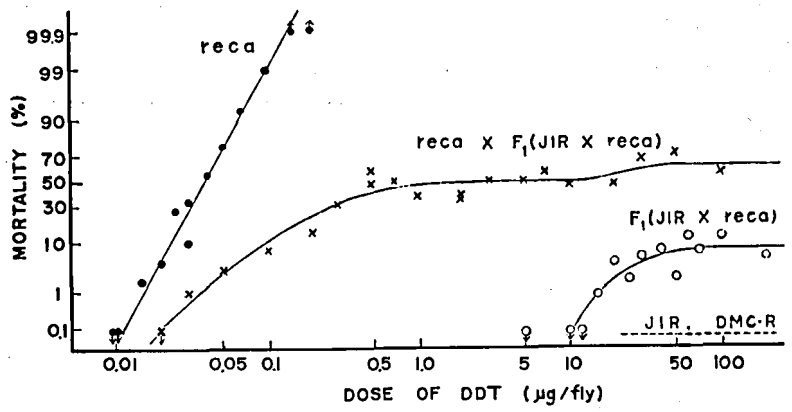

Figure 1. Resistance levels to DDT in the susceptible *ro*;*ext*;*cm*;*acv* ("reca") strain, the backcross progeny and the F_1 hybrid of Cross 1, and the resistant strains.

Table 2. Genetic analysis for dominant resistance factors in the following backcross (Cross 1): *ro*;*ext*;*cm*;*acv*♀♀ × F_1(JIR♀♀ × *ro*;*ext*;*cm*;*acv*♂♂)♂♂. Treated with a topical dose of DDT at 50 μg/fly.

(Phenotype) (2;4;5;6)	Exp. 1			Exp. 2			Pooled	Mean
	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Arc-sine survival		
+ ; + ; + ; +	108	102	76.36	124	119	78.42	154.78	77.39
ro ; + ; + ; +	96	53	47.99	102	52	45.56	93.55	46.78
+ ; *ext* ; + ; +	40	39	80.90	32	30	75.52	156.42	78.21
ro ; *ext* ; + ; +	32	14	41.41	16	10	52.24	93.65	46.83
+ ; + ; *cm* ; +	88	0	0	92	0	0	0	0
ro ; + ; *cm* ; +	100	0	0	99	0	0	0	0
+ ; *ext* ; *cm* ; +	53	0	0	29	0	0	0	0
ro ; *ext* ; *cm* ; +	36	0	0	23	0	0	0	0
+ ; + ; + ; *acv*	90	86	77.83	86	82	77.55	155.38	77.69
ro ; + ; + ; *acv*	88	35	39.10	77	32	40.14	79.24	39.62
+ ; *ext* ; + ; *acv*	52	50	78.69	41	40	81.01	159.70	79.85
ro ; *ext* ; + ; *acv*	37	18	44.23	32	11	35.90	80.13	40.07
+ ; + ; *cm* ; *acv*	81	0	0	66	0	0	0	0
ro ; + ; *cm* ; *acv*	77	0	0	59	0	0	0	0
+ ; *ext* ; *cm* ; *acv*	60	0	0	33	0	0	0	0
ro ; *ext* ; *cm* ; *acv*	59	0	0	39	0	0	0	0
Total	1097	397	486.51	950	376	486.34	972.85	486.44

somal resistance effect, the backcross progeny were treated with DDT at a dose of 50μg/fly. Table 2 gives the actual data and arc-sine-transformed survival rates. The evidence that no *cm* phenotyped flies could survive at the dose tested suggests the existance of at least one major dominant resistance factor in accordance to the information obtained from the shape of the ld-p line. Dominant effect of each chromosomal factor was then calculated from these data by factorial analysis and subsequent analysis of variance as summarized in Table 3. From this table, it is evident that in addition to the 5th chromosomal major resistance factor, the 2nd chromosomal factor also contributes to such a high level of DDT-resistance. Detailed examination of the shape of the ld-p line also suggests the existance of another plateau at a dose range of

Table 3. Analysis of variance for dominant effect of chromosomes on DDT-resistance in the JIR strain (Cross 1).

Source of variation	Resistance effect	Sum of squares	Degrees of freedom	Mean square	F
Total	—	34686.41	31	—	—
Phenotypes	(486.44)	34570.25	15	2304.68	297.62**
2	139.84	2444.40	1	2444.40	315.66**
4	−3.48	1.51	1	1.51	0.20
2×4	−2.48	0.77	1	0.77	0.10
5	486.44	29577.98	1	29577.98	3819.62**
2×5	139.84	2444.40	1	2444.40	315.66**
4×5	−3.48	1.51	1	1.51	0.20
2×4×5	−2.48	0.77	1	0.77	0.10
6	11.98	17.94	1	17.94	2.32
2×6	−15.86	31.44	1	31.44	4.06
4×6	1.74	0.38	1	0.38	0.05
2×4×6	0.94	0.11	1	0.11	0.01
5×6	11.98	17.94	1	17.94	2.32
2×5×6	−15.86	31.44	1	31.44	4.06
4×5×6	1.74	0.38	1	0.38	0.05
2×4×5×6	0.94	0.11	1	0.11	0.01
Replications	—	0.00	1	0.00	0.00
Error	—	116.16	15	7.74	—

** Significant at 1% level.

Table 4. Genetic analysis for both dominant and recessive resistance factors in the following intercross (Cross 2): JIR♀♀ × *ro*; *ext*; *cm*; *acv*♂♂ ⟶ F_1 ⟶ F_2. Treated with a topical dose of DDT at 50 μg/fly.

Phenotype (2;4;5;6)	Exp. 1			Exp. 2			Pooled	Mean
	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Arc-sine survival		
+ ; + ; + ; +	363	293	63.95	256	227	70.33	134.28	67.14
ro ; + ; + ; +	134	82	51.46	88	61	56.36	107.82	53.91
+ ; *ext* ; + ; +	51	43	66.67	29	26	71.24	137.91	68.96
ro ; *ext* ; + ; +	10	3	33.21	10	5	45.00	78.21	39.11
+ ; + ; *cm* ; +	103	27	30.80	102	23	28.35	59.15	29.58
ro ; + ; *cm* ; +	44	0	0	40	0	0	0	0
+ ; *ext* ; *cm* ; +	12	4	35.26	7	1	22.21	57.47	28.74
ro ; *ext* ; *cm* ; +	2	0	0	3	0	0	0	0
+ ; + ; + ; *acv*	111	85	61.06	79	63	63.26	124.32	62.16
ro ; + ; + ; *acv*	26	8	33.69	28	10	36.70	70.39	35.20
+ ; *ext* ; + ; *acv*	26	17	53.96	9	7	61.88	115.84	57.92
ro ; *ext* ; + ; *acv*	7	3	40.90	5	3	50.77	91.67	45.84
+ ; + ; *cm* ; *acv*	28	6	27.58	18	6	35.26	62.84	31.42
ro ; + ; *cm* ; *acv*	7	0	0	5	0	0	0	0
+ ; *ext* ; *cm* ; *acv*	10	1	18.44	4	1	30.00	48.44	24.22
ro ; *ext* ; *cm* ; *acv*	2	0	0	2	0	0	0	0
Total	936	572	516.98	685	433	571.36	1088.34	544.20

Table 5. Analysis of variance for combined (dominant & recessive) effect of chromosomes on DDT-resistance in the JIR strain (Cross 2)

Source of variation	Resistance effect	Sum of squares	Degrees of freedom	Mean square	F
Total	—	18429.45	31	—	—
Phenotypes	(544.20)	18045.66	15	1203.04	61.93**
2	196.08	4805.92	1	4805.92	247.41**
4	14.62	26.72	1	26.72	1.38
2×4	6.30	4.96	1	4.96	0.26
5	316.28	12504.13	1	12504.13	643.72**
2×5	−31.84	126.72	1	126.72	6.52*
4×5	−1.46	0.27	1	0.27	0.01
2×4×5	−9.78	11.96	1	11.96	0.62
6	30.68	117.66	1	117.66	6.06*
2×6	6.72	5.64	1	5.64	0.29
4×6	13.02	21.19	1	21.19	1.09
2×4×6	−37.86	179.17	1	179.17	9.22**
5×6	25.32	80.14	1	80.14	4.13
2×5×6	1.36	0.23	1	0.23	0.01
4×5×6	25.74	82.82	1	82.82	4.26
2×4×5×6	−25.14	79.00	1	79.00	4.07
Replications	—	92.41	1	92.41	4.75**
Error	—	291.37	15	19.42	—

* Significant at 5% level, ** Significant at 1% level.

30–100μg/fly. However, it is not sure whether this plateau can be ascribable to the 2nd chromosomal resistance factor or to the physiological saturation of DDT at a site of penetrance of the insecticide.

Cross 2: Both sexed F_1 hybrids of Cross 1 were intercrossed to produce the F_2 progeny. The resultant progeny were topically treated with DDT at a dose of 50μg/fly, and the survival rates were calculated for each visible phenotype (Table 4) in order to submit them to factorial analysis. Results of the statistical analyses (Table 5) also indicate that DDT-resistance of the JIR strain is due to a multifactorial genetic system in which both the 5th and the 2nd chromosomal factors are major ones. The 6th chromosomal effect was also statistically significant. In this cross, however, it is impossible to distinguish the dominant effect from the recessive effect of the resistance genes because most of the heterozygotes can survive at this high dose of DDT.

Cross 3: In order to estimate the recessive effect of resistance factor(s), a special resistant strain R(*bwb*; *ocra*; *ar*; *ac*) of which the 2nd, 3rd, 5th and 6th chromosomes were marked with visible mutants was established. Virgin females of the marked resistant strain were mated with males of the susceptible Lab strain (wild type) in a clean cage for 5–6 days. Then females of the resultant F_1 flies were tested for their resistance level to DDT, and male survivors at higher doses of DDT were mated to virgin females of the resistant parent strain. The mated females were also treated with DDT at a dose of 50μg/fly and the survivors were transferred into a cage for oviposition.

One-day-old flies of the backcross progeny were topically treated with DDT at a dose of 100μg/fly where a mixture of acetone and olive oil (3:1) was employed as a solvent of DDT. Table 6 gives the relation between visible phenotypes of the progeny and survival rates. Results of factorial analysis to detect effects of each homozygous resistant chromosome or interactions between them were summarized in a usual way (Table 7). From these tables, it is clear that in this case the effect of the 2nd chromosomal resistance factor is most responsible for the resistance,

Table 6. Genetic analysis for recessive resistance factors in the JIR strain. Cross 3 : R(*bwb*;*ocra*;*ar*;*ac*)♀ ♀× F_1{R(*bwb*;*ocra*;*ar*;*ac*)♀♀ × Lab♂♂}♂♂.
Treated with a topical dose of DDT at 100 μg/fly.

Phenotype (2;3;5;6)	Exp. 1			Exp. 2			Exp. 3			Pooled	Mean
	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Arc-sine survival		
bwb ; *ocra* ; *ar* ; *ac*	19	19	90.00	44	43	81.34	44	44	90.00	261.34	87.11
+ ; *ocra* ; *ar* ; *ac*	34	14	39.92	32	17	46.80	35	21	50.77	137.49	45.83
bwb ; + ; *ar* ; *ac*	27	27	90.00	38	37	80.67	51	50	81.95	252.62	84.21
+ ; + ; *ar* ; *ac*	42	28	54.74	50	35	56.79	56	34	51.19	162.72	54.24
bwb ; *ocra* ; + ; *ac*	47	38	64.05	42	30	57.69	37	32	68.43	190.17	63.39
+ ; *ocra* ; + ; *ac*	54	2	11.09	23	4	24.64	30	2	14.97	50.70	16.90
bwb ; + ; + ; *ac*	60	53	70.03	44	37	66.49	51	46	71.76	208.28	69.43
+ ; + ; + ; *ac*	58	3	13.14	47	10	27.48	38	4	18.94	59.56	19.85
bwb ; *ocra* ; *ar* ; +	23	23	90.00	33	32	79.97	61	60	82.64	252.61	84.20
+ ; *ocra* ; *ar* ; +	40	15	37.76	46	36	62.21	35	13	37.54	137.51	45.84
bwb ; + ; *ar* ; +	35	34	80.26	34	34	90.00	33	33	90.00	260.26	86.75
+ ; + ; *ar* ; +	56	23	39.86	48	44	73.23	36	18	45.00	158.09	52.70
bwb ; *ocra* ; + ; +	40	30	60.00	45	39	68.59	33	28	67.09	195.68	65.23
+ ; *ocra* ; + ; +	49	6	20.48	47	7	22.70	35	1	9.74	52.92	17.64
bwb ; + ; + ; +	55	45	64.75	49	41	66.18	51	46	71.76	202.69	67.56
+ ; + ; + ; +	65	4	14.36	34	7	26.98	40	6	22.79	64.13	21.38
Total	704	364	840.44	656	453	931.76	666	440	874.57	2646.77	882.26

Table 7. Analysis of variance for recessive effect of chromosomes on DDT-resistance in the JIR strain (Cross 3).

Source of variation	Resistance effect	Sum of squares	Degrees of freedom	Mean square	F
Total	—	30884.45	47	—	—
Phenotypes	(882.26)	29019.49	15	1934.63	36.30**
2	333.50	20854.17	1	20854.17	391.31**
3	−29.98	168.53	1	168.53	3.16
2×3	13.94	36.44	1	36.44	0.68
5	199.50	7462.55	1	7462.55	140.03**
2×5	−46.18	399.86	1	399.86	7.50*
3×5	0.14	0.00	1	0.00	0.00
2×3×5	17.30	56.12	1	56.12	1.05
6	−0.34	0.02	1	0.02	0.00
2×6	1.14	0.24	1	0.24	0.00
3×6	0.98	0.18	1	0.18	0.00
2×3×6	2.50	1.17	1	1.17	0.02
5×6	4.14	3.21	1	3.21	0.06
2×5×6	−3.46	2.24	1	2.24	0.04
3×5×6	6.82	8.72	1	8.72	0.16
2×3×5×6	11.50	24.80	1	24.80	0.47
Replications	—	266.14	2	133.07	2.50
Error	—	1598.81	30	53.29	—

* Significant at 5% level, ** Significant at 1% level.

although the 5th chromosomal resistance factor is also a major one.

Therefore, from the results obtained from Crosses 1, 2 and 3, it has been confirmed that at least two major factors are responsible for DDT-resistance of the JIR strain: one is the 5th chromosomal dominant factor and the other is the 2nd chromosomal incompletely recessive factor.

Cross 4: Another DDT-resistant strain DMC-R, originated from the nearctic region, was used in genetic analysis. Since the *ro*;*cm*;*acv* strain was employed as the susceptible strain, only the dominant effect of the 2nd, 5th and 6th chromosomes could be examined.

The F_1 progeny of the cross between the DMC-R and *ro*;*cm*;*acv* strains showed to be resistant to DDT as in the case of the JIR strain, indicating dominance of the resistance character. Therefore the F_1 males were backcrossed to the susceptible marker strain, and the resultant progeny were topically treated with 30μg/fly. Results shown in Table 8 also indicate that the 5th chromosomal factor is the major resistance gene, in accordance to the results obtained in the Japanese resistant strain JIR. Factorial analysis of these data, however, showed that no 2nd chromosomal dominant factor contributes to the resistance of this American strain (Table 9).

Experiments with DDT+DMC: Both the resistant strains, JIR and DMC-R, are highly resistant not only to DDT but also to a high

Table 8. Genetic analysis for dominant DDT-resistance factors in the DMC-R strain. Cross 4: *ro*;*cm*;*acv*♀♀ × F_1(DMC-R♀♀ × *ro*;*cm*;*acv*♂♂)♂♂. Treated with a topical dose of DDT at 30 μg/fly.

Phenotype (2;5;6)	Exp. 1			Exp. 2			Exp. 3			Exp. 4		
	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Are-sine survival
+ ; + ; +	100	60	50.77	97	66	55.57	123	75	51.34	89	66	59.45
ro ; + ; +	90	63	56.79	89	56	52.49	91	45	44.69	76	42	48.02
+ ; *cm* ; +	64	0	0	80	0	0	86	0	0	78	0	0
ro ; *cm* ; +	65	0	0	50	0	0	64	0	0	80	0	0
+ ; + ; *acv*	71	37	46.21	83	60	56.64	78	49	52.43	70	43	51.61
ro ; + ; *acv*	66	39	50.23	86	52	51.04	46	22	43.76	60	37	51.75
+ ; *cm* ; *acv*	54	0	0	65	0	0	70	0	0	82	0	0
ro ; *cm* ; *acv*	42	0	0	57	0	0	51	0	0	47	0	0
Total	552	199	204.00	607	234	215.74	609	191	192.22	582	188	210.83

Table 9. Analysis of variance for dominant effect of chromosomes on DDT-resistance in the DMC-R strain (Cross 4).

Source of variation	Resistance effect	Sum of squares	Degrees of freedom	Mean square	F
Total	—	21440.49	31	—	—
Phenotypes	(205.70)	21212.08	7	3030.30	335.90**
2	6.30	19.85	1	19.85	2.20
5	205.70	21158.30	1	21158.30	2345.37**
2×5	6.30	19.85	1	19.85	2.20
6	3.86	7.49	1	7.49	0.83
2×6	1.26	0.81	1	0.81	0.09
5×6	3.86	7.49	1	7.49	0.83
2×5×6	1.26	0.81	1	0.81	0.09
Replications	—	38.96	3	12.99	1.44
Error	—	189.45	21	9.02	—

** Significant at 1% level.

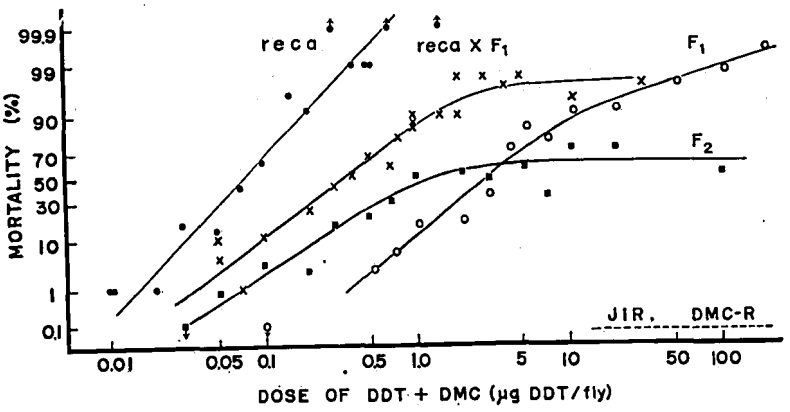

Figure 2. Resistance levels to DDT+DMC in the susceptible strain, backcross progeny, F_1, F_2 and the resistant strains.

dose range of synergized DDT with DMC. When compared to the results with DDT alone, however, the F_1 hybrids of the R×S crosses were not so resistant to DDT+DMC as their parental resistant strains. These observations suggest that at least a recessive resistance factor is responsible for the major part of DDT+DMC resistance. Figure 2 shows the resistance levels of the susceptible *ro*; *ext*; *cm*; *acv* strain, F_1 hybrids, backcross progeny (Cross 1) and the F_2 progeny (Cross 2) respectively. A wide plateau observed on the ld-p line for the F_2 flies also suggests the existance of an incomplete recessive factor.

In order to obtain more detailed information on the role of DMC in DDT-resistance, the genetic analysis has been performed for DDT+DMC-resistance by using the JIR strain. Namely, some fractions of the F_2 flies from Cross 2 were treated with a DDT+DMC (1 : 1) mixture at a dose of 50μg DDT/fly. Since only a few portions of the heterozygotes can survive at this dose, the analysis is effective to detect any recessive effect of the resistance factor(s). As shown in Table 10, almost all the *ro* flies (the 2nd chromosomal marker) were killed by the combination of DDT and DMC, whereas some of the *cm* flies (the 5th chromosomal marker) could survive. Results of factorial analysis of the data shown in Table 11 indicate more exactly that the 2nd chromosomal resistance factor is the major one, and that the effect of the 5th chromosomal DDT-resistance factor is considerably depressed by DMC, although the effect is still significant at 5% level.

A special experimental strain *ro*;DMC-R;*acv* (2;5;6) was established from Cross 4 by substituting the susceptible 5th chromosome with the resistant one. In other words, apart from the unmarked 3rd or 4th chromosome, the 5th chromosome of the resistant strain was inserted into the susceptible *ro*; *cm*; *acv* strain. The resistance level of this special strain was not so resistant to DDT+DMC, while it was highly resistant to non-synergized DDT. These observations also strongly indicate that the 5th chromosomal DDT-resistance factor does not contribute to the high resistance level of the parental DMC-R strain to a DDT+DMC combination.

Estimation of gene locus

In the previous section, each major resistance factor of the resistant strains of different origins has been associated with the 5th chromosome. A question consequently arises whether these major genes are allelic to each other. However, direct crossing experiments between these strains do not seem to be suitable for the elucidation of this problem, because the character DDT-resistance is genetically dominant. Therefore, Crosses 5 and 6 were designed to estimate the gene locus for these 5th chromosomal resistance factors.

Cross 5: As shown in Figure 3, the F_1 hybrid of the JIR strain sometimes contains a fyaction of less resistant individuals although some of

Table 10. Genetic analysis for recessive DDT+DMC-resistance factors in the JIR strain (Cross 2). Treated with 50 μg DDT+ 50μg DMC/fly.

Phenotype (2;4;5;6)	Exp. 1			Exp. 2			Pooled	Mean
	No. of flies tested	alive	Arc-sine survival	No. of flies tested	alive	Arc-sine survival		
+ ; + ; + ; +	507	113	28.17	467	164	36.34	64.51	32.26
ro ; + ; + ; +	159	0	0	165	3	7.75	7.75	3.88
+ ; *ext* ; + ; +	84	35	40.20	55	25	42.39	82.59	41.30
ro ; *ext* ; + ; +	20	0	0	14	0	0	0	0
+ ; + ; *cm* ; +	304	71	28.90	157	37	29.04	57.94	28.97
ro ; + ; *cm* ; +	73	0	0	44	0	0	0	0
+ ; *ext* ; *cm* ; +	38	12	34.19	25	6	29.33	63.52	31.76
ro ; *ext* ; *cm* ; +	15	0	0	5	0	0	0	0
+ ; + ; + ; *acv*	128	32	30.00	99	26	30.83	60.83	30.42
ro ; + ; + ; *acv*	45	0	0	33	0	0	0	0
+ ; *ext* ; + ; *acv*	38	15	38.92	23	5	27.79	66.71	33.36
ro ; *ext* ; + ; *acv*	10	0	0	15	0	0	0	0
+ ; + ; *cm* ; *acv*	57	10	24.76	43	7	23.79	48.55	24.28
ro ; + ; *cm* ; *acv*	22	0	0	12	0	0	0	0
+ ; *ext* ; *cm* ; *acv*	7	0	0	11	3	31.48	31.48	15.74
ro ; *ext* ; *cm* ; *acv*	5	0	0	3	0	0	0	0
Total	1512	288	225.14	1171	276	258.74	483.88	241.97

Table 11. Analysis of variance for recessive effect of chromosomes on DDT+DMC-resistance in the JIR strain (Cross 2).

Source of variation	Resistance effect	Sum of squares	Degrees of freedom	Mean square	F
Total	—	8285.58	31	—	—
Phenotypes	(241.97)	7649.70	15	509.98	12.74**
2	234.21	6856.79	1	6856.79	171.25**
4	−2.35	0.69	1	0.69	0.02
2×4	−10.11	12.78	1	12.78	0.32
5	40.47	204.73	1	204.73	5.11*
2×5	32.71	133.74	1	133.74	3.34
4×5	−13.85	23.98	1	23.98	0.60
2×4×5	−21.61	58.37	1	58.37	1.46
6	34.37	147.66	1	147.66	3.69
2×6	26.61	88.51	1	88.51	2.21
4×6	−13.55	22.95	1	22.95	0.57
2×4×6	−21.31	56.76	1	56.76	1.42
5×6	−7.05	6.21	1	6.21	0.16
2×5×6	−14.81	27.42	1	27.42	0.68
4×5×6	9.11	10.37	1	10.37	0.26
2×4×5×6	1.35	0.23	1	0.23	0.01
Replications	—	35.28	1	35.28	0.88
Error	—	600.59	15	40.04	—

* Significant at 5% level, **Significant at 1% level.

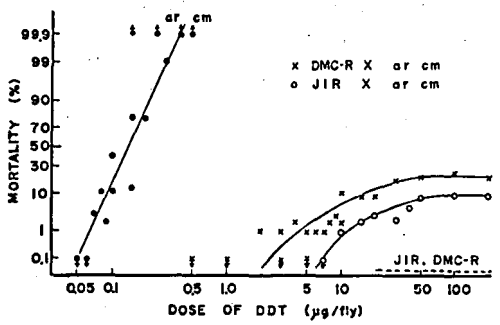

Figure 3. Resistance levels to DDT in the susceptible strain, F_1 progenies and the resistant strains.

them seem to be an accidental mortality. In order to confirm the use of true resistant individuals in crossing experiments, all the mated females were treated with DDT at a dose of 50μg/fly prior to their ovipositions and hence only the survivors could produce their offspring.

The resultant progeny of the F_1 females back-crossed to susceptible *ar cm* males were treated with DDT at a dose range of 50μg/fly, and both the survivors and dead flies were examined for their visible phenotypes at 24 hours after the treatment. Table 12 gives the data obtained with the JIR strain. Out of about ten thousand flies tested, only one *ar cm* fly could survive after the treatment of DDT. From this, it was assumed that the gene order on the chromosome were *ar-R-cm*. Therefore, the calculations of recombination values were carried out by the formulae described in a separate paper[28] as follows:

$$ar-R: \frac{1}{1+\sqrt{(AH-CF)/(BG-DE)}}$$
$$=0.0576(\text{or } 5.8\%)$$
$$R-cm: \frac{1}{1+\sqrt{(AH-BG)/(CF-DE)}}$$
$$=0.0139(\text{or } 1.4\%)$$
$$ar-cm: \frac{1}{1+\sqrt{[(A+E)(D+H)]/[(B+F)(C+G)]}}$$
$$=0.0701 \text{ (or } 7.0\%)$$

where each Capital letter is an observed number of flies corresponding to that in Table 12. Namely, the DDT-resistance gene on the 5th chromosome is located near the *cm* mutant (Figure 4). A new symbol *R-DDT* has been given for this resistance gene.

Table 12. Determination of locus for the DDT-resistance gene on the 5th chromosome in the JIR strain (Japan) of the housefly.

$$\frac{+\ R\ +}{ar\ +\ cm}\ ♀ \times \frac{ar\ +\ cm}{ar\ +\ cm}\ ♂$$

DDT 50μg/fly	Phenotype	Crorrover type	Cord sign	Observed
Alive (R)	+ +	(0 0)	A	4078
	ar +	(1 0)	B	205
	+ *cm*	(0 1)	C	34
	ar cm	(1 1)	D	1
Dead (R+S)	+ +	(1 1)	E	998
	ar +	(0 1)	F	141
	+ *cm*	(1 0)	G	356
	ar cm	(0 0)	H	4673

Cross 6: A similar crossing experiment was designed to estimate the locus of the 5th chromosomal resistance gene of the DMC-R strain in a heterozygous condition.

The results obtained were tabulated in Table 13.

Table 13. Determination of locus for the DDT-resistance gene on the 5th chromosome in the DMC-R strain (U. S. A) of the housefly.

$$\frac{+\ +\ R}{ar\ cm\ +}\ ♀ \times \frac{ar\ cm\ +}{ar\ cm\ +}\ ♂$$

DDT 30–50μg/fly	Phenotype	Crossover type	Cord sign	Observed
Alive (R)	+ +	(0 0)	A	5670
	ar cm	(1 0)	B	14
	ar +	(0 1)	C	18
	+ *cm*	(1 1)	D	5
Dead (R+S)	*ar* +	(1 1)	E	20
	+ *cm*	(0 1)	F	39
	+ +	(1 0)	G	154
	ar cm	(0 0)	H	5179

Contrary to the case of the JIR strain, *cm* flies seemed to be double crossover class. Namely the order of *ar-cm-R* was suggested.

The calculation of recombination values were carried out by the following formulae:

$$R-cm: \frac{1}{1+q\sqrt{[(A+C)(F+H)]/[(B+D)(E+G)]}}*$$
$$* \sqrt[4]{ACEG/BDFH}=0.0054 \text{ (or } 0.5\%)$$

where q is an estimate of the viability for the *cm* flies:

$$q \fallingdotseq \frac{B+D+F+H}{A+C+E+G}=0.8934$$

$$ar-cm: \frac{1}{1+\sqrt[4]{ABGH/CDEF}}$$
$$=0.0314(\text{or } 3.1\%)$$

These values are considerably lower than those

obtained in the JIR strain (Figure 4).

Discussion

Results of the present experiments with DDT have indicated that the kill-resistance (mortality-resistance) to DDT in two DDT-resistant strains is dependent upon at least two major resistance factors: the 5th chromosomal dominant gene and the 2nd chromosomal incompletely recessive gene (Tables 3, 5 and 7).

Although the biochemical or physiological mechanisms of DDT-resistance controlled by the 5th or the 2nd chromosomal resistance factors are still unknown, the dehydrochlorination of DDT to a non-toxic DDE is the most plausible explanation among the possible mechanisms of DDT-resistance proposed by various investigators. Therefore, an assumption may be possible that one of either 5th or 2nd chromosomal resistance factors is responsible for the detoxification of DDT. According to Lovell and Kearns[15], the activity of DDT-dehydrochlorinase *in vitro* in heterozygotes of the housefly was intermediate of those of their parent strains. Because the 2nd chromosomal resistance factor is rather recessive, it is unlikely that this factor is responsible for the metabolism of DDT *in vivo*.

Results of genetic analysis with a DDT+DMC (1:1) mixture (Table 11), however, have evidently showed that the recessive effect of the 5th chromosomal resistance factor is considerably inhibited by the addition of the synergist DMC but that of the 2nd chromosomal one is not when compared to the corresponding experiments with DDT alone (Table 5). As the synergist DMC is well known to be one of the effective inhibitors of the DDT-dehydrochlorinase *in vitro*, these evidences strongly indicate that the DDT-DDE dehydrochlorination, or more exactly the activity of the DDT-dehydrochlorinase, may be genetically controlled by the 5th chromosomal resistance gene, while the 2nd chromosomal one is independent of the dehydrochlorination. Therefore, the biochemical investigations on insecticide-resistance in future should be accompanied by genetical analysis or genetically prescribed materials. For this purpose, several experimental special strains, such as JIR; *ext*; *cm*; *acv* and *ro*; *ext*; JIR; *acv* (2;4;5;6), have also been established. Since these special strains have a common susceptible background except for the unmarked 3rd chromosome, differences, if any, found among these strains would be only due to differences of the specified chromosome derived from the resistant strain. Of these special strains, the JIR;*ext*;*cm*; *acv* was resistant both to DDT and to DDT+DMC whereas the *ro*; *ext*; JIR; *acv* strain was resistant to DDT but not to DDT+DMC. These observations also support the assumption on the role of the 5th chromosomal resistance gene.

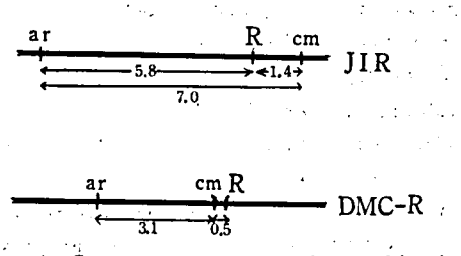

Figure 4. Gene arrangements and recombination values for the 5th chromosomal DDT-resistance genes in two resistant strains.

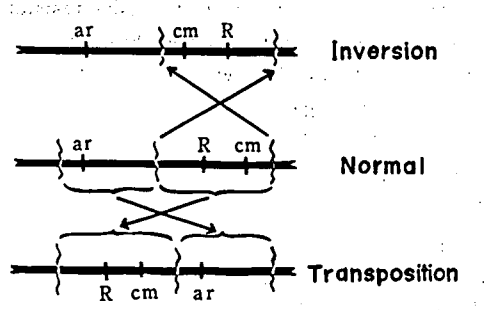

Figure 5. Plausible explanation by chromosomal aberrations for discrepancy in the gene order and recombination values shown in Figure 4.

As shown in Figure 4, the experiment with the JIR strain indicated the gene order of the marker mutants and the resistance gene to be *ar-R-cm* with the recombination values of 5.8% and 1.4% respectively, whereas the experiment with the DMC-R strain resulted in the different gene arrangement of *ar-cm-R* and lower recombination values: 3.1% and 0.5% respectively. The reason why the discrepancy occurred between the results with two resistant strains is unknown. The most simple answer to this may be that these resistance genes are non-allelic to each other. However,

this answer cannot explain the decreased recombination values, even between the standard markers *ar* and *cm*, in data with the DMC-R strain. Another assumption, therefore, seems to be more likely that the 5th chromosomal resistance gene of the DMC-R strain is an allele of that of the JIR strain but the normal crossing-over may be suppressed in the DMC-R strain by an unknown chromosomal aberration such as an inversion or a transposition involving the *R-cm* region (Figure 5). Moreover, as shown already, the high resistance level of the special experimental strain *ro*;DMC-R;*acv* to DDT was also depressed by the synergist DMC, indicating the genetic control of DDT-dehydrochlorinase activity by the 5th chromosomal resistance gene of the DMC-R strain. Such an evidence also supports the assumption on the allelism of these resistance genes.

For the 2nd chromosomal recessive resistance gene (*r-DDT*) of the JIR strain, no crossing experiment has been carried out to determine its locus on the chromosome. Milani and his co-workers[22,23] described two recessive resistance genes on the 2nd chromosome. Since no direct crossing test was also yet carried out between these resistance genes and the present resistance gene, final conclusion on the genetic relation between these resistance genes could not be drawn.

During the present investigations are under way, Lichtwardt (in press) has also investigated the genetics of DDT-resistance in an American (Illinois) strain of the housefly. According to her pre-publication manuscript[14], a dominant resistance factor to DDT-mortality links not to the 2nd chromosome but to the 5th chromosome of the resistant strain. In addition, the recombination value between the resistance gene and a marker gene *car* (carnation eye-color) has been estimated to be about 10% in both intercross and backcross data. Apart from the 2nd chromosomal resistance factor, the findings of Lichtwardt are also in accordance with the present results. Therefore, it may easily be inferred that the 5th chromosomal dominant DDT-resistance genes in these three strains of different origins are allelic to each other.

Summary

Genetics of DDT(kill)-resistance and DDT+DMC-resistance in the two strains, JIR (Japan) and DMC-R (U. S. A.), of the housefly have been investigated by using various visible mutant markers.

The results obtained from genetic analyses with DDT alone indicate that at least two major factors, i. e. 5th chromosomal dominant gene and 2nd chromosomal incomplete recessive gene, are responsible for high levels of resistance to DDT.

Map position of the dominant resistance gene (*R-DDT*) on the 5th chromosome has been estimated to be near the carmine eye-color mutant (*cm*). Although some discrepancy has been observed between the two resistant strains, the most likely assumption is that the 5th chromosomal resistance gene in these strains may be allelic to each other.

The results obtained from experiments with a DDT+DMC(1:1) mixture indicate that the gene action of the 5th chromosomal DDT-resistance gene is inhibited by the synergist DMC, whereas that of the 2nd chromosomal resistance gene remains unaffected. From these observations, it is assumed that the metabolism of DDT to DDE in the housefly is genetically controlled by the 5th chromosomal resistance gene and that the 2nd chromosomal resistance gene may concern to an unknown resistance mechanism which is independent of the dehydrochlorination of DDT.

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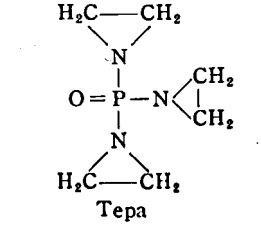
抄　　録

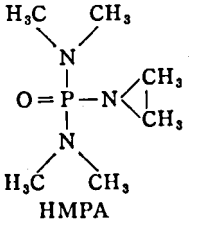
哺乳動物に低毒性の昆虫不妊剤

Insect Chemosterilants with Low Toxicity for Mammals. S. H. Chang, P. H. Terry and A. B. Borkovec, *Science*, **144**, 57～8 (1964)

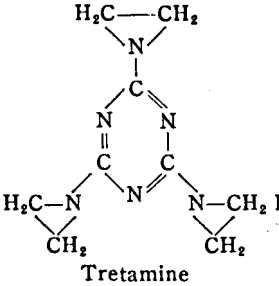
Triethylene phosphoramide (Tepa), Tretamine などの aziridine (ethylenimine) 誘導体が昆虫の雄に対する強力な不妊剤であることはすでに明らかにされている．しかし，これらは突然変異や癌を発生させる (mutagenic and carcinogenic) 可能性が考えられるために，不妊剤として昆虫の撲滅のために実用に供することには難点がある．突然変異をおこさせないで，哺乳類に対して毒性の少ない不妊剤を開発することは現在の研究課題である．この意味から，Tepa および Tretamine にそれぞれ化学構造がよく似ている hexamethyl phosphoramide (HMPA) および hexamethyl melamine (HMM) の雄のイエバエに対する不妊剤として試験された結果はそれぞれ Table 1 及び 2 に示す通りである．HMPA の場合注射法では水



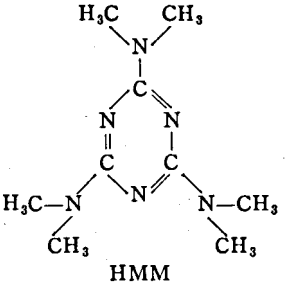
Tepa



HMPA



Tretamine



HMM