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【Research report】

調節基因在家蠅代謝性抗藥機制的作用【研究報告】

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Abstract

摘要

在家蠅身上，控制不同代謝性抗藥機制的基因，幾乎全座落于第二對染色體上。在抗性品系裡，該染色體普遍地有異常之現象發現，這個異常是屬於一種染色體倒置。實驗證明：染色體的倒置明顯地提高了家蠅抗DDT，有機磷和氨基甲酸鹽類殺蟲藥劑之幅度。這個發現所導出之研究一方面澄清了以往毒理學家們對抗性基因位點爭論之疑竇，另一方面也替調節基因在抗藥作用中所可能扮演之角色，提供了一些初步的證據。

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GENETICS OF METABOLIC RESISTANCE TO INSECTICIDES IN THE HOUSE FLY: EVIDENCE FOR THE ROLE OF A MAJOR REGULATORY GENE

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ABSTRACT

In house fly, *Musca domestica* L., genes conferring most of the metabolic resistance to insecticide were reported on chromosome II. A rearrangement, most possibly an inversion, on chromosome II was consistently occurred in resistant strains tested but not in susceptible strains. The chromosome rearrangement was found responsible for the high level of resistance to DDT, OPs and carbamates in house fly. Our study does not only elucidate the inconsistent results on resistance gene mapping which have been presented by other authors previously but also provide some primary evidences supporting the presumption that the regulatory gene does play a role in insecticide resistance.

Enzymatic mechanisms conferring metabolic resistance to organophosphates (OPs) and DDT in the house fly, *Musca domestica* L., include altered ali-esterase, high levels of glutathione transferase (GSH), high levels of microsomal mixedfunction oxidase (MFO) and high levels of DDT dehydrochlorinase (DDTase). All are controlled by genes on chromosome II (Oppenoorth *et al.* 1972, Khan *et al.* 1973, Tate *et al.* 1974, Devonshire and Sawicki 1975, Oppenoorth and Wellins 1976, Plapp 1976). Another gene on chromosome II, one for altered acetylcholinesterase involves a target resistance mechanism rather than increased detoxification (Oppenoorth *et al.* 1977). Investigations in which the relative contribution of all chromosomes to resistance was measured also showed that factors on chromosome II played the major role for insecticide resistance (Tsukamoto and Sukuzi 1966, Plapp and Casida 1969, Georghiou 1971). However, measurable levels of resistance are controlled by genes on other chromosomes, usually III and V.

Discovery that most OP and DDT resistance genes are located on the same chromosom was surprising. However, it was not really new. Research on *Drosophila melanogaster* showed very similar results 20 years ago. In *D. melanogaster*, resistance genes for different insecticides including DDT, BHC, OPs, and carbarmates were reported to be on the same chromosome, again, chromosome II (Kikkawa 1964a, 1964b). What is more, the reports all showed that the positions of the resistance gene were very close to the marker vestigial wing (vg), locating at 67.0, 66.0, and 64.5. These early studies indicated that the same or a series of very closely related genes conferred resistance to all types of insecticide in *D. melanogaster*. No hypotheses were advanced concerning the possible biochemical mechanisms involved with these phenomena.

Why are all resistance genes in *Drosophila* located on the same chromosome and linked so closely? Is this just chance? Are they a group of linked genes which have related functions? Or are they a common site which occurs in all resistant strains?

Compared to *Drosophila*, genetics in house fly is still in its infancy. However, genetic theory indicates we should expect that most of the phenomena found in *Drosophila* also occur in the house fly. But, when the location on chromosome II of different resistance genes was investigated, not all were at the same location as in *Drosophila*. Instead, they fell into 2 groups. One group of resistance genes is very close to the aristapedia gene (ar) (Franco and Oppenoorth 1962, Lichtdwardt 1964, Tsukamoto and Suzuki 1964, Sawicki 1974 Wang and Plapp 1980). Another group is about 30 units away from the ar gene (Tsukamoto and Suzuki 1966, Khan *et al.* 1973).

Thus, it seems that there are at least 2 different locations of resistance genes on chromosome II in house fly. It is worth noting that DDT resistance, presumably always involving only one mechanism, DDTase occurred at both positions in different studies. So did diazinon resistance, although the mechanisms were not specified. However, the measurement of resistance map position depends on the availability of visible recessive mutant markers. Several studies have shown inconsistancies in the location of these genes (Tsukamoto and Suzuki 1964, 1966, Hiroyoshi 1977). We wonder, therefore, whether the different map positions of resistance genes are due to differences in resistance genes or due to differences in relationship to the recessive marker genes.

Consequently, before we started to investigate the resistance genes, we established a house fly strain with 3 visible recessive markers on chromosome II in the area near the resistance gene(s). This strain was designated as ar-stw-car, indicating the 3 markers it possessed. With this strain, we accurately measured the precise map positions and gene order of these 3 mutants by using 3-point testcrosses to analyze about 10,000 flies. The gene order we found is ar-stw-car. The stw gene is located in the middle. This result is different from some former reports (Nickel and Wagoner 1970, Khan *et al.* 1973). However, it is the same of Hiroyoshi's suggestion in 1977 (Hiroyoshi 1977). The distance between the ar and stw genes is 13.49 units and that between the stw and car genes, 6.40 units.

The ar-stw-car strain was shown to be an OP an DDT susceptible strain from bioassay and enzyme assay data. Not only was it susceptible to insecticides, but it had typical susceptible strain levels of ali-esterase, GSH and MFO. Induction studies showed that it was more inducible for GSH and MFO activity than several resistant strains. Data from tests using phenobarbital as an inducer of these enzymes are summarized in Tables 1 and 2.

Based on the above tests, we were able to use the ar-stw-car strain to measure the map position of resistance genes in several resistant strains in relation to the mutants and to do biochemical studies on the inheritance of resistance.

The resistant strains we used in this experiment included DDT-resistance (DDT-R), Parathion-resistant (Para-R), Propoxur-resistant (Prop-R), Diazinon-resistant (Diaz-R), and Tetrachlorvinphos-resistant (Tetra-R). The biochemical characters for these strains are shown in Table 3.

For these tests, resistant strain females were crossed with the ar-stw-car males. F–1 females were backcrossed to the males of the ar-stw-car. Number of flies of each

Table 1. GSH activity in the ar-stw-car, stw; bwb; ocra and R-Tetrachlorvinphos strains of house fly.

Strain	Activity[a/]		Inducibility[c/]
	Control	Induced[b/]	
ar-stw-car	1.050	3.886	370%
stw; bwb; ocra[d/]	1.302	2.487	191%
R-Tetra	21.825	22.353	102%

a/ Activity expressed as O.D./min./g fly
b/ Flies exposed to 1% phenobarbital for 3 days
c/ Inducibility = Activity in induced flies / Activity in control flies
d/ The standard susceptible strain in our laboratory

Table 2. MFO activity in the ar-stw-car, stw; bwb; ocra and R-Diazinon strains of house fly.

Strain	Activity[a/]		Inducibility[c/]
	Control	Induced[b/]	
ar-stw-car	30.32	245.24	809%
stw; bwb; ocra[d/]	26.46	102.58	390%
R-Diaz	100.14	254.24	250%

a/ Activity expressed as picomoles of parathion metabolized/fly/15 min.
b/ Flies exposed to 1% phenobarbital for 3 days
c/ Inducibility = Activity in induced flies / Activity in control flies
d/ The standard susceptible flies in our laboratory

Table 3.
Enzymatic mechanisms conferring metabolic resistance to OPs and DDT and their apparent genetic location in the house fly

Enzymes	Apparent gene location
altered ali-esterase	Chromosome II
DDT dehydrochlorinase	Chromosome II
glutathione transferase	Chromosome II
microsomal oxidase	Chromosome II
microsomal oxidase	Chromosome V

Table 4.

Genetic map positions of R, ar, stw, and car genes in 5 resistant strains of the house fly.

Strain	Gene Distance			Fly numbers examined
	R to ar % crossing over	ar to stw % crossing over	stw to car % crossing over	
R-Parathion	2.79	0	2.89	3,080
R-Diazinon	1.80	0.08	5.13	5,005
R-Diazinon	2.15[a]	–	–	
R-Propoxur	3.58	0.04	2.95	4,469
R-Propoxur	6.10[a]	–	–	
R-Tetrachlor.	6.53	2.71	4.81	3,844
R-DDT	25.59[a]	11.92	4.21	4,035

[a]= R to ar distance for DDT

phenotype and resistance in each phenotype of the backcrosses were determined. The overall data are summarized in Table 4.

The map positions of the resistance genes we obtained from these 5 strains also fell into 2 groups. The map positions of resistance gene in OP resistant strains, i.e., Para-R, Prop-R, Diaz-R, and Tetra-R, which are also resistant to DDT are all very close to the ar gene. This is true for both OP and DDT resistance, regardless of the specific biochemical mechanisms involved. However, in a strain resistant to DDT, but OP susceptible, i.e., DDT–R, the resistance gene is about 27 units away from the ar gene. These results are similar to those in previous reports mentioned above in that resistance genes are either very close to the ar gene or 20–30 units from it. The resistance genes we measured were all on the left side of the ar gene, which is opposite to the side that the stw and car genes are located. Thus, the gene order is R-ar-stw-car.

In all the OP-resistant strains we tested, an unexpected result was observed (Table 4). The % crossing over between the ar and stw genes was greatly reduced. In the Tetra-R strain it was reduced from 13.49% to 2.71%. In the Prop-R and Diaz-R strains, a value smaller than 0.1% was found. There was no crossing over at all in the Para-R strain. In the DDT-R strain, which is OP-susceptible, no significant reduction in ar-stw crossing over was observed. The value of % crossing over between the ar and stw genes in this strain is very similar to those in the susceptible strain described previously.

These results confirmed our previous report (Wang and Plapp 1980). We suggested then that a chromosome aberration, most likely an inversion, probably was responsible for this phenomenon in that strain. We now think the inversion occurs in all OP resistant strains.

Recently, in work done in cooperation with Dr. J. Spencer Johnston, Plant Science Department, Texas A & M University, we have obtained cytological evidence for the occurrence of an inversion on chromosome II. In slides made of meiotic chromosome

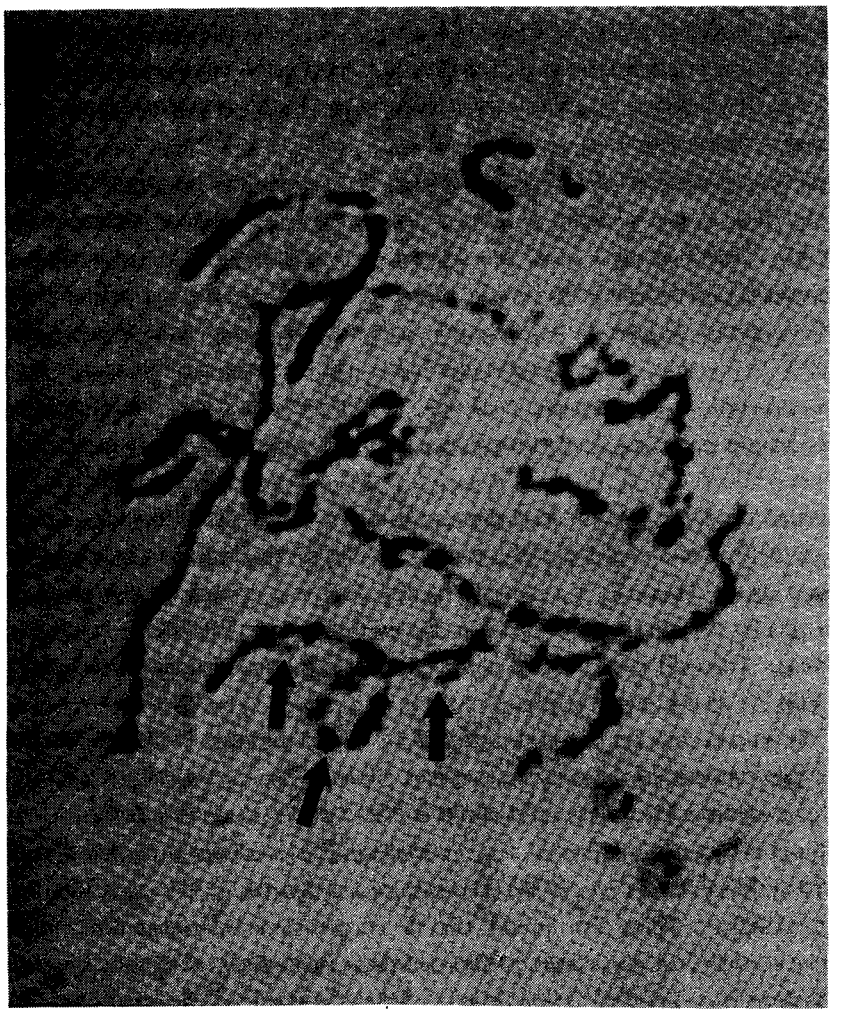

Fig. 1. Pachytene stage of testicular cells of house fly, *Musca domestica* L., showing heterozygous inversion loops on chromosome II (from Dr. J. S. Johnston).

preparations from the testes of F-1s of the cross between Diaz-R and a susceptible mutant strain, we can observe the inversion on chromosome II (Fig. 1).

The next question is, what is the function of the observed inversion? At first we thought that the inversion we observed in all OP resistant strains probably functioned for keeping linked resistance genes together to confer high levels of insecticide resistance. It is very common in *Drosophila* that populations with high frequencies of chromosome inversions in some particular section of chromosome are more tolerant of adverse conditions than those of the same species lacking the inversions. This theory was proposed by the late Dr. Dobzhansky.

However, as we examined the genetic map positions of the resistance gene which we had measured in all of our experiments, this hypothesis became confusing. This is because

all the map positions of the resistance gene which we observed seemed to be outside the inverted section of chromosome II. How could the inversion enhance insecticide resistance by keeping the linkage of related resistance genes if the resistance genes were not all contained in this section?

Is the association of inversion between the ar and stw genes and OP resistance in house fly just a coincidence? In order to clarify this, we have prepared 2 resistant substrains. One possessed both the resistance gene and the inverted section from the resistant strain. The second substrain possessed only the resistance gene but lacked the inverted section. The strain with both the resistance gene and the inverted section was similar to the resistant strain in resistance level. The one with the resistance gene only but no inverted section on chromosome II was only slightly resistant. Therefore, the inverted section, or the inversion itself, in resistant strains is important for the full expression of resistance.

Based on these results, we propose an alternative explanation for the relationship between the inversion and high OP resistance. This is, the inversion increases the level of resistance in the house fly by rearranging the relative positions of the genes. Probably it does this by bringing heterochromatin closer to or moving it farther from an important gene locus. This will work because heterochromatin is known to inactivate genes proximal to it. This is called position effect.

The precise mechanism we suggest is to inactivate genes which in susceptible flies repress the synthesis of detoxifying enzymes. In other words, resistant strains are resistant because there are more or less constitutive for detoxifying enzyme activity.

Similar observations have been reported on the peach-potato aphid *Myzus persicae* (Sulzer) by Blackman *et al.* (1978), Devonshire and Sawicki (1979), and Bunting and Van Emden (1980). They also found that a chromosome rearrangement enhanced the resistance by a position effect. The rearrangement they observed, however, was a translocation from another chromosome. Our observation, on the other hand, is for inversion of chromosome II.

Devonshire and Sawicki (1979) suggested gene duplication was the precise mechanism of resistance. They found lines of malathion resistant aphids having 2, 4, 6, 8, and 16 times the amount of esterase presented in susceptible aphids. These findings were confirmed by Bunting and Van Emden (1980). So far, we have no evidence for the same phenomenon in the house fly in our own work, although Walker and Terriere (1970) long ago proposed that gene amplification was involved in the house fly.

The information resulting from our experiments is not sufficient enough to confirm which type of gene, regulator or structure, we were dealing with.

However, it seems that a regulatory gene is most likely. The evidence in favor of this is:

1) All the map positions of resistance genes we measured in OP resistant strains were the same, i.e., it is actually one locus. The reason why we did not set precisely identical map position values for all the strains might be that there are different breakpoints involved in the chromosome rearrangement in different strains.

2) The relative position of the resistance gene determines the level of resistance in all the strains we tested.

3) The results of our enzyme assays are in agreement with this hypothesis. Resistant strains have high basal levels of detoxifying enzymes, but are less inducible than susceptible strains. That means in susceptible strain, the structural gene is regulated by the product from regulatory gene and is inducible. In resistant strains, the regulatory gene is inactivated by heterochromatin and the structural gene becomes constitutive, synthesizing the detoxifying enzyme all the time, and is less inducible.

According to our results, heterochromatin, which inactivates the regulatory gene conferring resistance probably is located between the ar and stw genes and is very close to the stw gene on the left side. Therefore, the ar gene is always included in the inverted section of chromosome. The inversion brings the heterochromatin away from the stw gene and locates it close to the resistance gene, resulting in inactivation.

There is probably more than one breakpoint between the resistance and ar gene. If the breakpoint between the resistance and ar genes is close to the resistance gene, the heterochromatin will be brought proximal to the resistance gene and cause a higher level of inactivation, and thus make the house fly more resistant. This is also true for the position of breadpoint between the ar and stw genes.

If the breakpoint occurs between the ar and stw genes, i.e., if not all heterochromatin is moved toward the resistance gene, the degree of inactivation, and thus the level of resistance would be less than maximum. If both the ar and stw genes were included in the inverted section of the chromosome, i.e., if the breakpoint lay beyond the stw gene, all the heterochromatin would be transferred to close proximity to the resistance gene. In this case, the degree of inactivation would be maximum and the strain would be highly resistant.

Hisey *et al.* (1979), in their research on *Drosophila*, also reported the importance of the position and amount of heterochromatin proximal to the target gene. The more

Table 5. Individual test cross of the R-Diazinon strain of the house fly.

Group	% crossing over between at-stw	% wild type flies killed
1	0	0
2	0	0
3	0	0
4	0	2
5	1.4	22
6	2.24	46
7	3.52	66
8	10.28	76

and closer the heterochromatin located to the target gene, the higher the level of inactvation.

In our experiments, resistance levels in some strains were not found to be uniform. With one strain, individual fly testcrosses were performed. The results are shown in Table 5. The high level of OP resistance is always associated with the reduced recombination between the ar and stw genes. This not only confirms the observations which were described previously, but also shows evidence for the involvement of heterochromatin in the action of insecticide resistance. As we statistically transformed the data, the relationship between the % crossing over and resistance level became linear (Fig. 2). In other words, the longer the distance between the ar and stw genes in a strain, the more susceptible it is. This is the same as those phenomenon of position effect described by Hisey *et al.* (1979).

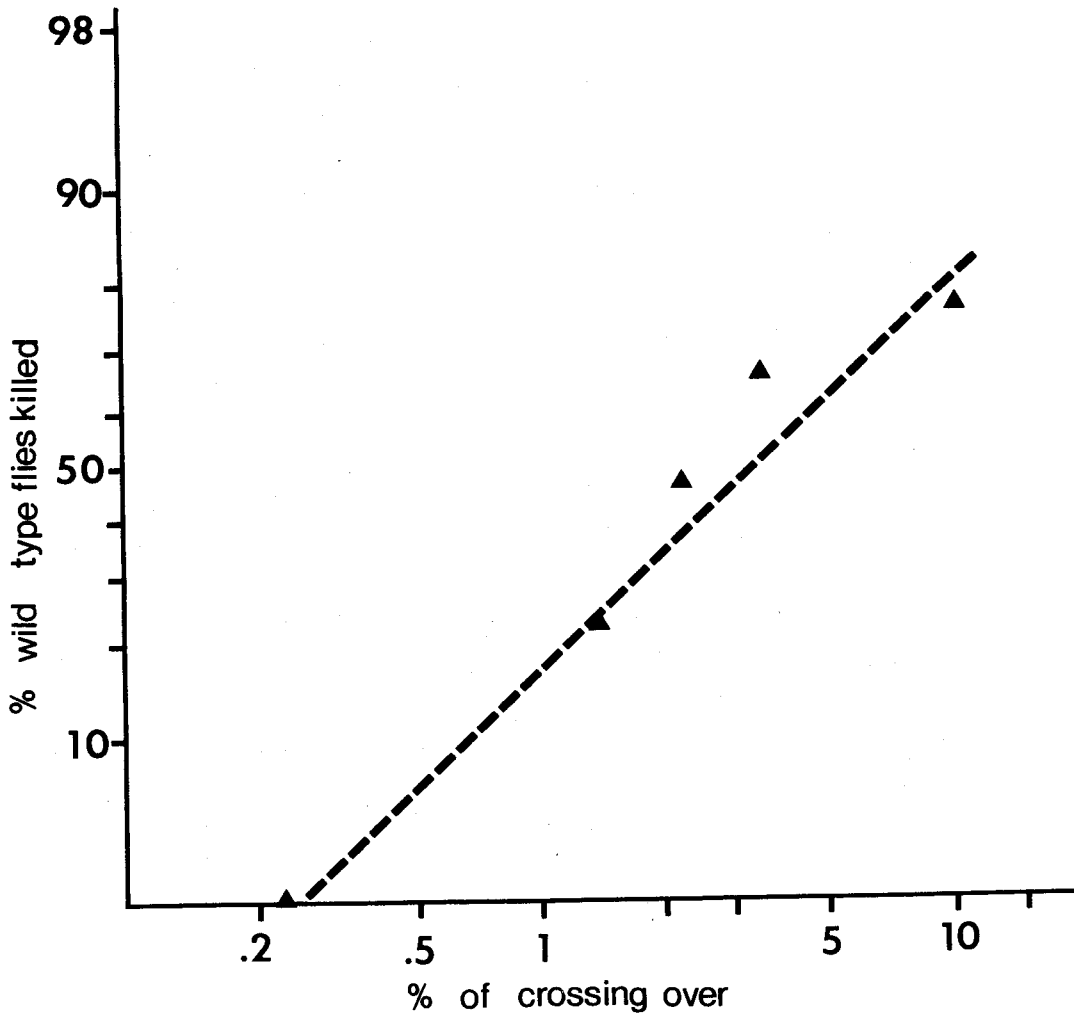

Fig. 2. Relationship between and of crossing over of ar-stw and the level of susceptibility in R-Diazinon strain of the house fly.

In summary, we wish to make the following points:

1. The different genetic map positions on chromosome II which have been reported by various researchers were probably all correct. The differences are caused by chromosome rearrangement in different strains.
2. The resistance gene on chromosome II is very probably regulatory in nature. Many different resistant strains have this common site for the regulation of the structural gene(s) for insecticide detoxification.
3. The inversion in the OP resistant strains increases the level of resistance by means of a position effect.
4. In the house fly, just as in *Drosophila,* heterochromatin is very possibly involved in the position effect of insecticide resistance by means of inactivating the regulatory gene.
5. The different positions of breakpoints in the rearrangement of the inverted chromosome also cause variation in the level of resistance.

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調節基因在家蠅代謝性抗藥機制的作用

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在家蠅身上，控制不同代謝性抗藥機制的基因，幾乎全座落于第二對染色體上。在抗性品系裡，該染色體普遍地有異常之現象發現，這個異常是屬於一種染色體倒置。實驗證明：染色體的倒置明顯地提高了家蠅抗DDT，有機磷和氨基甲酸鹽類殺蟲藥劑之幅度。這個發現所導出之研究一方面澄清了以往毒理學家們對抗性基因位點爭論之疑竇，另一方面也替調節基因在抗藥作用中所可能扮演之角色，提供了一些初步的證據。

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