susceptible direction.

The chromosome, the locus and the property of this resistance gene were in accordance with the ones which were found in various resistant strains of the world. This fact suggests strongly that the majority of resistant strains may be due to polyphyletic origins.

Lastly it was pointed out that the phenomenon of cross-resistance to various insecticides and a negatively correlated effect for PTU, might be due to pleiotropic expressions of a single gene.

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The Genetic Study on the Resistance to Sevin in *Drosophila melanogaster*. Hideo KIKKAWA (Department of Genetics, Medical School, Osaka University, Osaka) Received July 16, 1964 *Botyu-Kagaku*, **29**, 42, 1964

9. キイロショウジョウバエにおけるセビン抵抗性に関する遺伝学的研究

吉川秀男（大阪大学医学部遺伝学教室） 39. 7. 16 受理

最近セビン，ピロラン等のカーバメート系殺虫剤がカやシラミの駆除に広く用いられるようになった．興味のあることはカやシラミではカーバメート系殺虫剤と DDT 等の間に逆相関の効果，すなわち DDT に強いものは却ってカーバメート系殺虫剤に弱いという現象があるらしいといわれている．

キイロショウジョウバエではまだカーバメート系殺虫剤の抵抗性についての研究がないと思われるので，上記の逆相関の現象と結びつけて実験を行った．カーバメート系殺虫剤としてはセビンを用いたが，実験の結果キイロショウジョウバエのセビン抵抗性は DDT やパラチオン抵抗性と同じく第2染色体の 64.5 の位置にある一つの優性遺伝子によってひきおこされることがわかった．つまりショウジョウバエでは一つの遺伝子の変化に伴って塩素系，有機燐系およびカーバメート系のどの殺虫剤に対しても交差抵抗性のおこることが見出された．従ってカやシラミに見出されたような DDT とカーバメート系殺虫剤との逆相関の関係は存在しないものと思われる．

Introduction

Within the past few years, carbamate insecticides have become widely used for the control of vectors such as mosquitoes and body lice. Recent work suggests the possibility of a negatively correlated effect between carbamate insecticides and chlorinated hydrocarbons[1,2]. Such a negatively correlated effect was found by Ascher and his coworkers[3,4] in *Musca domestica*, viz., some drugs such a cetylbromoacetate were more effective to DDT-resistant flies than to susceptible ones. Subsequently, Ogita[5,6] found in *Drosophila melanogaster* that phenylthiourea and its halogen derivatives were more effective to DDT-resistant larvae than to susceptible ones. A review concerning this phenomenon has been reported by Brown[7]. Since there seems no available datum as to the resistance to carbamate insecticides in *D. melanogaster*, a genetic study was undertaken in connection with the problem of a negatively correlated effect mentioned above.

Materials and Methods

As a carbamate insecticide, Sevin (1-naphthyl-

methyl carbamate) supplied from the Nihon Agricultural Drug Company Inc., was used. It was dissolved in 99% ethylalcohol in a concentration of 10,000 p. p. m. and was mixed with a medium to the desired consistency.

The test method was the same as that employed in my previous paper[8]. A certain number of first instar larvae of *D. melanogaster* were placed on a medium composed of 2% agar, 4% sucrose, 3% yeast powder and a known concentration of Sevin, and the rate of emergence of adult flies was examined.

The Hikone strain which had been selected by increasing concentrations of Sevin for ten generations was used as a Sevin-resistant strain. As susceptible lines, three strains with the following visible markers were used: the first was homozygous for brown (*bw*; 2-104.5); scarlet (*st*; 3-44.0); shaven-naked (sv^n; 4-0.0+), the second for cinnabar (*cn*; 2-57.5); spineless (*ss*; 3-58.5); grooveless (*gvl*; 4-0.0+), and the third for cinnabar (*cn*; 2-57.5) vestigial (*vg*; 2-67.0) brown (*bw*; 2-104.5).

Experimental Results

Rates of emergence of these strains in probits are shown in Figure 1. The LD_{50} of Hikone is about 80 p. p. m., whereas that of the *cn*; *ss*; *gvl* strain and of the *cn vg bw* strain is slightly less than 10 p.p.m. The LD_{50} of the *bw*; *st*; sv^n strain seems to be very low, viz., it is about 1 p. p. m.

In order to examine whether the resistance to Sevin is dominant or not, and also whether it is sex-linked or not, the following matings were made. The results are shown in Tables 1 and 2.

From these results, it may be concluded that the resistance to Sevin is dominant to the susceptibility, and that the major gene or genes responsible for the resistance to Sevin, seem to be not sex-linked. However, in a high concentration of Sevin as 100 p. p. m., the level of resistance in the resistant × susceptible matings, is nearly half that of the homozygous resistant strain (Hikone). This result suggests that the resistance gene or genes are not completely dominant. Furthermore, the fact that the number of males in the mating of resistant ♀ × susceptible ♂ is always larger than that in the reciprocal mating, suggests that there may be involved a minor resistance gene or genes on the X-chromosome of the Hikone strain. However, this problem has not yet been analyzed thoroughly.

Next, in order to determine the chromosome to which the major resistance gene belong, the Hikone strain was mated to the *bw*; *st*; sv^n; Sevin-susceptible strain, and its F_1 males were back-crossed to the multi-chromosomal mutant

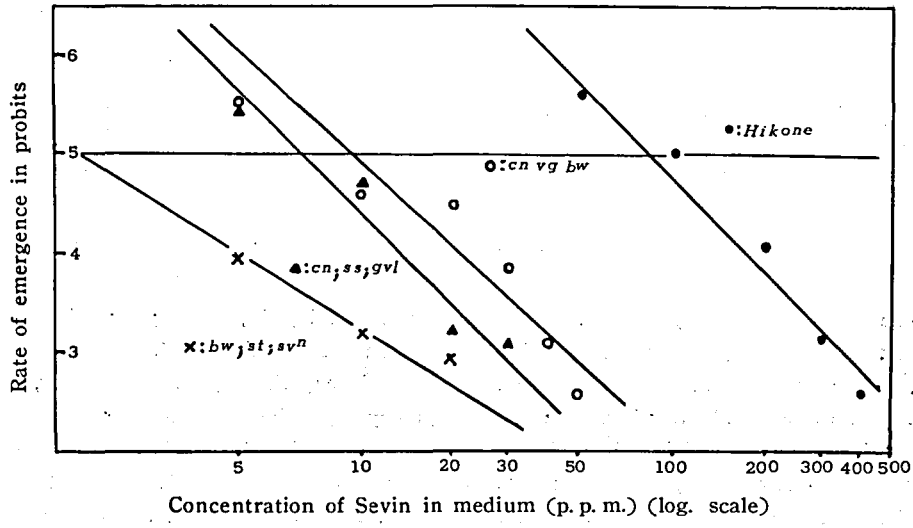

Fig. 1. Levels of resistance to Sevin in resistant and susceptible strains of *D. melanogaster*.

Table 1. Matings of Hikone (*H*) (resistant) and *cn* ; *ss* ; *gvl* (susceptible) strains. 250 larvae were used for each test.

Cross	Concentration of Sevin (p. p. m.)	♀	♂	Total
H♀×*H*♂	0	91	86	117
	50	57	56	113
	100	59	66	125
H♀×*cn* ; *ss* ; *gvl*♂	0	110	103	213
	50	71	102	173
	100	13	55	68
cn ; *ss* ; *gvl*♀×*H*♂	0	95	126	221
	50	54	60	114
	100	41	17	58
cn ; *ss* ; *gvl*♀× *cn* ; *ss* ; *gvl*♂	0	81	108	189
	50	0	1	1
	100	0	0	0

Table 2. Matings of Hikone (*H*) (resistant) and *bw* ; *st* ; sv^n (susceptible) strains. 200 larvae were used for each test.

Cross	Concentration of Sevin (p. p. m.)	♀	♂	Total
H♀×*H*♂	0	60	77	137
	50	86	78	164
	100	77	86	163
H♀×*bw* ; *st* ; sv^n♂	0	77	87	164
	50	80	83	163
	100	41	57	98
bw ; *st* ; sv^n♀×*H*♂	0	82	76	158
	50	64	66	130
	100	53	23	76
bw ; *st* ; sv^n♀× *bw* ; *st* ; sv^n♂	0	55	59	114
	50	5	13	18
	100	0	0	0

strain. The first instar larvae derived from the back-cross were raised on media with or without Sevin. The result is shown in Table 3.

As shown in this Table, under the selection of Sevin, the frequency with which the brown character appears is considerably less than the frequency of the control. This fact indicates clearly that the major gene or genes responsible for the Sevin-resistance are on the second chromosome.

A similar experiment was carried out by using another Sevin-susceptible multi-chromosomal mutant strain, viz., the *cn* ; *ss* ; *gvl* strain. The result is shown in Table 4.

In this experiment, strange to say, the frequency with which each mutant character appears in the offspring is considerably less than the expected frequency (50%), even in the control series. Although the reason for this is not clear, it is assumed that sublethal genes might be

Table 3. *bw* ; *st* ; sv^a ♀ × *bw*/*H* ; *st*×/*H* ; sv^a/*H*♂. Selected with 100 p. p. m. Sevin.

Phenotype	Control	Experiment
bw ; + ; +	38	8
bw ; *st* ; +	31	4
bw ; + ; sv^a	27	2
bw ; *st* ; sv^a	28	2
+ ; *st* ; +	49	27
+ ; *st* ; sv^a	37	21
+ ; + ; sv^a	46	22
+ ; + ; +	52	38
Total	308	124
Larvae tested	500	1800
Rate of emergence	60.2%	6.9%
Frequency of appearance of each mutant character (%)		
bw :	40.3	12.9
st :	47.1	43.5
sv^a :	44.8	37.9

Table 4. *cn* ; *ss* ; *gvl* ♀ × *cn*/*H* ; *ss*/*H* ; *gvl*/*H*♂. Selected with 100 p. p. m. Sevin.

Phenotype	Control	Experiment
cn ; + ; +	76	1
cn ; *ss* ; +	39	0
cn ; + ; *gvl*	72	2
cn ; *ss* ; *gvl*	45	0
+ ; *ss* ; +	51	13
+ ; *ss* ; *gvl*	81	21
+ ; + ; *gvl*	111	65
+ ; + ; +	334	128
Total	809	230
Larvae tested	1100	1300
Rate of emergence	73.5%	17.7%
Frequency of appearance of each mutant character (%)		
cn :	28.7	1.3
ss :	26.7	14.8
gvl :	30.2	38.3

involved in each chromosome of the *cn* ; *ss* ; *gvl* flies used in this experiment.

In spite of abnormal segregations in the control series, the very low frequency of appearance of the *cn* character in the experimental series indicates that the major resistance gene or genes must be on the second chromosome.

In order to determine the locus (loci) of the Sevin-resistance gene (genes) on the second chromosome, the Hikone strain was mated to the *cn vg bw* Sevin-susceptible strain, and the F_1 females were back-crossed to the *cn vg bw* males. The first instar larvae derived from the back-cross were raised on media with or without Sevin. The result is shown in Table 5.

Table 5. *cn vg bw*/*H* ♀ × *cn vg bw*♂. Selected with 100 p. p. m. Sevin.

Phenotype			Control	Experiment
cn	*vg*	*bw*	245 (0*)	4 (1, 2*)
+	+	+	364 (0)	304 (0)
cn	+	+	42 (1)	23 (1)
+	*vg*	*bw*	35 (1)	5 (2)
cn	*vg*	+	152 (2)	12 (1, 2, 3)
+	+	*bw*	152 (2)	92 (3)
cn	+	*bw*	7 (1, 2)	2 (1, 3)
+	*vg*	+	8 (1, 2)	3 (2, 3)
Total			1005	445
Larvae tested			1450	3350
Rate of emergence			69.3%	13.3%

Recombination value

cn-vg : 9.2 *cn*-RI** : 9.2

vg-bw : 31.7 *RI-vg* : 5.4

vg-bw : 24.5

* Showing crossover region.

** *RI* : Resistance gene for Sevin.
Locus of *RI* : 61.6-66.7

As seen in this Table, it is concluded that the major resistance gene (*RI-Sevin* or *RI*) is located near the *vg* gene, although the result seems to be slightly disturbed by some surviving susceptible (non-*RI-Sevin*) individuals. If the locus of the *RI-Sevin* gene is computed by a proportional scale, the gene will be located at 63.5. This locus is well in accordance with that of the parathion- or DDT-resistance gene (2-64.5), reported previously[5].

Discussion

As shown in the preceding section, the major gene responsible for Sevin-resistance in *D. melanogaster* behaves like the parathion- or the DDT-resistance gene. This fact implies that the dominant gene located at 64.5 on the second chromosome shows cross-resistance not only for chlorinated hydrocarbon insecticides like DDT and organophosphorus insecticides like parathion,

but also for carbamate insecticides like Sevin. This conclusion is supported further by the fact that the parathion-resistance gene induced by the X-ray treatment is also resistant to Sevin[9]. Thus no negatively correlated effect has been found between DDT- and Sevin-resistance in *D. melanogaster*. In this respect, the mechanism of resistance to various insecticides in *Drosophila* seems to be different from that in mosquitoes or body lice.

Summary

The resistance to Sevin in *D. melanogaster* is mainly controlled by a dominant gene located near 64.5 on the second chromosome, which is also responsible for the resistance to DDT and parathion. No negatively correlated effect has been found between DDT- and Sevin-reistance.

Acknowledgment: This work has been supported by the grant of WHO and NIH(GM-10154), respectively.

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Toxicity of Malathion to the Common House Fly Evaluated by the Impregnated Filter Paper Method. Studies on the Biological Assay of Insecticides. XLVII. Sumio Nagasawa and Michiyo Shiba (Ihara Agricultural Chemicals Institute, Shimizu). Received July 18, 1964. *Botyu-Kagaku*, **29**, 46, 1964 (with English summary, 50).

10. 濾紙法による malathion のイエバエに対する毒性の評価

殺虫剤の生物試験に関する研究 第47報 長沢純夫・柴三千代（イハラ農薬研究所）39. 7. 18 受理

イエバエに対する malathion の毒性を，アセトンでとかした場合と，これにゴマ油を加用した場合とにわけて，沪紙法により評価比較した．プロビットに変換した致死率と，塗附薬量および曝露時間の対数の3者からなるプロビット平面をえがき，Finney の方法によりこれを解析した．この実験の範囲内ではゴマ油をアセトンに加用すると，malathion の毒性はかなり低下すること，塗附薬量は曝露時間より致死を決定する要因として，はるかに重要であることをしりえた．

沪紙に薬物をしみこませ，これに昆虫を接触させてその有効度を評価する，いわゆる impregnated filter paper method は殺虫剤の残留毒性の検出定量，あるいは残留毒を利用することによって害虫を駆除しようとする場合，その基礎資料をえる上に有効簡便な方法である．ところで，この試験法において反応率に最も大きく影響すると考えられる要因は供試薬剤の塗布量と供試個体をこれに接触させておく時間のふたつであろう．それ故この両要因を同時に解析考察しうる実験を計画し施行するならば，それぞれを個々に比較考察するより結論の抽出はより容易になるはずである．今回そうした考えのもとに malathion のイエバエに対する毒性を，溶媒にアセトンをもちいた場合と，このアセトンにゴマ油を加用した場合とにわけて比較実験した．その結果をここにのべる．本文にはいるに先だち，供試昆虫の飼育を担当して戴いた伏見圭子嬢に謝意を表する．

実験材料および方法

供試昆虫：この実験にもちいたイエバエ *Musca domestica vicina* Macq. は，常法の豆腐粕培基によりその幼虫期を，砂糖と水をあたえてその成虫期を飼育した，いわゆる高槻系と称される累代飼育系統で，とくにその羽化後4～5日目の雌個体をもちいた．