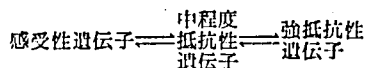


Genetical Studies on the Resistance to Parathion in *Drosophila melanogaster*. III. Induction of a susceptible gene from its resistance allele. Hideo KIKKAWA (Department of Genetics, Medical School, Osaka University, Osaka) Received November 6, 1967. *Botyu-Kagaku*, 32, 101, 1967.

11. キイロショウジョウバエにおけるパラチオン抵抗性の遺伝学的研究 III. 抵抗性遺伝子より感受性遺伝子の誘発. 吉川秀男 (大阪大学医学部遺伝学教室) 42. 11. 6 受理

すでに第I報でのべたようにキイロショウジョウバエにおけるパラチオン抵抗性は第2染色体の64.5の位置に存在する優性遺伝子によってひきおこされる。この優性遺伝子をホモまたはヘテロの状態にもつ個体は有機磷剤だけでなく、塩素系やカーバメート系殺虫剤に対しても交差抵抗性を示す。しかしフェニールチオ尿素およびそのハロゲン誘導体にはかえって感受性となる。

著者は第II報でパラチオン抵抗性遺伝子がはたして突然変異によって感受性遺伝子から誘発されるかどうかをみるため、現在もっとも感受性と思われる Canton-S 系統の雄に X 線を照射し中程度の抵抗性を示す系統を得ることに成功した。この系統に含まれる中間抵抗性の遺伝子は非常に不安定で自然的に、もっと強い抵抗性遺伝子になったり、反対にもとの Canton-S と同程度の感受性遺伝子に復帰することがあった。その関係は次のように表わされる。



この中破線で示した段階、すなわち強い抵抗性遺伝子は上記のように中程度の抵抗性遺伝子をへて感受性遺伝子に変化するものか、或は一挙に感受性遺伝子に変化するかはまだ調査されていなかった。

そこで本報では現在もっとも強いパラチオン抵抗性遺伝子をもつと考えられる 彦根系統の雄に 2000R の X 線を照射し、それを第2染色体に可視遺伝子をもつパラチオン感受性の雌と交配し、その F₁ の 1 齢幼虫を 3mM のフェニールチオ尿素を含んだ餌で飼育した。それはもし彦根系統の第2染色体に含まれる抵抗性遺伝子が感受性遺伝子に変化した場合、その個体はこのような餌でも生存できるという想定の下に行なわれたものである。

実験の結果 2 頭の中間程度の抵抗性を示す個体が得られた。この 2 頭に由来した系統に含まれる中間抵抗性遺伝子は前報の場合と同じく不安定で、特に感受性遺伝子に容易に変化することが確かめられた。以上の結果から殺虫剤抵抗性遺伝子も感受性遺伝子も一般の可視形質を支配する遺伝子と同じく突然変異によってひきおこされるが、キイロショウジョウバエの場合は特に不安定な中間程度の抵抗性遺伝子をへて強抵抗性、或は感受性遺伝子に変化するものと考えられる。

Introduction

It is generally considered that the appearance of insects showing resistance to a certain insecticide is due to mutation and selection. There are many experiments concerning selection for resistance, but there are few experiments in which special attention has been paid to the question of mutation of the resistance gene.

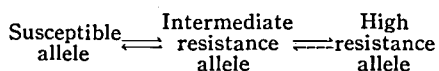
Since 1961, I have engaged in the study of parathion-resistance, using *Drosophila melanogaster* as the test insect. As I reported in my first paper¹⁾, resistance to parathion in *D. melanogaster* is caused by a dominant gene at the locus 64.5 of the second chromosome.

It is interesting that a fly having the dominant

gene in homozygous or heterozygous state shows resistance not only to organophosphorus compounds, but also to chlorinated hydrocarbon insecticides such as DDT and Lindane, and to carbamate insecticides like Sevin^{2,3)}. However, as Ogita^{4,5)} in our laboratory found in 1958, such a fly is susceptible to phenylthiourea and its halogen derivatives, the cross-resistance being negatively correlated in this respect.

Now, as reported in my second paper²⁾, by treating males of the Canton-S strain which was the most susceptible to parathion in our laboratory with X-rays, a sub-strain named *R18* showing intermediate resistance to parathion was obtained. Of interest is the fact that the parathion-resistance gene involved in the *R18* sub-strain was unstable,

and the gene was apt to mutate spontaneously to an allele determining high resistance, or to the original susceptible one. The process may be represented as follows.



In this schematic representation, however, the step indicated by a dotted line, *i. e.*, the step from high resistance to intermediate resistance, has not yet been examined. Namely, the question remains whether an allele for high resistance mutates to a susceptible one *via* an intermediate resistance allele, or mutates directly to a susceptible one in one step. In order to examine this point, the following experiments have been performed.

Experimental Results

The material employed consisted of males of the Hikone strain which showed the highest resistance to parathion in our laboratory. They were treated with X-ray (2000R), and were mated to virgin parathion-susceptible females carrying two recessive visible markers, cinnabar (*cn*, 2-57.5) and brown (*bw*, 2-104.5) on the second chromosome. The F₁ first instar larvae were reared in a medium containing 3mM phenylthiourea by the procedure described in my first paper¹⁾. If a mutation from a high resistance gene in the Hikone strain to a susceptible allele had been induced by the X-ray treatment, the fly carrying such a susceptible allele would be able to survive even in the medium containing 3mM phenylthiourea, because of the negatively correlated effect. In this way, 7 adult females were obtained from 5510 larvae tested (Table 1).

In *Drosophila*, in order to establish a strain

Table 1. Induction of a parathion-susceptible allele from its resistant one of the Hikone strain by X-ray treatment (2000R).

$\frac{cn}{+} + \frac{bw}{RI^*}$ larvae were tested by rearing them in a medium containing 3mM phenylthiourea (*RI gene derived from the Hikone strain).

Larvae tested	Survivors
5510	7 (♀7, ♂0)

homozygous for a certain gene, it is more advantageous to use males than females, because no crossing over takes place in males. Unfortunately, since eligible males were not obtained in this experiment, only females could be used for such a purpose. The 7 females obtained were first mated to the *cn bw* parathion-susceptible males respectively. But among them, 2 died before laying eggs. From the progeny of the remaining 5 females, only wild type males were selected and they were mated individually to *cn Cy/Pm* females. From the F₁ adults in each vial, *Cy* phenotype flies were taken and crossed among themselves. From their progeny, wild type flies were selected and sib-matings were performed. Thus, 5 sub-strains which were assumed to be homozygous for the second chromosome of the Hikone strain were established from each original 5 surviving females.

These sub-strains were tested for parathion-resistance level with the original Hikone strain and with the Canton-S strain. As the experimental results, it was found that the sub-strains derived from 3 original females showed resistance nearly same as the original Hikone strain. Probably, the 3 original females from which these sub-strains were derived, had survived in the medium con-

Table 2. Percent survival of adult flies to parathion in the sub-strains established from the offspring of 2 of the survivors shown in Table 1.

Strain	Concentration of parathion (ppm)					
	0.0	0.5	1.0	2.0	3.0	
No. 1	a	63	71	54	21	9
	b	70	63	30	6	0
	c	89	70	59	1	0
	d	66	52	32	6	0
	e	84	77	73	5	0
No. 2	a	74	0	0	0	0
	b	67	61	34	14	4
	c	85	0	0	0	0
	d	73	59	35	23	9
	e	78	48	41	46	32
Hikone	70	60	72	65	56	
Canton-S	70	0	0	0	0	

(100 larvae were used in each test).

taining 3mM phenylthiourea by accident and not because of changes of their genotypes. However, the sub-strains derived from the remaining 2 original females did show intermediate resistance (Table 2).

As seen in this table, all 5 sub-strains derived from the original female No. 1 showed intermediate resistance to parathion, while among the 5 sub-strains derived from the female No. 2, two sub-strains (No. 2-a and No. 2-c) showed complete susceptibility.

There is a possibility that these two susceptible sub-strains of No. 2 had been derived from the incorporation of a susceptible gene which was involved in the second chromosome carrying cinnabar and brown mutant markers by double crossing over between *cn-RI⁺* and *RI⁺-bw* regions, when the original female was mated to the *cn bw* male. However, the theoretical frequency with which such a double crossing over should take place between these regions is only 2%. As shown later, it is more plausible to assume that the 2 susceptible sub-strains might have been derived from successive or stepwise mutation *via* an intermediate resistance allele to a susceptible one during the course of establishing the homozygous sub-strains.

In order to examine this point more minutely, the following experiments were carried out with the sub-strains No. 1-d and No. 2-b, both having intermediate resistance alleles. Males of these sub-strains were mated to females of the parathion-susceptible strain carrying cinnabar and brown markers. From the F₁ progeny, 1000 first instar larvae were reared in a medium containing 3ppm parathion, and other 1000 in a medium containing 5mM phenylthiourea. The results are shown in Table 3.

Table 3. Experiments to obtain more resistant or susceptible alleles from intermediate ones (No. 1-d and No. 2-b).

Source	3 ppm parathion		5 mM phenylthiourea	
	Larvae tested	Survivors	Larvae tested	Survivors
<i>cn bw</i> × No. 1-d	1000	0	1000	25 (♀ 16, ♂ 9)
<i>cn bw</i> × No. 2-b	1000	8 (♀ 5, ♂ 3)	1000	15 (♀ 7, ♂ 8)

As seen in this table, no survivor was obtained in the medium containing 3 ppm parathion in the experiment of *cn bw* × No. 1-d, while 8 survivors appeared in the experiment of *cn bw* × No. 2-b. In the phenylthiourea medium, on the other hand, a considerable number of survivors was obtained in both experiments. Out of the 8 survivors in

Table 4. Levels of parathion-resistance of the established sub-strains derived from the surviving males in Table 3.

Strain Sub-strains derived from 2 males obtained in the medium containing 3 ppm parathion	Concentration of parathion (ppm)			
	0	1	2	3
No. 2-b-A-1	59	52	6	4
No. 2-b-A-2	66	33	39	1
No. 2-b-A-3	61	58	1	0
No. 2-b-B-1	63	56	45	4
No. 2-b-B-2	66	22	8	2
No. 2-b-B-3	52	51	15	17
Sub-strains derived from 3 males obtained in the medium containing 5 mM phenylthiourea	Concentration of parathion (ppm)			
	0	1	2	3
No. 1-d-A-1	72	0	0	0
No. 1-d-A-2	67	0	0	0
No. 1-d-A-3	69	0	0	0
No. 1-d-B-1	63	0	0	0
No. 1-d-B-2	45	0	0	0
No. 1-d-B-3	66	0	0	0
No. 1-d-C-1	79	0	0	0
No. 1-d-C-2	75	0	0	0
No. 1-d-C-3	51	0	0	0
No. 2-b-A-1	73	0	0	0
No. 2-b-A-2	46	0	0	0
No. 2-b-A-3	57	0	0	0
No. 2-b-B-1	78	0	0	0
No. 2-b-B-2	87	0	0	0
No. 2-b-B-3	59	0	0	0
No. 2-b-C-1	72	0	0	0
No. 2-b-C-2	70	0	0	0
No. 2-b-C-3	75	0	0	0
Hikone	77	64	38	25
Canton-S	65	0	0	0

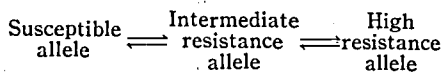
(100 larvae were used in each test).

the parathion-experiment of *cn bw* × No. 2-b, 3 males were mated individually to *cn Cy/Pm* females, and 3 sub-strains homozygous for the second chromosome in question were established from 2 original males by the procedure described before, for 1 original male failed to mate to the female. On one hand, 3 males which survived in the phenylthiourea medium were chosen arbitrarily in both experiments, and 3 sub-strains homozygous for the second chromosome in question were established by the same way as before. These sub-strains were then tested for their levels of parathion-resistance (Table 4).

It is seen that the sub-strains which were derived from the males that had survived in the medium containing 3ppm parathion showed intermediate resistance to parathion as before. On the other hand, all the sub-strains derived from the males which had survived in the medium containing 5mM phenylthiourea showed complete susceptibility to parathion. These results suggest that mutation from the intermediate resistance allele to the susceptible allele had occurred more frequently than to the high resistance one. This conclusion would account for the appearance of the completely susceptible sub-strains in Table 2 (No. 2-a and No. 2-c), and also for the fact that there was a greater number of survivors in the medium containing phenylthiourea than that containing parathion (Table 3).

Discussion

As shown in these experiments, it was ascertained that the mutation from a parathion-resistance gene to its susceptible allele actually takes place, and that the mutation seems to occur *via* an unstable allele showing intermediate resistance. Thus the following scheme will be obtained.



The reason why the intermediate resistance allele is unstable remains at present unknown, though several assumptions are proposed by Auerbach⁶⁾ and Alexander⁷⁾ *etc.*

From all the results reported in my three papers, the following implications similar to those de-

scribed in my second paper²⁾ may be derived.

(1) There are many instances where various degrees of resistance to a certain insecticide are existent in natural population of insects. Most investigators assume that this phenomenon is due to the existence of polygenic factors, and in fact some cases may be completely explained by this assumption. However, it may also be possible to assume that there are some multiple alleles which show different degrees of resistance in the population. If one or more of these alleles are unstable and tend to mutate to alleles for higher resistance or for lower susceptibility as was found in *Drosophila*, the resistance level in that population will change from generation to generation depending on the environmental conditions. Thus the variations in resistance levels in natural population of insects may be still explained by a single gene hypothesis.

(2) As I reported in my first paper¹⁾, all parathion-resistant strains of *Drosophila* collected from various countries such as Japan, Sweden, U. S. A. and Korea, were determined by a dominant resistance gene at the locus 64.5 on the second chromosome. In my opinion, such resistance genes in the different population might have been caused independently by separate mutations from their susceptible alleles, since our experiments have shown that mutations to resistance alleles and *vice versa* actually do take place just as those for visible characters. However, in a limited region, it is also possible to assume that flies with certain resistance genes would have migrated and conquered susceptible flies in that region, in case where the resistant flies have factors with a greater advantage than susceptible ones.

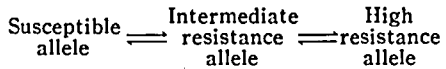
(3) It has been demonstrated that various pleiotropic phenomena associated with the parathion-resistance gene, *e.g.*, cross-resistance to chlorinated hydrocarbon insecticides and carbamate insecticides and enhanced susceptibility to phenylthiourea, are caused by the mutation of a single gene.

Summary

Treatment of males of a highly parathion-resistant Hikone strain with X-ray (2000R) resulted in a few offspring which produced sub-strains of

intermediate parathion-resistance.

The intermediate resistance gene involved in these sub-strains was apparently unstable, since evidence was obtained for the tendency of mutating to the susceptible allele. Thus, combining with experimental results shown in my second paper²⁾, the following scheme will be obtained.



Certain questions concerning resistant and susceptible alleles can be understood with light of these results. Most important is the demonstration that insecticide-resistant or susceptible genes are produced by mutation in the same way as other mutant genes for visible characters.

Acknowledgments: I wish to express my hearty thanks to Miss Takako Okuno for her great assistance in performing the experiments. I would like to also express my gratitude to Prof.

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開花ホルモン研究の現状 滝本 敦 (京都大学農学部)

開花ホルモンの存在が予想されてから今年で丁度30年になるが未だにその実体がつかめない。果して開花ホルモンは存在するのであろうか？現在ではそのような疑問を持つ人も多い。しかしこれから述べるようにいろいろの実験事実を総合するとやはり開花ホルモンの存在を仮定するのが妥当であり、ごく最近の生化学的なレベルでの研究にも注目すべきものが出て来た。

以下開花ホルモンに関してこれまで行なわれてきた研究の概要を述べるが、開花ホルモンは正確には花芽形成ホルモンと呼ぶべきであって、実際に植物の開花(花が開くこと)を支配するホルモンではない。これまでの習慣で花芽形成を開花と呼ぶことが多く混乱を招きやすいとは思いますが、以下でもしばしば開花という言葉を用いる。しかしこれらはいずれも花芽形成を意味するのであって開花ホルモンもまた、栄養生長から生殖生長への転換、すなわち花芽形成を支配するホルモンを意味する。この点あらかじめ誤解のないようお断りしておく。

1) 光周性と開花ホルモン

ある種の植物は日長がある一定の長さ(限界日長)より短くならないと花芽を形成せず、ある種の植物は反対に日長が一定の長さ以上にならないと花芽を形成しない。前者を短日植物と呼び、イネ、キク、ダイズ、アサガオ、シソ、オナモミなどがこれに属する。後者は長日植物と呼ばれるもので、コムギ、ルドベキア、ムシトリナデシコ、アブラナ、ドクムギ、ホーレンソウなどがこれに属する。このように日の長さ、すなわち光周期 (photoperiod) に感応して花芽を形成する現象を光周性 (photoperiodism) と呼ぶ。ただし光周性は花芽形成以外の発育反応にも見られる。

植物が光周刺激をうける場所は葉であって、葉だけに適当な光周期を与えると植物は花芽を形成する。葉の面積を小さくすると光周反応は徐々に弱まり、全部の葉を除いた植物に適当な光周期を与えても花芽形成は見られない。実際に花芽を形成する場所は芽であるから、葉で受けとった光周刺激は芽に伝えられて、そこで花芽分化をおこすことになる。おそらくは葉である種の物質がつくられ、この物質が芽に運ばれてそこ