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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

荻田善一・笠井 勉* (大阪大学医学部遺伝学教室) 40. 10. 31 受理

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

* Visiting Research Fellow from Japan Agricultural Chemicals and Insecticides Co., Ltd.

insecticides with that of synthetic insecticides such as chlorinated hydrocarbons and organophosphates, and to know to which group botanical resistance belongs, for resistance to the botanical insecticides seems to have developed only rarely in spite of their use against a wide variety of insect species for many years.

The present paper deals with the cross-resistance pattern of a nicotine sulfate resistant strain in house flies, and with a genetic analysis of nicotine sulfate-resistance. The relations between insecticide resistance genes in the house fly and *Drosophila*, and the homologies of their chromosome elements are discussed.

Materials and Methods

Insecticide resistant and susceptible strains of the house fly reared at Osaka University were used. They included a DDT-resistant strain (*K-3926*), a BHC-resistant strain (*Hikone-R*), a diazinon-resistant strain (*203d*), and a susceptible strain (*Lab*). Marker strains in which one or more chromosomes were marked with visible mutant markers were employed for genetic analysis. They included *ro* ; *ct* ; *cm* (2 ; 4 ; 5), *car* (5) and *ar* (5) strains. Their characteristics have been reported[3]. Two nicotine sulfate-resistant strains of the house fly, *NS-R* and *NS* (*ro* ; *ct* ; *cm*), were established by selection pressure with nicotine sulfate. Adult flies newly emerged from pupae were fed with powdered milk and nicotine sulfate solution for 48 hours. The concentration of nicotine-sulfate used was 0.4～2%, which brought about 50～80% mortality. The survivals were reared with untreated food. The selection was continued through succesive generations, and the flies used in these experiments were the result of 18～22 generations of selection. The *NS-R* strain was obtained from a mixed population of 8 wild type strains, and the *NS* (*ro* ; *ct* ; *cm*) strain was obtained from the *ro* ; *ct* ; *cm* (a multichromosomal mutant strain established by Dr. Hiroyoshi of Osaka University) strain by selecting them with nicotine sulfate.

A 40% solution of nicotine sulfate, technical grade of DDT, γ-BHC, Diazinon and Sevin used in these experiments were supplied by Japan Agricultural Chemicals and Insecticides Co., Ltd.

Levels of resistance to insecticides were estimated by two methods. (a) Topical application : one μl of acetone solution of insecticides was topically applied to dorsum of the thorax of ether anesthetized flies and mortality counts were taken 24 hours later. Moribund flies were counted as dead. (b) Contact method : filter paper (No. 2, Toyoroshi Co.) was put into a petri dish, 9 cm in diameter and 2 cm high, and 1 ml of 4% nicotine sulfate solution was pipetted onto the filter paper. Ten flies were confined in the petri dish. Knock down counts were performed at appropriate intervals. For the determination of the linkage group of nicotine sulfate resistance a crossing experiment was performed. Males of F_1 hybrid obtained from a cross (*ro* ; *ct* ; *cm* ♀ × *NS-R* ♂) were backcrossed to female *ro* ; *ct* ; *cm* flies. F_2-progeny from the backcross were separated into each phenotype and levels of resistance to nicotine sulfate were estimated with the contact method.

Results

Several wild and mutant strains which had been cultured for many years in the laboratory, were tested for their resistance to nicotine sulfate (Table 1). *NS-R* and *NS* (*ro* ; *ct* ; *cm*) strains were highly resistant, showing no mortality after 24 hours. Though *K-3926*, *Hikone-R* and *203d* strains were also highly resistant, they showed a few knock-down flies after 24 hours. These strains were assumed to consist of flies mostly resistant to nicotine sulfate, but some susceptible flies were included. These strains showed only a few flies knocked down after 24 hours, KT-50 values being over 24 hours. *Lab*, *ro* ; *ct* ; *cm*, *car* strains were susceptible, KT-50 being 100～130 minutes with the contact method, whereas *ar* strain was intermediate. As *K-3926*, *Hikone-R*, *203d* strains exhibited resistance to nicotine sulfate, it was not clear to which group (DDT, BHC or organophosphate groups) nicotine sulfate-resistance belongs, or whether it was independent and uncorrelated from these insecticidal groups. Investigation of cross-resistance patterns of nicotine sulfate resistance was performed by comparing *NS* (*ro* ; *ct* ; *cm*) strain with its original unselected *ro* ; *ct* ; *cm* strain on the levels of resistance to various insecticides (Table 2). The LD-50 value of *NS*

Table 1. Levels of resistance to nicotine sulfate in several strains of house flies.

Strain	No. of insects tested	Time (min.)—Knock-down (%) 60	90	120	180	300	600	1440	KT-50 (min.)
Lab	31	6.5	42	67.6	83.9	100			100
car	30	10	23.3	43.3	90	100			130
ar	30				6.7	20	33.3	43.3	1440<
NS(*ro* ; *ct* ; *cm*)	50							0	1440<
K-3926	50						2	6	1440<
Hikone-R	50					6	12	18	1440<
203d	50						0	4	1440<
ro ; *ct* ; *cm*	50	14	42	64	84	100			100
F_1(*ro* ; *ct* ; *cm*♀ × *NS-R*♂)	50					2	18	26	1440<
NS-R	50							0	1440<

Table 2. Resistance levels of *ro*;*ct*;*cm* strain and its nicotine sulfate selected strain, *NS* (*ro*;*ct*;*cm*), to several insecticides.

Insecticide	LD-50 (μg/fly) by topical application *ro*;*ct*;*cm* (A)	*NS*(*ro*;*ct*;*cm*) (B)	Resistance ratio (B)/(A)
DDT	0.12	0.15	1.25
BHC	0.48	0.89	1.85
Diazinon	0.011	0.085	7.64
Sevin	1.5	4.5	3.0
Nicotine sulfate*	100	1440<	14.4<

* Based on KT-50 obtained with the contact method.

(*ro* ; *ct* ; *cm*) strain to Diazinon was about 8 fold larger than those of *ro* ; *ct* ; *cm* strain, whereas LD-50 for DDT, γ-BHC and Sevin were about 1~3 times as large in *NS* (*ro* ; *ct* ; *cm*) strain than in *ro* ; *ct* ; *cm* strain. These results suggest that there may be common factor(s) in nicotine sulfate resistance and Diazinon resistance, and that the slightly larger LD-50 values to DDT, γ-BHC and Sevin might be caused by a vigor tolerance of the *NS* (*ro* ; *ct* ; *cm*) strain.

A crossing experiment was done to determine whether the inheritance of nicotine sulfate resistance was controlled by dominant or recessive genes. As shown in Fig. 1, it was clear that the resistance to nicotine sulfate of F_1 flies emerged from a cross of susceptible with resistant strains showed an intermediate level. As female flies of F_1-progeny showed almost the same level of

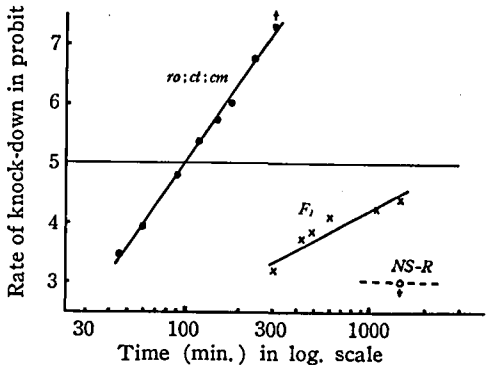

Fig. 1. Time-Knock down regression lines of nicotine sulfate with the contact method against susceptible and resistant strains of house flies.

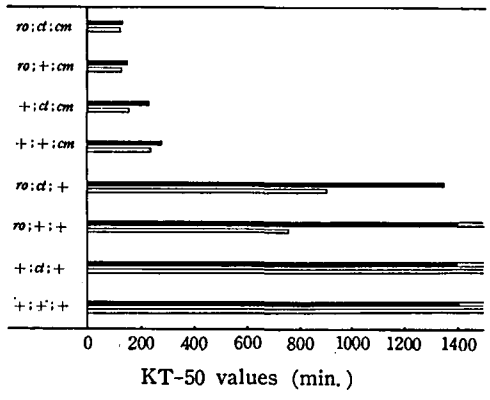

Fig. 2. KT-50 of each phenotypic F_2-progeny obtained from a backcross {*ro* ; *ct* ; *cm*♀ × F_1 (*ro* ; *ct* ; *cm*♀ × *NS-R*♂) ♂} with nicotine sulfate treatment. Thirty to 60 flies of each phenotype were used, ■ ; ♀, □ ; ♂

resistance as males, it is assumed that the co-dominant gene or genes are not sex-linked. To analyze which chromosome is responsible for resistance, a backcross was carried out by using susceptible multichromosomal mutant strains as shown in the previous section. From the backcross, flies of 8 different phenotypes emerged in the F_2-generation. They were separated into each phenotype and tested for insecticidal action of nicotine sulfate (Fig. 2). From Fig. 2 it is clear that phenotypes *ro*; *ct*; *cm*, *ro*; +; *cm*, +; *ct*; *cm*, and +; +; *cm* were more susceptible than *ro*; *ct*; +, *ro*; +; +, +; *ct*; +, and +; +; + phenotypes. This fact shows clearly that the major gene responsible for the nicotine sulfate-resistance is linked with the *cm* character located on the 5th chromosome. However, *ro*; *ct*; *cm* and *ro*; +; *cm* flies are slightly more susceptible than +; *ct*; *cm* and +; +; *cm* flies, *ro*; *ct*; + and *ro*; +; + are also slightly more susceptible than +; *ct*; + and +; +; + flies. This fact implies that a minor factor linked with *ro* character (2nd chromosome) is also involved in nicotine sulfate resistance.

Discussion

Genetic studies on insecticide-resistance in *Drosophila* and house flies have been performed by many investigators. Tsukamoto and Ogaki[4] indicated that DDT-resistance is controlled mainly by a gene located at 65± on the second chromosome in *Drosophila melanogaster*. This gene is also responsible for the resistance to BHC[5], parathion[2] and Sevin[6]. Another gene located at 50± on 3rd chromosome is responsible for nicotine sulfate-resistance[7]. Oshima and Hiroyoshi[8] studied resistance to DDT and nicotine sulfate in *D. virillis* and indicated that two factors might be responsible for the resistance to the insecticides, namely, the factors on the 5th and 2nd chromosomes, which were assumed to be homologous chromosomal elements from 2nd and 3rd chromosomes of *D. melanogaster*. This fact implies that the mechanism of resistance to DDT and nicotine sulfate in these two species of insects may be the same.

However, cross-resistance patterns in house flies were somewhat different from *Drosophila*. Resistance to DDT, BHC and parathion, which

Table 3. The homology of chromosome elements and the location of insecticide-resistance genes.

	Chromosome elements							Reference
	A	B	C	D	E	F	G	
D. melanogaster		X	2L	2R	3L	3R	4	
DDT				●		○		(5)
BHC				●		○		(5)
Nicotine-sulfate				○		●		(7)
Parathion				●		○		(2)
Sevin				●		○		(6)
D. virilis		X	4	5	3	2	6	
DDT				●		●		(8)
Nicotine sulfate				○		●		(8)
M. domestica	X	2	3	4		5	6	
DDT		○				●		(11)
BHC		●		○				(9, 10)
Nicotine sulfate		○				●		
Diazinon						●		(9)
Sevin		○		○		●		(10)

The homology of the chromosome elements between *Musca* and *Drosophila* was suggested by Dr. Hiroyoshi.

● : the major resistance gene, ○ : the modifier gene.

was due to a single gene in *D. melanogaster*, was controlled by separate genes in house flies ; γ-BHC-resistance was controlled mainly by the factor on the 2nd chromosome[9)10)]. Major genes for DDT-resistance and Diazinon-resistance were located at different loci on the 5th chromosome[11)].

Hiroyoshi has compared analogous mutant genes on each chromosome element in *Drosophila* and house flies and he has assumed that these insect species have the homologies of chromosome elements as shown in Table 3 (Hiroyoshi, unpublished). The authors have provided some evidence on gene homologies between *Drosophila* and house flies. Namely, genetic studies on esterases showed that occurrance of esterases is controlled by the alleles on the 3rd chromosome in *D. melanogaster* (Ogita[12)] 1961, Wright[13)] 1963) and on the 5th chromosome in house flies (Ogita[14)] 1962, Ogita and Kasai[3)]1965). Oshima and Hiroyoshi[8)]suggested that genes responsible for the resistance to DDT or nicotine sulfate in *D. virillis* and those in *D. melanogaster* are assumed to have evolved from the same original gene. However, as shown in Table 3, the major gene responsible for resistance to DDT, or to BHC in house flies are not located on a homologous chromosome with *Drosophila*, whereas the major gene responsible for resistance to nicotine sulfate is located on the homologous chromosome, *i. e.*, 3rd chromosome in *D. melanogaster* and 5th chromosome in the house fly.

Thus, the genetic study of insecticide-resistance might bring about a convenient method for classification of insecticides as to their cross-resistance patterns. Classification of the insecticides is useful for the rotation in insect control program to avoid the devolopment of insecticide-resistance. As resistance to botanical insecticides seems to develop rarely, it may be very useful, if the mechanism or the cross-resistance pattern of the botanical insecticides are clarified. Further studies on comparisons between botanical and synthetic insecticides are now going on from biochemical and genetic bases.

Summary

In order to classify insecticides as to their cross-resistance patterns, a genetic study on resistance to nicotine sulfate in house flies was performed. A nicotine sulfate resistant strain showed a moderate resistance to Diazinon whereas it showed only very slight resistance to DDT, γ-BHC and Sevin. A genetic analysis indicated that inheritance of nicotine sulfate resistance is controlled by codominant and multiple factors, *i. e.*, depending upon the main gene on the 5th chromosome and modifiers on other chromosomes. The homology of chromosome elements was compared between house flies and *Drosophila*, and it was assumed the main resistance gene for nicotine sulfate was evolved from the same original gene among these insects.

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