原　著

Genetic Studies of Resistance to DDT and Nicotine Sulfate in *Drosophila virilis*. Chozo Oshima and Toshiki Hiroyoshi(Genetical Laboratory, Faculty of Science, Osaka University, Osaka, Japan) Received July 10, 1956. *Botyu-Kagaku* **21**, 65～70, 1956.

15. クロシヨウジヨウバエの DDT 及び硫酸ニコチン抵抗性の遺伝学的研究

大島長造・広吉寿樹（大阪大学　理学部　生物学教室） 31. 7. 10 受理

クロシヨウジヨウバエの野生型の19系統及び突然変異型の5系統の DDT 抵抗性を調べた。それらの抵抗性の程度は DDT を 25μg/cm^2 の割合に滲み込ませた濾紙に 25°C 湿度 75% のもとで24時間ハエを接触させてその生存率によつて知ることが出来た。一方飼料中に DDT 30～40 μg/ml，ニコチン 750～900 ppm の割合に混ぜてそれから羽化してくるハエと全く殺虫剤を含まない飼料から羽化してくるハエの羽化数を比較して統計学的な分析を行つた。抵抗性の彦根系統（滋賀県彦根市にて採集）の DDT 抵抗性をあらわす優性遺伝子は第2及び第5染色体にあることがわかつた。それら抵抗性遺伝子のあらわす効果は殆んど同じで，それらの間には相互作用が認められた。又同系統の硫酸ニコチン抵抗性をあらわす優性遺伝子も第2及び第5染色体にあることが認められた。効果の点では第2染色体の遺伝子の方が強かつたが両染色体の遺伝子間の相互作用は認められなかつた。しかし抵抗性をあらわす優性遺伝子がいくつあるか及び両殺虫剤抵抗性遺伝子が共通のものであるかどうかはこの実験では明かにすることは出来なかつた。DDT 及び硫酸ニコチン抵抗性に関する主要な優性遺伝子がキイロシヨウジヨウバエとクロシヨウジヨウバエの互に相同と考えられた染色体にあるということは興味あることである。又これら抵抗性遺伝子が両殺虫剤に対してある共通の生理的作用をあらわすことも考えられる。このような推測は交叉抵抗性や抵抗性の獲得過程を説明するには有益である。

Genetical experiments to determine the locations of DDT resistant genes on chromosomes of *D. melanogaster* have been performed by Tsukamoto, Ogaki and Crow et al. Recently Tsukamoto has reported the genic analysis of nicotine sulfate resistant genes in the same species.

In this paper, the linkage relation of dominant genes responsible for resistance to DDT and nicotine sulfate in *D. virilis* was studied and the genetic nature of resistance to insecticides was discussed together with the experimental results reported in *D. melanogaster*.

Materials, methods of mating and of testing for resistance.

DDT resistance of adult flies in wild and mutant strains of *D. virilis* and F_1 adult flies between some strains was investigated by the procedure (1) stated below. The most highly resistant strain (Hikone) obtained in the experiment, was mated to the multichromosomal mutant strain (*b*; *t*; *cd*; *es*), which had a recessive mutant gene on each autosome, as shown in the following mating formula.

The degree of resistance of each phenotyped fly in the F_2 generation was examined by the procedure (2) stated below and the linkage relation of dominant genes to DDT and nicotine sulfate was assumed.

Mating.

(Hikone strain) ♀ × *b*; *t*; *cd*; *es* ♂

↓

b; *t*; *cd*; *es* ♀ × F_1 ♂

↓

F_2

+; +; +; +	+; +; *cd*; +	+; +; +; *es*	+; +; *cd*; *es*
b; +; +; +	*b*; +; *cd*; +	*b*; +; +; *es*	*b*; +; *cd*; *es*
+; *t*; +; +	+; *t*; *cd*; +	+; *t*; +; *es*	+; *t*; *cd*; *es*
b; *t*; +; +	*b*; *t*; *cd*; +	*b*; *t*; +; *es*	*b*; *t*; *cd*; *es*

In the F_2 generation, sixteen phenotypes are segregated.

The symbol + means heterozygous chromosomes derived from resistant and mutant strains.

b : broken, wing vein mutant gene located on the second chromosome.

t : telescope, eye and thorax shape mutant gene located on the third chromosome.

cd : cardinal, eye color mutant gene located on the fourth chromosome.

es : eosinoid, eye color mutant gene located on the fifth chromosome.

(1) DDT-resistance test of adult flies.

Flies lived on a culture medium of malted rice, sugar, agar and yeast throughout their lives. The adult flies were collected on emergence and withheld for 4 or 5 days before testing them for resistance. The degree of resistance was determined from survival rates of flies exposed to DDT for 24 hours.

A piece of filter paper (2.5×6 cm^2) was impregnated with DDT-acetone solution. The DDT content after drying was 25 $\mu g/cm^2$.

The filter paper was then fastened with starch to the bottom of a glass tube (diameter 2 cm, height 5 cm). Just prior to testing, the filter paper was moistened with 0.3ml distilled water. Approximately forty adult flies were introduced into the tube which was then stoppered with a cotton plug. The glass tube was placed in a large dessicator, in which humidity was maintained at 75% by means of a saturated solution of sodium chloride and at a constant temperature of 25°C. This test was repeated about three times in a strain.

(2) DDT and nicotine sulfate resistance test of flies and the analysis of variance of dominant resistance factors.

For detecting the linkage of dominant resistant genes, larvae having different genotypes were cultivated on the same medium as described above, mixed with an insecticide by means of homogenizer. As shown in the mating, five multichromosomal mutant female flies were mated with five F_1 male flies. These pairs were kept on the culture medium for five days, during which the females continued egg-laying. The character of F_2 flies emerged from the culture medium were observed. The sequence of vials was divided into two groups of even and odd numbered vials in order to evaluate the variation due to chance. The number of each phenotyped fly emerged in each group of vials was counted and totaled.

The percentages of each phenotyped fly emerged from the medium containing an insecticide to each phenotyped fly emerged from the normal medium were transformed to arc sin units.

The main effects and various interactions of dominant resistance factors were calculated and the analysis of variance was performed.

Experimental results.

Adult flies of wild and mutant strains of *D. virilis*, collected in Japan and in America or developed in our laboratory, were tested for DDT-resistance. The result is shown in table 1.

Table 1. DDT-resistance of wild and mutant strains of *D. virilis*.

Strain	Survival rate (%)
American strain	
New York	7.0
Pasadena	32.0
San Antonio	19.0
Japanese strain	
Maruyama	25.0
Otaniike	12.0
Fukushima	5.0
Niigata	17.0
Kitakata	12.0
Kanazawa	16.0
Daishoji	4.0
Tokyo	7.0
Ashitakayama	6.0
Hikone	81.6
Otsu	17.0
Daisen	6.0
Hibayama	8.0
Matsuyama	4.0
Kochi	7.0
Fukuoka	5.0
Mutant strain	
cn es	16.4
b; t; cd; es	7.0
Sb	0.8
v; es pe	88.8
st B pe	83.9

Most strains, except Hikone and some mutant strains, were not resistant to DDT. The resistence of F_1 adult flies between some strains was observed and the result is shown in Table 2.

The DDT-resistance of F_1 flies was almost intermediate in degree between their parent's resistance. Female flies showed higher resistance than male flies. No cytoplasmic character for DDT-resistance was recognized.

Table 2. DDT-resistance of F_1 flies from mating between some strains.

P ♀		♂	F_1	Survival rate (%)
cn es	×	Pasadena		13.3
Kochi	×	*v; es pe*		52.5
v; es pe	×	Kochi		60.5
st B pe	×	*v; es pe*		84.3
b; t; cd; es	×	*st B pe*	♀ 63.4 ♂ 34.1	48.5
st B pe	×	*b; t; cd; es*	♀ 50.3 ♂ 25.9	37.6

The experiment, using a highly resistant Hikone strain and a susceptible multichromosomal mutant strain, was performed in order to determine the linkage of dominant DDT resistant genes.

At the same time, analyses of dominant nicotine sulfate resistant genes were performed with the aim of obtaining knowledge of the interrelation between DDT and nicotine sulfate resistances.

The culture medium was mixed homogeneously with the insecticide: DDT (30-40 μg/ml) or nicotine (750-900 ppm). The culture medium containing no insecticide was used as the control experiment.

These results are shown in table 3.

These results were statistically analysed as stated in paragraph (2). The results are shown in tables 4 and 5.

From these results, it would be supposed that dominant DDT resistant genes are located on the second and fifth chromosomes respectively and dominant nicotine sulfate resistant genes are also located on the same chromosomes of Hikone strain. In the case of DDT-resistance, the main effects of both dominant factors were almost equivalent to each other and the interaction between them was highly significant, but the main effect of dominant factor to nicotine sulfate on the second chromosome was larger than that on the fifth chromosome and the interaction between them was not significant.

Discussion.

Analyses of the linkage of genes related to insecticide resistance have been effectively

Table 3. Each number of F_2 offspring, having different phenotypes, emerged from the mating of *b; t; cd; es* ♀ × F_1 (Hikone ♀ × *b; t; cd; es* ♂) ♂.

Sort of medium	Control			30 - 40 μg/ml DDT			750 - 900 ppm Nicotine		
Phenotype of F_2 flies	♀	♂	Total	♀	♂	Total	♀	♂	Total
+; +; +; +	268	265	533	154	139	293	297	224	521
b; +; +; +	241	228	469	33	24	57	67	41	108
+; *t*; +; +	134	173	307	102	118	220	165	124	289
b; *t*; +; +	147	145	292	22	13	35	25	12	37
+; +; *cd*; +	221	213	434	128	95	223	272	182	454
b; +; *cd*; +	191	185	376	25	12	37	49	29	78
+; *t*; *cd*; +	193	217	410	103	130	233	215	164	379
b; *t*; *cd*; +	169	161	330	17	10	27	27	22	49
+; +; +; *es*	248	218	466	20	17	37	237	168	405
b; +; +; *es*	185	179	364	3	2	5	39	20	59
+; *t*; +; *es*	202	200	402	40	41	81	203	161	364
b; *t*; +; *es*	168	159	327	5	4	9	26	18	44
+; +; *cd*; *es*	203	193	396	48	17	65	164	130	294
b; +; *cd*; *es*	175	154	329	6	9	15	14	16	30
+; *t*; *cd*; *es*	200	191	391	41	39	80	160	115	275
b; *t*; *cd*; *es*	170	148	318	11	0	11	2	1	3
Total	3115	3089	6204	758	670	1428	1962	1427	3389
Number of vials	25			50			45		

advanced in *Drosophila* species in which many mutant genes are found.

Crow, Stott and Burrington[1] have analysed the DDT-resistance of *D. melanogaster* by use

Table 4. The percentages and transformed arc sin units of each phenotyped fly emerged from the medium containing an insecticide to each phenotyped fly emerged from the normal medium. The number of vials was revised to 25 vials in all cases.

Phenotype	DDT				Nicotine sulfate			
	Percent in odd numbered vials	Arc sin unit	Percent in even numbered vials	Arc sin unit	Percent in odd numbered vials	Arc sin unit	Percent in even numbered vials	Arc sin unit
+; +; +; +	26.3	30.85	28.7	32.39	52.9	46.66	55.7	48.27
b; +; +; +	7.0	15.34	5.1	13.05	16.4	23.89	9.8	18.24
+; *t*; +; +	30.6	33.58	41.0	39.82	45.3	42.30	59.3	50.36
b; *t*; +; +	6.2	14.42	5.8	13.94	7.2	15.56	6.9	15·23
+; +; *cd*; +	24.4	29.60	27.0	31.31	56.7	48.85	59.7	50·59
b; +; *cd*; +	4.8	12.66	5.1	13.05	15.7	23.34	7.5	15.89
+; *t*; *cd*; +	27.6	31.69	29.3	32.77	51,2	45.69	51.5	45.86
b; *t*; *cd*; +	4.9	12.79	3.3	10.47	12.1	20.36	4.2	11.83
+; +; +; *es*	4.1	11.68	3.9	11.39	48,5	44.14	48.1	43.91
b; +; +; *es*	0.6	4.44	0.8	5.13	12.1	20.36	5.8	13.94
+; *t*; +; *es*	9.5	17.95	8.0	16.43	40.5	39.52	47.2	43·39
b; *t*; +; *es*	0.3	3.14	2.5	9.10	10.4	18.81	4.3	11.97
+; +; *cd*; *es*	7.6	16.00	8.8	17.26	41.4	40.05	40.9	39.76
b; +; *cd*; *es*	2.1	8.33	2.4	8.91	6.7	15.00	3.3	10·47
+; *t*; *cd*; *es*	10.7	19.09	9·7	18.15	39.9	39.17	38.4	38.29
b; *t*; *cd*; *es*	0.6	4.44	2.8	9.63	0.3	3.14	0.6	4.44

Table 5. The analysis of variance of dominant resistant factors.

Chromosome	DDT dominant resistant factors					Nicotine sulfate dominant resistant factors				
	Effect	S.S.	D.F.	M.S.	F	Effect	S.S.	D.F.	M.S.	F
2	115.54	417.17	1	417.	107.2**	230.70	1692.17	1	1692.	147,5**
3	−13.00	5.28	1	5.	1.3	28.76	25.85	1	26.	2.3
4	−1.74	0.10	1	0.	0.0	23.78	17.67	1	18.	1,6
5	93.34	272.26	1	272.	69.9**	48.32	72.96	1	73.	6,4*
2-3	−15.94	7.82	1	8.	2.1	−10.60	3.51	1	4.	0,4
2-4	−0.04	0.00	1	0.	0.0	−11.08	3.84	1	4.	0,4
2-5	40.72	51.82	1	52.	13.4**	2.52	0.20	1	0.	0,0
3-4	−11.10	3.85	1	4.	1,0	−6.42	1.29	1	1.	0,1
3-5	1.78	0.10	1	0.	0.0	−0.22	0.00	1	0.	0,0
4-5	20.80	13·52	1	14.	3.6	−21.88	14.96	1	15.	1,3
2-3-4	−2.88	0.26	1	0.	0.0	4.10	0.53	1	1.	0,1
2-3-5	−0.24	0.02	1	0.	0.0	3.38	0.36	1	0.	0,0
2-4-5	3.50	0·38	1	0.	0.0	7.18	1.61	1	2.	0,2
3-4-5	2.04	0.13	1	0.	0.0	5.12	0.82	1	1.	0,1
2-3-4-5	−1.30	0.05	1	0.	0.0	13.00	5.28	1	5.	0,4
E		62.28	16	3.89			183.55	16	11.47	

* Significant: 5%　　** Highly significant: 1%

of some dominant mutant markers and concluded that there are one or more dominant factors on each of the major autosomes and on the X chromosome and one or more recessive factors on each of the major autosomes. On the other hand, Tsukamoto and Ogaki[4)7)8)] have reported in their papers that the DDT-resistance in Japanese *D.melanogaster* strains may be controlled by a dominant gene located near *sca* or *vg* genes on the right arm of the second chromosome. Oshima (unpublished) has analysed DDT-resistance of a highly resistant strain used by the previous authors and presumed that this character may be polygenic. However, the effect of dominant factor on the second chromosome was larger than that on the third chromosome, and the interaction between these chromosomal factors was found to be significant.

Tsukamoto[9)] has investigated the genic analysis of nicotine sulfate resistant gene in *D. melanogaster* by using a DDT resistant strain and showed that a dominant gene near the spindle fiber attachment of the third chromosome is concerned with the character, together with some dominant modifying genes on other chromosomes.

Recently, he and Hiroyoshi[6)] have studied the location of the major dominant resistant gene on the third chromosome and reached the conclusion that it is located on the right arm of the third chromosome.

Both *D. virilis* and *D. melanogaster* belong to the same genus, but their karyotypes are remarkably different from each other.

Sturtevant and Novitski[5)] and several other workers have compared analogous mutant genes on each chromosome element in the two species, and they heve assumed that the two species have the homologies of chromosome elements as shown in the following figure.

The dominant gene, responsible for the resistance to DDT on the fifth chromosome of *D. virilis* and that on the right arm of the second chromosome of *D. melanogaster* are assumed to have evolved from the same original gene.

Similarly, the dominant gene, responsible for the nicotine sulfate resistance on the second

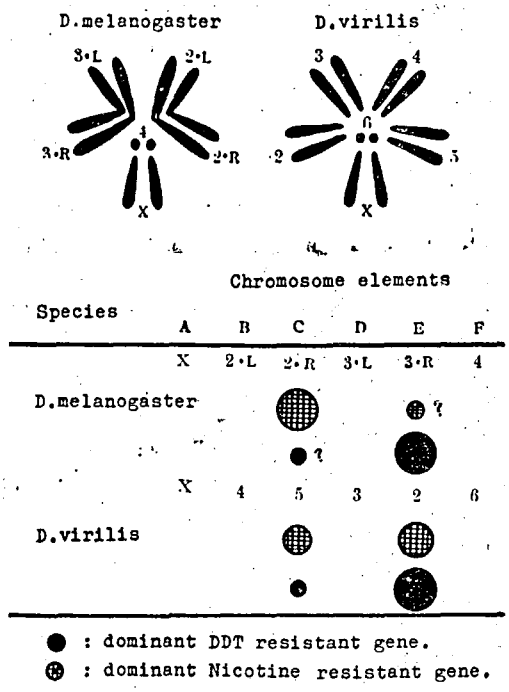

Figure 1. Karyotypes of *D, melanogaster* and *D. virilis* and the homologies of their chromosome elements and the location of dominant DDT and nicotine sulfate resistant genes.

chromosome of *D. virilis* and that on the right arm of the third chromosome of *D. melanogaster* are also assumed to have evolved from the same original gene. If this assumption is affirmed, it may be expected that a dominant resistant gene of DDT is found on the right arm of the third chromosome and a modifying dominant resistant gene of nicotine sulfate on the right arm of the second chromosome of *D. melanogaster*. These phenomena are very interesting for the study of the problem of evolution in *Drosophila* species.

The questions con erning the number of dominant resistant genes on a chromosome and whether or not the genes of DDT-resistance are identical to those of nicotine sulfate, could not be actually proved by this experiment, but it is very important to succeeding physiological investigations of the insecticide resistance that these questions be clarified. Nevertheless, it may be inferred by the following facts that these resistant genes to DDT and nicotine sulfate have some common parts of physiological actions

for these unrelated chemical compounds.

Milani[3] has recognized that house flies which are resistant to a particular chemical compound become resistant to unrelated chemical compounds more rapidly than susceptible flies.

Metcalf[2] has reviewed that the development of resistance to a certain insecticide and other chemical compounds is often induced simultaneously in house flies.

The character of resistance to insecticides may be generally polygenic. The positive interaction between dominant resistant factors of DDT on the two major autosomes in *D. melanogaster*, which was found by Crow et al. and by Oshima, was also recognized similarly in *D. virilis*. On the contrary, the interaction was not noticed between the dominant resistant factors of nicotine sulfate on the two autosomes.

This difference may express the characteristic nature of the chemical compounds. The existence of recessive genes responsible for the resistance to insecticides may be assumed in *D. virilis*, but the locations of these genes on chromosomes will be shown by further studies.

Summary.

(1) DDT and nicotine sulfate resistance in *D. virilis* was investigated. The degree of resistance of adult flies was determined by their survival rate when in contact with a filter paper containing DDT (25 μg/cm^2) during 24 hours, at 75% humidity, 25°C. In another resistance test, the rate of adult emergence from larvae reared on the medium mixed with the insecticide: DDT (30-40 μg/ml) or nicotine (750-900 ppm) was calculated and statistically analysed in comparison with the rate of adult emergence from the normal medium.

(2) The dominant genes responsible for the DDT-resistance in *D. virilis* of Hikone strain were confirmed to be located on the second and fifth chromosomes. It was recognized by the statistical analysis that the main effects of two chrmosomal factors were almost equivalent to each other and their interaction was positive. The dominant genes relating to the nicotine sulfate resistance in the same strain were confirmed to be located on the second and fifth chromosomes, but the main effect manifested by the former was larger than the latter and their interaction between them was not significant. However, the question concerning the number of dominant resistant genes on chromosomes and whether or not the resistant genes of the two insecticides are identical, could not be proved by this experiment.

(3) Of significance to an understanding of the processes of evolution is the finding that DDT and nicotine sulfate dominant resistant genes have been located on homologous chromosome elements of *D. virilis* and *D. melanogaster*. It would be assumed that these genes (or one gene) might have some common physiological actions to both insecticides.

This conjecture may be useful in explaining cross resistance and developmental states of resistance to insecticides.

Acknowledgment.

We appreciate to Prof. Kikkawa for his reading our manuscript and to Mr. Okamoto in the Mathematical Department for his valuable suggestions of the statistical analysis.

Literature.

1) Crow, J.F., G. Stott and J. Burrington: Progress report to Medical Research and Development Board Office of the Surgeon General Department of the Army Washington **25**. D. C. (1953)
2) Metcalf, R. L.: Physiol. Rev., **35**: 197-232 (1955)
3) Milani, R.: Ist International Symposium of the Control of Insect Vectors of Disease. 252-274 (1954)
4) Ogaki, M. and M. Tsukamoto: Botyu-Kagaku, **18**: 100-104 (1953)
5) Sturtevant, A. H. and E. Novitski: Genetics, **26**: 517-541 (1941)
6) Tsukamoto, M. and T. Hiroyoshi: Botyu-Kagaku, **21**: 71~76 (1956)
7) Tsukamoto, M. and M. Ogaki: Botyu-Kagaku, **18**: 39-44 (1953)
8) Tsukamoto, M. and M. Ogaki: Botyu-Kagaku, **19**: 25-32 (1954)
9) Tsukamoto, M.: Botyu-Kagaku, **20**: 73-81 (1955)