原　著

Genetic Analyses of Diazinon-Resistance in the House Fly. * Masuhisa TSUKAMOTO** and Reiko SUZUKI (Department of Genetics, Osaka University, Medical School, Osaka) Received October 31, 1965. *Botyu-Kagaku* 31, 1. 1965.

1. イエバエにおけるダイアジノン抵抗性の遺伝学的分析*

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ダイアジノンに極めて抵抗性の鉾田系統のイエバエを用いてそのダイアジノン抵抗性の遺伝を研究した．その結果，この系統のダイアジノン抵抗性は第5染色体の不完全優性遺伝子のほかにいくつかの劣性および優性遺伝子によって支配されていることがわかった．また，この主要遺伝子の染色体上の位置は *ar* ミュータントから左へ約 30% の組換値を示す所，すなわち第5染色体左腕の末端部附近であることが明らかとなった．

Results of genetic studies on insecticide-resistance in insect pests of medical or agricultural importance have been reviewed by various authors in recent years (Milani[13], Crow[3], Brown[1,2], Davidson and Mason[4], Oppenoorth[18]). Some investigators have interpreted their results to indicate a monofactorial mode of inheritance of the type of resistance investigated, and some investigators have reported multifactorial systems of resistance. Except for experiments in which visible mutants are available, however, most of these investigations were based upon the results of toxicological tests, at an appropriate discriminating dose or increasing scalar doses, conducted on the progeny of crossing experiments between genetically unmarked strains which possessed different susceptibility levels to insecticides. By such a toxicological method alone, the results obtained are too fragmentary to estimate the whole picture of the mode of inheritance, and it may be practically effective merely in such cases where the resistance character is due to a monofactorial system and the degree of resistance of each segregant genotype (i. e., the homozygous susceptible, the heterozygous hybrids, and the homozygous resistant) differs sufficiently to be recognizable by a "plateau" or "plateaux" on a dosage-response curve in filial generations. When the dosage-mortality regression lines for each segregant genotype overlap to any extent, no typical plateau may be observed even if the resistance is due to a simple Mendelian factor, and hence some of the investigators may be misled to conclude that the resistance is due to a multifactorial genetic system. The shape of dosage-mortality curves has been discussed in relation to genetics of insecticide-resistance in a previous paper.[22]

In the house fly *Musca domestica* L., the formal genetics of this species has recently been advanced by Milani[12,14], Milani and Franco[15], Hiroyoshi[9,10], Tsukamoto, Baba and Hiraga[25], Franco[5], etc., and now many visible mutants of the house fly have become available as markers fully as good as those in *Drosophila*, for genetic analyses of physiological or quantitative characters including resistance to insecticides. The first report on the genetics of resistance to diazinon in the house fly was made by Oppenoorth[17], giving results suggesting the presence of at least two diazinon-resistance genes in a Danish strain, namely a single autosomal gene *a* which was responsible for both the resistance and low aliesterase activity, and another gene which did not affect the esterase activity. Since his crossing experiments were carried out by unmarked wildtype strains, however, no further informative aspect on the genetics of the resistance was obtained therefrom. Subsequently, Franco and Oppenoorth[6] have

* This work was supported in part by a grant from the World Health Organization, United Nations, and by a PHS research grant (GM 10154) from the National Institutes of Health, Public Health Service, U.S.A.

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reported, using an American resistant strain, that a diazinon-resistant gene is linked with the 5th chromosome. Independently of these investigations, more detailed genetic analyses were being performed in a Japanese diazinon-resistant strain with the aid of several visible mutant markers, and some of these results were preliminarily reported[20,21] in 1962 that the resistance levels of the F_1 hybrids in crosses between a diazinon-resistant strain and susceptible multichromosomal mutant strains were intermediate between those of their parents, and that a dominant major gene responsible for the resistance was located on the 5th chromosome, in agreement with the results of Franco and Oppenoorth[6].

The purpose of present paper is to describe the results of a complete set of genetic analyses of both the dominant and recessive effects of diazinon-resistance factors in a highly-resistant strain of the house fly of Japanese origin.

Materials and methods

The insecticide used was a purified sample of *O, O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate (diazinon) which was kindly supplied by Dr. K. Kojima, Institute for Agricultural Chemicals, Toa Noyaku Co., Ltd., Odawara, and a technical sample (96.69%) which was supplied by Dr. T. Kasai, Japan Agricultural Chemicals Co. Ltd., Osaka, Japan.

The susceptible and diazinon-resistant strains of the house fly employed were as follows:

Lab em-7-em···A highly-susceptible laboratory strain obtained from Mrs. E. T. Lichtwardt, University of Kansas, Lawrence, Kansas, U.S.A., as one of substrains of the IS-1 strain inbred by her[11], originally derived from the NAIDM 1948 strain. The LD_{50} by topical application approximates 0.02 μg/fly. The abbreviation *Lab* is used for this strain in the present paper.

pcv; *ocra*; *ar*; *acv*···A susceptible multichromosomal mutant strain in which the 2nd, 3rd, 5th and 6th chromosomes are marked respectively with the mutants posterior-crossveinless (*pcv*), ochre eyes (*ocra*), aristapedia (*ar*) and anterior-crossveinless (*acv*). The topical LD_{50} approximates 0.04 μg/fly.

bwb; *ocra*; *ar*; *ac*···A non-resistant mutant strain in which the 2nd, 3rd, 5th and 6th chromosomes are marked respectively with brown-body (*bwb*), ochre eyes, aristapedia, and ali curve (*ac*). The LD_{50} approximates 0.04 μg/fly.

ro; *ext*; *cm*; *acv*···A susceptible strain marked with the mutants rough eyes (*ro*), extended wings (*ext*), carmine eyes (*cm*) and anterior-crossveinless (*acv*) respectively for the 2nd, 4th, 5th and 6th chromosomes. The LD_{50} approximates 0.03 μg/fly.

ar car···A non-resistant strain in which the 5th chromosome is marked with the two recesssive mutants aristapedia (*ar*) and carnation eyes (*car*). The LD_{50} approximates 0.025 μg/fly.

Hokota···A diazinon-resistant strain, originally collected from the field in 1960 and selected for diazinon-resistance for several generations in the National Institute of Health, Tokyo (Yasutomi[26]), and further selected in the laboratory at the Osaka University for 4 years. Phenotypically wild-type, its LD_{50} approximates 5 μg/fly.

R (*ar car*)···A diazinon-resistant substrain derived from the Hokota strain by marking the 5th chromosome with *ar* and *car* genes. The LD_{50} approximates 2.5 μg/fly.

R (*pcv*; *ocra*; *ar*; *acv*)···A diazinon-resistant marker strain synthesized from the Hokota and *pcv*; *ocra*; *ar*; *acv* (2; 3; 5; 6) strains. The LD_{50} approximates 2.5 μg/fly.

R (*bwb*; *ocra*; *ar*; *ac*)···A diazinon-resistant marker strain synthesized from the Hokota and *bwb*; *ocra*; *ar*; *ac* (2; 3; 5; 6) strains by repeated backcrossings and selections with diazinon. The LD_{50} approximates 4.5 μg/fly.

The development or maintenance of diazinon-resistance was accomplished by feeding the newly-emerged flies on a bait consisting of powdered milk and diazinon.

Susceptibility tests were performed on one-day-old flies, usually in groups consisting of 50 females plus 50 males, by topical application of about one μl of a solution of diazinon in acetone onto the dorsum of the thorax. The flies had been anaesthetized first with CO_2 gas and then with diethyl ether. After treatment with the insecticide, the flies were put into a glass vial of capacity $10\times10\times12cm^3$ provided with a pad of cotton wool soaked with milk. Before, during, and after the treatment, the vials were kept in a constant-

temperature room at 25±1°C and 50±10% relative humidity.

Mortality counts were made 24 hours later, and the moribund flies were combined with the dead. Practically negligible mortality was observed in the control group in which flies were topically treated with one μl of acetone alone, and hence no correction of observed data was made for the control mortality. In segregation tests in crossing experiments, almost all the flies emerging from the same experimental group were treated with the insecticide usually in groups comprising a 50 : 50 sex ratio but otherwise of any phenotype. In order to obtain suffcient numbers of progeny, a system of mass mating was employed in all the crossing experiments.

The design of crossing experiments for the determination of linkage group was based on the F_1 male-backcross[23] to a resistant parent strain for detecting any recessive resistance factor or to a susceptible parent strain for detecting any dominant factor, since crossing-over is lacking in the male house fly[10]. Thus each chromosome behaves as a single factor in such a crossing system. Furthermore, since in preliminary experiments the resistance levels appeared to involve no sex-linked genes, and maternal effects were negligible, reciprocal crosses were not made where the analyses were designed only for autosomal factors.

In determining the linkage group for the resistance factors, the percentage survival for each genotype was transformed into the arc-sine unit and submitted to statistic analyses based on the factorial arrangement described by Yates[27]. In determining the locus of the resistance gene on the chromosome, exactly all the flies emerged from each vial were treated with diazinon at an appropriate dose or doses, because the calculation of recombination values requires the estimation of the viability for some marker gene. Details on the application of the factorial analysis to genetics of insecticide-resistance and the method of calculation of recombination values have been descrived in previous papers.[23,24]

Results

Analyses for dominant effect of resistance factors : Males of the F_1 hybrid of the susceptible (S) × resistant (R) cross were backcrossed to females of the susceptible marker strain used. The crosses made to analyse the dominant (or heterozygous) effect of autosomal resistance factors are shown as Crosses 1, 2 and 3.

Both in Crosses 1 and 2, the 2nd, 3rd, 5th and 6th chromosomal factors can be analysed for the reristance. In Cross 3, however, the 2nd, 4th, 5th and 6th chromosomal dominant factors can be analysed. Thus, these crossing systems can effectively cover all the autosomes.

Adult flies of the resultant backcross progeny were then tested for their resistance levels by topical application with diazinon. The log dosage-probit mortality (ld-p) lines for these parent strains, the hybrids, and their backcross progeny in Cross 2 are illustrated in Figure 1 as an example, in which each mortality point is based on group of 50 females plus 50 males. The intermediate resistance levels shown by the F_1 hybrids indicate that the diazinon-resistance in the Hokota strain is incompletely dominant over susceptibility, or alternatively involves both dominant and recessive resistance factors. Although the shape of the ld-p lines for these backcross progeny is not exactly coincident with that expected on a monofactorial hypothesis, it is inferred that at least one major dominant factor is involved in the resistance.

Cross 1. *pcv* ; *ocra* ; *ar* ; *acv*♀ × F_1 (*pcv* ; *ocra* ; *ar* ; *acv*♀ × Hokota♂) ♂
Cross 2. *bwb* ; *ocra* ; *ar* ; *ac*♀ × F_1 (*bwb* ; *ocra* ; *ar* ; *ac*♀ × Hokota♂) ♂
Cross 3. *ro* ; *ext* ; *cm* ; *acv*♀ × F_1 (Hokota♀ × *ro* ; *ext* ; *cm* ; *acv*♂) ♂
Cross 4. R (*pcv* ; *ocra* ; *ar* ; *acv*) ♀ × F_1 (*pcv* ; *ocra* ; *ar* ; *acv*♀ × Hokota♂) ♂
Cross 5. R (*bwb* ; *ocra* ; *ar* ; *ac*) ♀ × F_1 {R(*bwb* ; *ocra* ; *ar* ; *ac*) ♀ × Lab♂} ♂
Cross 6. Hokota♀ × *ro* ; *ext* ; *cm* ; *acv*♂ ⟶ F_1♀♂ ⟶ F_2
Cross 7. F_1 {R(*ar car*)♀ × Lab♂} ♀ × *ar car*♂
Cross 8. F_1 {R(*ar car*)♀ × Lab♂} ♀ × R(*ar car*)♂

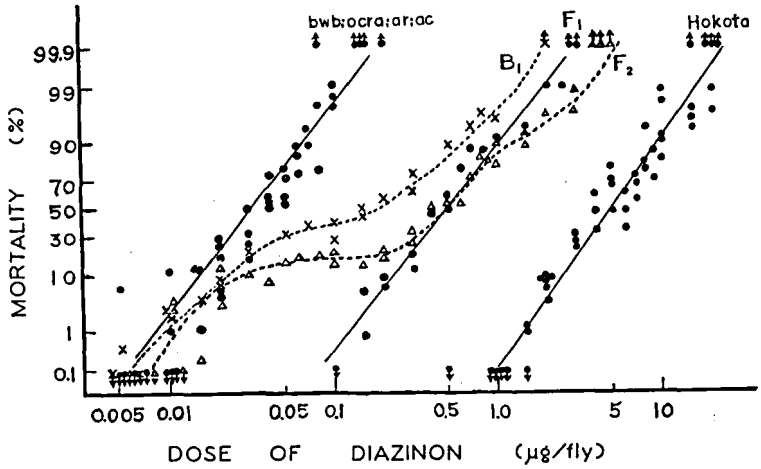


Fig. 1. Resistance levels to topical diazinon in the susceptible mutant strain, the resistant Hokota strain, and their progeny.
F_1 : *bwb* ; *ocra* ; *ar* ; *ac*♀ ×Hokota♂
F_2 : F_1♀ ×F_1♂
B_1 : *bwb* ; *ocra* ; *ar* ; *ac*♀ ×F_1♂

Table 1. Analysis for dominant factors: Relation between the diazinon resistance and the chromosome make-up in backcross progeny. Cross 1. *pcv* ; *ocra* ; *ar* ; *acv*♀ ×F_1 (*pcv* ; *ocra* ; *ar* ; *acv*♀ ×Hokota♂)♂

Phenotype (2;3;5;6)	Dosage of diazinon 0.1~0.3 μg/fly				0.4~0.5 μg/fly				Arc-sine Survival (θ)	
	No. of flies		Survival rate		No. of flies		Survival rate			
	Tested	Alive	%	θ	Tested	Alive	%	θ	Pooled	Mean
+ ; + ; + ; +	162	160	98.77	83.63	151	117	77.48	61.67	145.30	72.65
pcv ; + ; + ; +	158	148	93.67	75.43	162	61	37.65	37.85	113.28	56.64
+ ; *ocra* ; + ; +	140	113	80.71	63.95	130	18	13.85	21.85	85.80	42.90
pcv ; *ocra* ; + ; +	111	65	58.56	49.93	100	5	5.00	12.92	62.85	31.43
+ ; + ; *ar* ; +	85	27	31.76	34.31	85	12	14.12	22.08	56.39	28.20
pcv ; + ; *ar* ; +	80	10	12.50	20.70	60	3	5.00	12.92	33.62	16.81
+ ; *ocra* ; *ar* ; +	75	0	0	0	46	0	0	0	0	0
pcv ; *ocra* ; *ar* ; +	43	0	0	0	33	0	0	0	0	0
+ ; + ; + ; *acv*	101	97	96.04	78.52	103	61	59.22	50.31	128.83	64.42
pcv ; + ; + ; *acv*	125	106	84.80	67.05	144	49	34.04	35.69	102.74	51.37
+ ; *ocra* ; + ; *acv*	69	54	78.26	62.21	94	10	10.64	19.04	81.25	40.63
pcv ; *ocra* ; + ; *acv*	65	26	40.00	39.23	67	5	7.46	15.85	55.08	27.54
+ ; + ; *ar* ; *acv*	32	9	28.13	32.03	28	4	14.29	22.21	54.24	27.12
pcv ; + ; *ar* ; *acv*	61	8	13.11	21.23	50	4	8.00	16.43	37.66	18.83
+ ; *ocra* ; *ar* ; *acv*	36	0	0	0	27	0	0	0	0	0
pcv ; *ocra* ; *ar* ; *acv*	23	0	0	0	18	0	0	0	0	0
Total	1366	823		628.22	1298	349		328.82	957.04	478.54

Table 2. Analysis for dominant factors : Relation between the diazinon resistance and the chromosome make-up in backcross progeny. Cross 2. *bwb* ; *ocra* ; *ar* ; *ac*♀ ×F_1 (*bwb* ; *ocra* ; *ar* ; *ac*♀ ×Hokota♂) ♂

Phenotype (2;3;5;6)	Dosage of diazinon 0.03～0.1 μg/fly				0.15～0.3 μg/fly				0.5～1.0 μg/fly			
	No. of flies		Survival rate		No. of flies		Survival rate		No. of flies		Survival rate	
	Tested	Alive	%	0	Tested	Alive	%	0	Tested	Alive	%	0
+ ; + ; + ; +	224	212	94.64	76.64	207	169	81.64	64.63	160	37	23.13	28.75
bwb ; + ; + ; +	226	204	90.27	71.82	161	103	63.98	53.12	133	10	7.52	15.91
+ ; *ocra* ; + ; +	175	160	91.43	72.98	146	84	57.53	49.33	124	9	7.26	15.63
bwb ; *ocra* ; + ; +	157	127	80.89	64.07	136	44	32.35	34.67	105	4	3.81	11.26
+ ; + ; *ar* ; +	114	55	48.25	43.99	99	6	6.06	14.25	99	13	13.13	21.24
bwb ; + ; *ar* ; +	105	31	29.52	32.91	100	3	3.00	9.98	88	0	0	0
+ ; *ocra* ; *ar* ; +	71	12	16.90	24.27	83	0	0	0	72	0	0	0
bwb ; *ocra* ; *ar* ; +	79	6	7.59	15.99	55	0	0	0	43	0	0	0
+ ; + ; + ; *ac*	197	181	91.88	73.44	168	128	76.19	60.79	95	13	13.68	21.70
bwb ; + ; + ; *ac*	190	165	86.84	68.72	129	72	55.81	48.34	84	4	4.76	12.60
+ ; *ocra* ; + ; *ac*	164	149	90.85	72.42	115	70	60.87	51.28	97	4	4.12	11.71
bwb ; *ocra* ; + ; *ac*	166	128	77.11	61.42	119	37	31.09	33.88	76	4	5.26	13.26
+ ; + ; *ar* ; *ac*	103	33	32.04	34.47	109	6	5.50	13.56	62	0	0	0
bwb ; + ; *ar* ; *ac*	84	24	28.57	32.31	85	1	1.18	6.24	46	0	0	0
+ ; *ocra* ; *ar* ; *ac*	55	4	7.27	15.64	57	0	0	0	46	0	0	0
bwb ; *ocra* ; *ar* ; *ac*	57	0	0	0	58	0	0	0	39	0	0	0
Total	2167	1491		761.09	1827	723		440.07	1369	98		152.06

Table 3. Analysis for dominant factors : Relation between the diazinon-resistance and the chromosome make-up in backcross progeny. Cross 3. *ro* ; *ext* ; *cm* ; *acv* ♀ ×F_1 (Hokota♀ ×*ro* ; *ext* ; *cm* ; *acv*♂) ♂

Phenotype (2;4;5;6)	Replication at a dose of 0.2μg/fly 1				2				Arc-sine survival pooled
	No. of flies		Survival rate		No. of flies		Survival rate		
	Tested	Alive	%	0	Tested	Alive	%	0	
+ ; + ; + ; +	170	163	95.88	78.29	207	193	93.24	74.93	153.22
ro ; + ; + ; +	163	144	88.34	70.04	119	93	78.15	62.14	132.18
+ ; *ext* ; + ; +	60	59	98.33	82.58	48	46	95.83	78.22	160.80
ro ; *ext* ; + ; +	47	39	82.98	65.63	26	23	88.46	70.14	135.77
+ ; + ; *cm* ; +	161	10	6.21	14.43	127	4	3.15	10.23	24.66
ro ; + ; *cm* ; +	129	3	2.33	8.78	86	0	0	0	8.78
+ ; *ext* ; *cm* ; +	83	4	4.82	12.69	41	1	2.44	8.99	21.68
ro ; *ext* ; *cm* ; +	57	0	0	0	31	0	0	0	0
+ ; + ; + ; *acv*	137	131	95.62	77.92	107	99	92.52	74.16	152.08
ro ; + ; + ; *acv*	117	102	87.18	69.02	73	57	78.08	62.09	131.11
+ ; *ext* ; + ; *acv*	72	70	97.22	80.41	43	40	93.02	74.68	155.09
ro ; *ext* ; + ; *acv*	43	37	86.05	68.07	25	17	68.00	55.55	123.62
+ ; + ; *cm* ; *acv*	115	8	6.96	15.30	61	1	1.64	7.36	22.66
ro ; + ; *cm* ; *acv*	105	1	0.95	5.59	47	0	0	0	5.59
+ ; *ext* ; *cm* ; *acv*	62	3	4.84	12.71	36	1	2.78	9.59	22.30
ro ; *ext* ; *cm* ; *acv*	66	1	1.52	7.09	36	0	0	0	7.09
Total	1587	775		668.55	1113	575		588.08	1256.63

In order to determine the linkage group of the resistance factor or factors, both living and dead flies of the backcross progeny were examined for their visible phenotypes 24 hours after topical treatment of various discriminating doses of the insecticide. Tables 1, 2 and 3 represent the survival rate for each phenotype in Crosses 1, 2 and 3 respectively. In these tables, data for both males and females were pooled because no consistent intersexual difference in segregation of the resistance character was observed in preliminary experiments. As is shown in Tables 1 and 2, survival rates for phenotypically *ocra* and/or *ar* flies (homozygous for the susceptible 3rd or 5th chromosomes) are very lower than those for other phenotypes at several doses of diazinon. On the other hand, non-*ar* or non-*ocra* flies (genotypically heterozygous for the resistant chromosomes) show much greater survival than the corresponding mutant-type flies, suggesting the presence of major resistance factors on both the 5th and the 3rd chromosomes. Similarly, the result of Cross 3 also indicates the presence of the 5th and 2nd chromosomal resistance factors (Table 3). This multichromosomal interpretation for the diazinon-resistance in the Hokota strain was further confirmed by factorial analysis reported later.

Analyses for recessive effect of factors: In order to detect any recessive (or homozygous) effect of autosomal resistance factors, males of the F_1 hybrid of the S×R cross were backcrossed to females of the resistant marker strain. The crossing procedures employed are shown as Crosses 4 and 5.

Procedures on toxicological tests were similar to those described in the analyses for dominant effects. Since the resistance was incompletely dominant over the susceptibility, and the ld-p line for the hybrids overlapped that of the resistant parent strain, no clear-cut segregation may be expected to show in the ld-p line for the backcross progeny even when the inheritance is monofactorial. Therefore toxicological tests for linkage-group determination of the resistance factor were carried out at certain discriminating doses at which almost all the heterozygotes (*r*/+) are killed. The survival rates of treated flies belonging to each phenotype in Crosses 4 and 5 are given in Tables 4 and 5

Table 4. Analysis for recessive factors : Relation between the diazinon resistance and the chromosome make-up in backcross progeny. Cross 4. R(*pcv* ; *ocra* ; *ar* ; *acv*) ♀ × F_1 (*pcv* ; *ocra* ; *ar* ; *acv* ♀ × Hokota ♂) ♂

Phenotype (2;3;5;6)	Dosage of diazinon: 1.5 μg/fly				4.0 μg/fly			
	No. of flies		Survival rate		No. of flies		Survival rate	
	Tested	Alive	%	θ	Tested	Alive	%	θ
+ ; + ; + ; +	135	111	82.22	65.06	157	73	46.50	42.99
pcv ; + ; + ; +	96	46	47.92	43.81	129	8	6.20	14.42
+ ; *ocra* ; + ; +	89	58	65.17	53.83	124	24	19.35	26.10
pcv ; *ocra* ; + ; +	98	23	23.47	28.98	80	0	0	0
+ ; + ; *ar* ; +	77	18	23.38	28.92	73	3	4.11	11.84
pcv ; + ; *ar* ; +	55	2	3.64	10.99	57	0	0	0
+ ; *ocra* ; *ar* ; +	82	6	7.32	15.70	70	0	0	0
pcv ; *ocra* ; *ar* ; +	55	1	1.82	7.75	66	0	0	0
+ ; + ; + ; *acv*	90	64	71.11	57.49	100	29	29.00	32.58
pcv ; + ; + ; *acv*	93	25	26.88	31.23	98	4	4.08	11.65
+ ; *ocra* ; + ; *acv*	69	37	53.62	47.07	87	7	8.05	16.48
pcv ; *ocra* ; + ; *acv*	74	5	6.76	15.07	90	0	0	0
+ ; + ; *ar* ; *acv*	50	6	12.00	20.27	61	2	3.28	10.35
pcv ; + ; *ar* ; *acv*	60	0	0	0	76	1	1.32	6.59
+ ; *ocra* ; *ar* ; *acv*	33	2	6.06	14.25	53	0	0	0
pcv ; *ocra* ; *ar* ; *acv*	48	0	0	0	58	0	0	0
Total	1204	404		440.42	1379	151		173.00

Table 5. Analysis for recessive factors : Relation between the diazinon resistance and chromosome make-up in backcross progeny. Cross 5. R(*bwb* ; *ocra* ; *ar* ; *ac*) ♀ × F_1{R(*bwb* ; *ocra* ; *ar* ; *ac*) ♀ × Lab em-7-em♂} ♂

Phenotype (2;3;5;6)	Replication at a dose range of 1.5~2.0 μg/fly											
	1				2				3			
	No. of flies		Survival rate		No. of flies		Survival rate		No. of flies		Survival rate	
	Tested	Alive	%	θ	Tested	Alive	%	θ	Tested	Alive	%	θ
bwb ; *ocra* ; *ar* ; *ac*	101	89	88.12	69.84	110	102	92.73	74.36	78	62	79.49	63.07
+ ; *ocra* ; *ar* ; *ac*	90	40	44.44	41.80	100	60	60.00	50.77	87	37	42.53	40.71
bwb ; + ; *ar* ; *ac*	99	63	63.64	52.91	119	92	77.31	61.56	74	61	82.43	65.22
+ ; + ; *ar* ; *ac*	61	30	49.18	44.53	91	37	40.66	39.62	99	43	43.43	41.23
bub ; *ocra* ; + ; *ac*	93	44	47.31	43.46	93	39	41.94	40.38	84	42	50.00	45.00
+ ; *ocra* ; + ; *ac*	87	8	9.20	17.66	67	11	16.42	23.91	112	13	11.61	19.92
bwb ; + ; + ; *ac*	114	19	16.67	24.18	77	13	16.88	24.26	80	17	21.25	27.46
+ ; + ; + ; *ac*	35	7	20.00	26.56	72	4	5.56	13.63	108	10	9.26	17.72
bwb ; *ocra* ; *ar* ; +	98	64	65.31	53.92	116	90	77.59	61.74	74	56	75.68	60.46
+ ; *ocra* ; *ar* ; +	102	32	31.37	34.06	89	37	41.57	40.15	86	27	41.40	34.08
bwb ; + ; *ar* ; +	113	50	44.25	41.70	130	60	46.15	42.79	98	48	48.98	44.42
+ ; + ; *ar* ; +	90	13	14.44	22.32	79	21	26.58	31.03	89	29	32.58	34.81
bwb ; *ocra* ; + ; +	98	28	28.57	32.31	100	41	41.00	39.82	93	34	36.56	37.20
+ ; *ocra* ; + ; +	83	6	7.23	15.60	75	1	1.33	6.63	97	7	7.22	15.58
bwb ; + ; + ; +	90	8	8.89	17.35	56	1	1.79	7.69	84	4	4.76	12.60
+ ; + ; + ; +	85	0	0	0	61	1	1.64	7.36	94	2	2.13	8.59
Total	1439	501		538.20	1435	610		565.70	1437	492		568.07

Table 6. Analysis for recessive factors : Relation between the diazinon-resistance and the chromosome make-up in the intercross progeny. Cross 6. Hokota ♀ × *ro* ; *ext* ; *cm* ; *acv*♂ ⟶ F_1 ⟶ F_2

Phenotype (2;4;5;6)	Replication at a dose of 2.0 μg/fly								Arc-sine survival pooled
	1				2				
	No. of flies		Survival rate		No. of flies		Survival rate		
	Tested	Alive	%	θ	Tested	Alive	%	θ	
+ ; + ; + ; +	540	212	39.26	38.79	442	163	36.88	37.40	76.19
ro ; + ; + ; +	198	41	20.71	27.07	147	29	19.73	26.37	53.44
+ ; *ext* ; + ; +	108	25	23.15	28.76	84	19	22.62	28.39	57.15
ro ; *ext* ; + ; +	46	3	6.52	14.79	33	2	6.06	14.25	29.04
+ ; + ; *cm* ; +	151	6	3.97	11.50	162	7	4.32	12.00	23.50
ro ; + ; *cm* ; +	67	0	0	0	58	0	0	0	0
+ ; *ext* ; *cm* ; +	46	0	0	0	27	0	0	0	0
ro ; *ext* ; *cm* ; +	16	0	0	0	6	0	0	0	0
+ ; + ; + ; *acv*	142	47	33.10	35.12	127	41	32.28	34.62	69.74
ro ; + ; + ; *acv*	56	4	7.14	15.49	35	2	5.71	13.82	29.31
+ ; *ext* ; + ; *acv*	43	6	13.95	21.94	26	10	38.46	38.33	60.27
ro ; *ext* ; + ; *acv*	18	0	0	0	11	1	9.09	17.55	17.55
+ ; + ; *cm* ; *acv*	35	1	2.86	9.74	27	1	3.70	11.09	20.83
ro ; + ; *cm* ; *acv*	13	0	0	0	14	0	0	0	0
+ ; *ext* ; *cm* ; *acv*	26	0	0	0	13	0	0	0	0
ro ; *ext* ; *cm* ; *acv*	4	0	0	0	4	0	0	0	0
Total	1509	345		203.20	1216	275		233.82	437.02

Table 7. Factorial analysis of resistance effects by using the (2;3;5;6) multichromosomal mutant strains, *pcv* ; *ocra* ; *ar* ; *acv* and R (*pcv* ; *ocra* ; *ar* ; *acv*)

Chromosome	Dominant factors (Cross 1)				Recessive factors (Cross 4)			
	Effect	D. F.	M. S.	F	Effect	D. F.	M. S.	F
2	73. 30	1	671. 6	5. 1*	136. 22	1	2319. 5	40. 4**
3	193. 54	1	4682. 2	35. 5**	81. 48	1	829. 9	14. 5**
5	296. 62	1	10997. 9	83. 4**	180. 05	1	4052. 3	70. 6**
6	18. 72	1	43. 8	0. 3	43. 68	1	238. 5	4. 2
2-3	24. 18	1	73. 1	0. 6	14. 59	1	26. 6	0. 5
2-5	33. 94	1	144. 0	1. 1	60. 22	1	453. 3	7. 9*
2-6	4. 44	1	2. 4	0. 0	2. 27	1	0. 6	0. 0
3-5	11. 62	1	16. 9	0. 1	30. 22	1	114. 2	2. 0
3-6	6. 40	1	5. 1	0. 0	4. 19	1	2. 2	0. 0
5-6	20. 60	1	53. 0	0. 4	19. 94	1	49. 7	0. 9
2-3-5	−15. 18	1	28. 8	0. 2	−17. 01	1	36. 2	0. 6
2-3-6	7. 68	1	7. 4	0. 1	6. 10	1	4. 7	0. 1
2-5-6	−1. 76	1	0. 4	0. 0	2. 83	1	1. 0	0. 0
3-5-6	8. 28	1	8. 6	0. 1	−1. 15	1	0. 2	0. 0
2-3-5-6	1. 48	1	0. 3	0. 0	−5. 94	1	4. 4	0. 1
Error	—	15	131. 8	—	—	15	57. 4	—

* Significant at 5% level. ** Highly significant at 1% level.

Table 8. Factorial analysis of resistance effects by using the (2 ; 3 ; 5 ; 6) multichromosomal mutant strains, *bwb* ; *ocra* ; *ar* ; *ac* and R (*bwb* ; *ocra* ; *ar* ; *ac*)

Chromosome	Dominant factors (Cross 2)				Recessive factors (Cross 5)			
	Effect	D. F.	M. S.	F	Effect	D. F.	M. S.	F
2	60. 03	1	675. 7	5. 9*	138. 48	1	3595. 6	172. 0**
3	85. 87	1	1382. 6	12. 1**	96. 13	1	1732. 7	82. 9**
5	274. 51	1	14129. 2	123. 7**	207. 42	1	8066. 8	386. 0**
6	29. 91	1	167. 7	1. 5	89. 18	1	1491. 2	71. 3**
2-3	7. 59	1	10. 8	0. 1	48. 64	1	443. 6	21. 2**
2-5	13. 39	1	33. 6	0. 3	19. 44	1	70. 9	3. 4
2-6	7. 91	1	11. 7	0. 2	3. 96	1	2. 9	0. 1
3-5	−16. 17	1	49. 0	0. 4	−27. 57	1	142. 5	6. 8*
3-6	10. 83	1	22. 0	0. 2	−11. 11	1	23. 1	1. 1
5-6	−10. 37	1	20. 2	0. 2	6. 90	1	8. 9	0. 4
2-3-5	−7. 17	1	9. 6	0. 1	−17. 48	1	57. 3	2. 7
2-3-6	12. 07	1	27. 3	0. 2	−2. 64	1	1. 3	0. 1
2-5-6	−5. 25	1	5. 2	0. 0	9. 20	1	15. 9	0. 8
3-5-6	3. 39	1	2. 2	0. 0	−10. 13	1	19. 2	0. 9
2-3-5-6	−10. 89	1	22. 2	0. 2	−2. 32	1	1. 0	0. 0
Error	—	30	114. 2	—	—	30	20. 9	—

* Significant at 5% level. ** Highly significant at 1% level.

Table 9. Factorial analysis of resistance effects by using the *ro* ; *ext* ; *cm* ; *acv* (2 ; 4 ; 5 ; 6) multichrosomal mutant strain.

Chromosome	Dominant factors (Cross 3)				Recessive factors (Cross 6)			
	Effect	D. F.	M. S.	F	Effect	D. F.	M. S.	F
2	84. 18	1	885. 8	107. 2**	89. 18	1	994. 1	56. 7**
4	1. 97	1	0. 5	0. 1	54. 50	1	371. 3	21. 2**
5	515. 56	1	33225. 3	4020. 4**	174. 20	1	3793. 2	216. 3**
6	8. 78	1	9. 6	1. 2	20. 80	1	54. 1	3. 1
2-4	−9. 22	1	10. 6	1. 3	18. 34	1	42. 0	2. 4
2-5	14. 34	1	25. 7	3. 1	44. 84	1	251. 3	14. 3**
2-6	−0. 55	1	0. 0	0. 0	−14. 80	1	27. 4	1. 6
4-5	−8. 66	1	9. 4	1. 1	10. 16	1	12. 9	0. 7
4-6	−1. 38	1	0. 2	0. 0	12. 44	1	19. 3	1. 1
5-6	11. 30	1	16. 0	1. 9	18. 14	1	41. 1	2. 3
2-4-5	−5. 28	1	3. 5	0. 4	−26. 00	1	84. 5	4. 8*
2-4-6	−0. 58	1	0. 0	0. 0	−0. 20	1	0. 0	0. 0
2-5-6	−5. 83	1	4. 2	0. 5	−17. 46	1	38. 1	2. 2
4-5-6	−14. 28	1	25. 5	3. 1	9. 78	1	12. 0	0. 7
2-4-5-6	7. 09	1	6. 3	0. 8	−2. 86	1	1. 0	0. 1
Error	—	15	8. 3	—	—	15	17. 5	—

* Significant at 5% level. ** Highly significant at 1% level.

respectively. Here the decreases in survival rate are especially remarkable for *pcv*, *ocra*, and *ar* flies, indicating the presence of several recessive factors on the autosomes except for the 4th chromosome.

Another type of cross (Cross 6) was therefore carried out in order to detect the 4th chromosomal resistance gene, if any.

The F_2 progeny of this intercross was also treated at relatively higher doses of diazinon (2. 0μg/fly) at where only the resistant homozygotes can survive but the heterozygotes may be killed by the insecticide. Table 6 gives the result of the analysis. In this case, the 2nd and 5th chromosomal mutants, *ro* and *cm* flies, decrease extremely.

Statistical analyses: In order to confirm the results suggesting multifactorial inheritance of the diazinon-resistance, effect of each chromosomal factor and interaction between these factors were calculated from the arc-sine transformed survival rates by factorial analysis[23].

Table 7 summarizes both dominant and recessive effects of each resistant chromosome obtained from the data in Crosses 1 and 4 in which the 2nd, 3rd, 5th and 6th chromosomes were tested for the linkage group with the aid of the *pcv* ; *ocra* ; *ar* ; *acv* strain. Similarly, the effects and their significance in data from Crosses 2 and 5 were represented in Table 8 with the aid of the other mutant markers *bwb* ; *ocra* ; *ar* ; *ac* or R (*bwb* ; *ocra* ; *ar* ; *ac*). Table 9 represents the summarized results obtained from data in Crosses 3 and 6 where the susceptible marker strain *ro* ; *ext* ; *cm* ; *acv* was used for examining the 2nd, 4th, 5th and 6th linkage groups respectively. From these tables, it is clear that the diazinon-resistance in the Hokota strain of the housefly is mainly due to the 5th chromosomal factor. Besides this major factor, the influence of other dominant and recessive factors on both the 2nd and the 3rd chromosomes is statistically significant. Both the 4th and 6th chromosomal factors, if any, do not seem to contribute to the resistance considerably, whereas the effect of the 6th chromosomal recessive factor was highly significant statistically in Cross 5.

Estimation of gene locus for resistant factor: As shown above, both dominant and recessive effects of the 5th chromosome constituted the most important contribution to the diazinon-resistance. Therefore, analyses were then designed

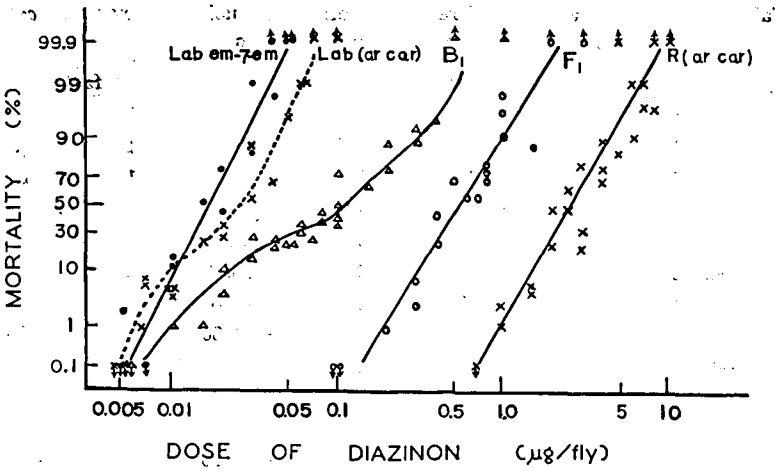


Fig. 2. Resistance levels to topical diazinon in two susceptible strains, the resistant marker strain, and their progeny.
F_1 : R (*ar car*) ♀×Lab em-7-em♂
B_1 : F_1♀×Lab (*ar car*) ♂

to ascertain, by determining the gene locus for these factors, whether the 5th chromosome bears one incompletely-dominant gene or at least two different genes, one dominant and one recessive. The crossing experiments (Crosses 7 and 8) were based on the F_1 female-backcross involving the 5th-chromosomal mutants *ar* and *car*.

Of these crossing systems, Cross 7 would detect the recombination value between the dominant resistance gene (*R*) and the mutant markers, and Cross 8 for the recessive resistance gene (*r*). The symbols *R* and *r* were used as the general terms for dominant and recessive resistance factors to a given insecticide. Figure 2 illustrates the ld-p lines for the susceptible and resistant strains, their hybrids, and backcross progeny of Cross 7.

For calculating the locus of the dominant resistance gene, both males and females of the backcross progeny were topically treated with diazinon at diagnostic doses of 0.1~0.2μg/fly at which all the susceptible flies cannot survive 24 hours after the topical application of the insecticide. Table 10 gives the relation between phenotypes and actual counts of flies obtained from Cross 7. It is obvious from these data that the phenotype category showing the least survival, namely +*car* in this case, belongs to the double-crossover class, and hence the *R-ar-car* arrangement is indicated on the 5th chromosome. The viability of each mutant allele against its wild-type allele varies from gene to gene, and mortality counts of resistant genotype flies also vary from dose of the insecticide used. In order to eliminate influences of these source of variation, recombination values were calculated from the data shown in Table 10 by the following formulae for a coupling system, described in a previous paper[24]:

For the *R—ar* region :

$$x=\frac{1}{1+\frac{1}{q}\sqrt{\frac{(A+C)(F+H)}{(B+D)(E+G)}}\sqrt[4]{\frac{ACEG}{BDFH}}} \quad (1)$$

$$=0.298$$

or

$$x=\frac{1}{1+\frac{1}{q}\sqrt{\frac{AC}{BD}}}=0.292 \quad (2)$$

where q is the viability term of the *ar* mutant to its wild allele and is estimated by A+C+E+G/B+D+F+H=0.790.

And for the *ar—car* region :

$$y=\frac{1}{1+\sqrt[4]{\frac{ABGH}{CDEF}}}=0.226 \quad (3)$$

or

$$y=\frac{1}{1+\sqrt{\frac{(A+G)(B+H)}{(C+E)(D+F)}}}=0.230 \quad (4)$$

Table 11 also gives actual data for determining the recessive resistance gene, *r*. Calculations of

Table 10. Linkage data for dominant effect of diazinon resistance factor in progeny from Cross 5. Selective doses : 0.1~0.2μg/fly

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

Response	Phenotype		Crossover type	Code sign	Number of flies observed		
					Females	Males	Pooled
Alive	*ar*	*car*	(0 0)	A	795	725	1520
	+	+	(1 0)	B	511	382	893
	ar	+	(0 1)	C	251	234	485
	+	*car*	(1 1)	D	145	80	225
Dead	*ar*	+	(1 1)	E	197	283	480
	+	*car*	(0 1)	F	454	473	927
	ar	*car*	(1 0)	G	648	943	1591
	+	+	(0 0)	H	1475	1641	3116
Total					4476	4761	9237

the recombination values are similarly as follows:

For the *r—ar* region,

x=0.335 from the formula (1)

or x=0.328 from the formula (2)

where q was estimated by A+C+E+G/B+D+F+H=0.814.

For the *ar—car* region,

y=0.197 from the formula (3)

or y=0.199 from the formula (4)

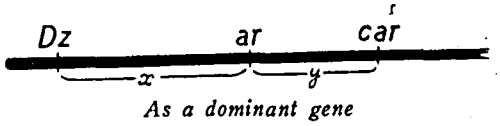
These recombination data indicate that both the dominant and the recessive factors for diazinon-resistance are located at the terminal region of the left arm of the 5th chromosome. Although the x values calculated above are not exactly coincident with each other, the discrepancy seems to be non-significant because the locus for each resistance factor is too distant from the *ar* marker to discuss the precise map position, and the y values for the *ar—car* region also vary probably within experimental errors. At present no visible marker at the terminal region is available for further genetic analysis.

Therefore, the assumption seems to be more likely that both the dominant and the recessive factors are located at one and the same position on the chromosome. In other words, the diazinon-resistance influence on the 5th chromosome is considered to be a single incompletely dominant gene, located at an approximate recombination value of 30% from the *ar* locus. A new symbol *Dz* (Diazinon-resistance) is therefore proposed here for this 5th chromosomal major gene responsible for the resistance. Figure 3 shows the map position of the *Dz* gene in relation to the marker genes employed.

Table 11. Linkage data for recessive effect of diazinon resistance factor in progeny from Cross 6. Selective dose : 1.5μg/fly

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

Response	Phenotype		Crossover type	Code sign	Number of flies observed
Alive	*ar*	*car*	(0 0)	A	361
	+	+	(1 0)	B	248
	ar	+	(0 1)	C	101
	+	*car*	(1 1)	D	53
Dead	*ar*	+	(1 1)	E	163
	+	*car*	(0 1)	F	241
	ar	*car*	(1 0)	G	639
	+	+	(0 0)	H	1011
Tatal					4370



As a dominant gene

Recombination value x calculated from :
Formula (1) 29.8%
Formula (2) 29.2%

Recombination value y calculated from :
Formula (3) 22.6%
Formula (4) 23.0%

As a recessive gene

Formula (1) 33.5%
Formula (2) 32.8%
Formula (3) 19.7%
Formula (4) 19.9%

Fig. 3. Relative position of the diazinon-resistance gene, *Dz*, to marker genes on the 5th chromosome of the house fly.

Discussion

From the results both expressed as ld-p lines and submitted to factorial analysis, it has been ascertained that the diazinon-resistance in the Hokota strain of the house fly is due to a multifactorial genetic system including both dominant and recessive factors, with a principal imcompletely-dominant gene on the 5th chromosome. Such multifactorial inheritance in the Hokota strain is somewhat different from the monofactorial or oligofactorial situation previously found in the house fly resistance to organphosphorus (OP) insecticides. Using three OP-resistant strains of different origins, Nguy and Busvine[16] showed that both malathion-resistance and parathion-resistance were inherited through single dominant gene pairs, and that these two resistant genes were associated with the same chromosome and possibly the same locus. However, their conclusion on the allelism of these two OP-resistance gene is uncertain because they did not describe whether the heterozygotes used in backcrosses were females or males. In an Australian strain, Hart[8] have also reported a dominant monofactorial inheritance of diazinon-resistance discovered by making repeated backcrosses of hybrids to the susceptible strain. According to his data, however, the LD_{50} value of diazinon for the "resistant" strain is only 0.5 μg/fly or so, while the resistance levels for usual diazinon-resistant strains reported in the world are about 3~5μg/fly in the topical LD_{50}. Harris, Wearden and Roan[7] have reported preliminary data on the genetics of malathion-resistance in an American strain. Their explanation for the 1:1 segregation ratio of R and S individuals in the F_1 hybrids was that malathion-resistance was inherited by two allelic groups. However, the data they reported were too fragmentary to allow any definite conclusion on the mode of inheritance of the resistance. Most of these early reports on the genetics of OP-resistance are based on rather insufficient data of or inadequate interpretation of the ld-p line unsupported by proper genetic analyses.

In the genetic analyses of the diazinon-resistance reported in the first sections of this paper, the crossing experiments were designed to detect only the linkage groups to which the resistant factors belong, and hence each chromosome derived from the resistant strain (i.e. each R chromosome) was inherited as a single unit contributing so much to the resistance. It therefore still remains unknown whether the ensemble of the diazinon-resistance in the homozygous Hokota strain is due to incompletely-dominant factors or to a combination of fully-dominant and recessive genes. For convenience in comparison, the relative values of both the dominant and recessive effects shown in the arc-sine unit in Tables 7, 8 and 9 are illustrted in Figure 4. From the quantitative difference between the size of the dominant effect and that of the recessive effect, it may be provisionally concluded that the 2nd and 4th chromosomal factors act as the incompletely-recessive gene, whereas the 3rd and 5th chromosomal factors as the incompletely-dominant ones. The major influence of the 5th chromosome manifested by both dominant and recessive effects is assumed to be due to the same locus and thus a single partially dominant gene. Although the 6th chromosomal effects and the chromosomal interactions are sometimes statistically significant (for example, 2-5 in Cross 4), their effects are rather smaller than those of these major resistance factors and may have little or no biological significance because the variance ratio, F, may vary with the mean square for error. In these crosses for detecting the recessive factors, the values of the mean square for error are smaller than those in the crosses for the dominant factors and this may

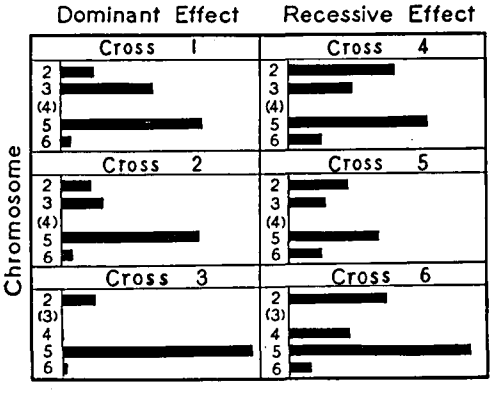


Fig. 4. Schematic illustration of relative chromosomal effects on diazinon-resistance.

be one of the causes for the overestimation of these interactions.

The observed shape of the ld-p lines for the backcross progeny of Crosses 1 and 2 are in accordance with those of hypothetical ld-p lines expected from the frequency of each R chromosome (or phenotype) adjusted from the observed data in Tables 1 and 2 and from relative resistance levels for each phenotype estimated from Tables 7 and 8. For example, in these backcrossing systems, 16 kinds of phenotypes were expected to segregating equal amounts in the backcross generation. However, in the observed figures, the mutant phenotypes showed lower numbers of emerged flies. This might be one of the causes of the deviation of the observed from the expected lines in usual unmarked experiments. Therefore, a combination of factorial analysis with the usual toxicological approach involving ld-p lines brings out the most efficient information on the mode of inheritance of insecticide-resistance.

Oppenoorth[17]and Oppenoorth and van Asperen[19] proposed the hypothesis that a mutation at one and the same locus brings about both increased OP-resistance and lower aliesterase activity in the housefly. Franco and Oppenoorth[6] further reported that both diazinon-resistance and lower aliesterase activity could be associated with the 5th chromosome in an American strain. However, they did not determine whether these two physiological chatacters were due to a single allele or different genes on the same chromosome.

The biochemical or physiological function of the diazinon-resistance genes in the Hokota strain, especially the *Dz* gene on the 5th chromosome, still remains undiscovered, although Ogita and Kasai (unpublished) showed that this diazinon-resistant strain had lower aliesterase activity. Therefore it remains important to know (1) whether Oppenoorth's *a* gene for lower aliesterase activity is truly responsible for the diazinon-resistance, and (2) whether the *Dz* and the *a* genes are alleles of each other. Determinations of linkage and gene location of these physiological characters would seem to offer the useful genetic approach to this aspect of insecticide-resistance research.

Summary

The genetic analyses of diazinon-resistance in a Japanese strain of the house fly were carried out on the basis of the F_1 male-backcross for the determination of linkage group and that of the F_1 female-backcross for the gene location of the resistance factor on a chromosome. Factorial analysis of the data from the F_1 male-backcrosses both to susceptible and resistant multichromosomal marker strains indicated that the diazinon-resistance was due to a multifactorial system in which the 5th chromosome exerted the major influence. The ranking of chromosomes for contribution to the dominant effect was 5th> 3rd> 2nd ; whereas that for the recessive effect was 5th>2nd>3rd. Analyses based on the F_1 female-backcrosses suggested that a single locus was responsible for both the dominant and the recessive effects of the 5th chromosome on the resistance. This incompletely-dominant major gene for the diazinon-resistance is denoted by the new symbol *Dz*, and it is located in the terminal region of the left arm at an approximate recombination value of 30% from the aristapedia (*ar*) locus.

Acknowledgements-The authors wish to express sincere appreciations to Prof. H. Kikkawa for his direction, and to Dr. T. Hiroyoshi for his useful suggestions throughout the course of this investigation. They are also greateful to Dr. K. Yasutomi of the National Institute of Health, Tokyo, for sending the resistant Hokota strain, to Prof. R. Milani, University of Pavia, Italy, for sending the *ar* ; *ac* strain from which multichromosomal mutant strains were synthesized, to Dr. K. Kojima and Dr. T. Kasai for supplying the samples of diazinon, and to Prof. A. W. A. Brown, University of Western Ontario, Ontario, Canada for kindly reading the original manuscript.

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A Genetic Study of Resistance to Nicotine Sulfate in House Flies. Zen-ichi Ogita and Tsutomu Kasai* (Department of Genetics, Medical School, Osaka University, Osaka). Received October 31, 1965. *Botyu-Kagaku*, 31, 14. 1966.

2. イエバエにおける硫酸ニコチン抵抗性の遺伝学的解析

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殺虫剤に抵抗性を示す昆虫を防除するための一つの方法として殺虫剤の交互使用 (rotation) が考えられる. この場合 ratation をおこなう薬剤は相互に交差抵抗性を示さないことが要求される. 硫酸ニコチンやロテノン等の植物性殺虫剤に対する抵抗性と有機合成殺虫剤に対する抵抗性との関係を明らかにするために, 硫酸ニコチン抵抗性の遺伝学的解析をおこなうことによって交差抵抗性を明らかにした. またイエバエとキイロショウジョウバエとの間の交差抵抗性を示す殺虫剤相互の差異から両種の間の染色体の相同性についても論議した.

It has often been suggested that a rotation of insecticides would be a mean of avoiding or delaying the development of resistance to insecticides in insects. It is necessary to use two or more insecticides exhibiting independent and uncorrelated action to be rotated. Investigation of the cross-resistance pattern revealed that, in house flies, there were three separate types of resistance within the insecticides available, namely, DDT-resistance, BHC-resistance and organophosphate-resistance[1]. This classification may be applied in several other insect species, such as mosquitoes, body lice, cockroaches[1]. However, only two types of resistance exist in *Drosophila melanogaster*[2]. Although many investigators have reported on resistance to various kind of insecticides in many insect species, few reports have been published on resistance to botanical insecticides such as nicotine, rotenone and pyrethrin. It is interesting to compare the mechanism of resistance to the botanical

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