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Genetic Control of Low Nerve Sensitivity to DDT in Insecticide-Resistant Houseflies†. Masuhisa Tsukamoto* (Department of Genetics, Osaka University Medical School, Osaka, Japan), Toshio Narahashi** and Teruo Yamasaki (Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Tokyo, Japan). Received October 31, 1965. Botyu-Kagaku 30, 128, 1965.

21. DDT 抵抗性イエバエにおける低神経感受性の遺伝†. 塚本増久*(大阪大学医学部 遺伝学教室)・楢橋敏夫**・山崎輝男 (東京大学農学部 害虫学教室) 40. 10. 31 受理

イエバエの DDT 抵抗性には 2 つの主要遺伝子が関与しており，そのうち第 5 染色体の優性遺伝子は DDT 脱塩酸酵素の活性を支配していることはすでに塚本・鈴木らによって報告されたが，第 2 染色体にある不完全劣性遺伝子の役割については不明であった．一方，山崎・楢橋らの研究によって DDT 抵抗性のイエバエは感受性の系統に較べてその DDT に対する神経感受性が低いことが示されている．その後，両者による共同研究の結果，DDT 抵抗性系統のイエバエの低神経感受性は第 2 染色体の不完全劣性遺伝子によって支配されており，神経組織での DDT の解毒とは切り離して考えられねばならないことが明らかとなった．

There are many possible mechanisms of resistance to insecticides in insects, such as reduced penetration of an insecticide into the insect body of sensitive organ, storage of the insecticide in insensitive tissues, enhanced metabolism of the insecticide, rapid excretion of the insecticide, reduced sensitivity of the nerve to the insecticide, enhanced cholinesterase activity, etc.[2,3,7,10,16,26] Among them, a parallelism between the resistance level to DDT and an increased ability to detoxify

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* Present address: Department of Entomology, University of California, Berkeley, California 94720, U. S. A.

** Present address: Department of Physiology, Duke University Medical Center, Durham, North Carolina 27706, U. S. A.

the insecticide has been established in the housefly, *Musca domestica* L., both *in vivo*[17,18] and *invitro*.[21] On the other hand, the nerves of DDT- and BHC-resistant strains of the housefly are less sensitive to directly applied DDT and BHC than are those of susceptible strains,[14,19,20,27,28] although this relation does not hold for diazinon-resistance.[14] The evidence that the nervous system of DDT-resistant flies is rich in DDT-dehydrochlorinase activity[13] suggested that the lower nerve sensitivity to DDT may be the result of local detoxification.

The inheritance of knockdown-resistance to DDT in the housefly is controlled by a single recessive gene pair[1,4,11] and this gene was located on a certain region of the 2nd chromosome.[12] The *in vitro* dehydrochlorination of DDT is inherited by a single incompletely dominant gene.[9] At that time, however, no further experiment was carried out to elucidate the relationship between the dehydrochlorination of DDT and the knockdown-resistance gene. Recent developments in the genetics of the housefly[5,6,11,23] made it possible to approach to the physiological mechanisms of insecticide resistance on the basis of genetics. By using visible mutants as markers, Tsukamoto and Suzuki[24] have shown that at least two major genes are responsible for high kill-resistance to DDT: an incompletely recessive resistance gene on the 2nd chromosome (*r-DDT*) and a dominant resistance gene on the 5th chromosome (*R-DDT*). The detoxification of DDT by dehydrochlorination has been associated with the 5th chromosomal resistance gene.[15,24] The role of the 2nd chromosomal resistance gene on the resistance mechanism has remained unknown.

In view of these available evidences, there are at least two possible loci of the genes responsible for low nerve sensitivity to DDT, i.e., the 2nd chromosomal gene and the 5th chromosomal one. The former possibility is suggested by the finding that a knockdown-resistance gene is located on the 2nd chromosome,[11,12] because knockdown is to a certain extent an indication of the nerve response to insecticides. The latter possibility is

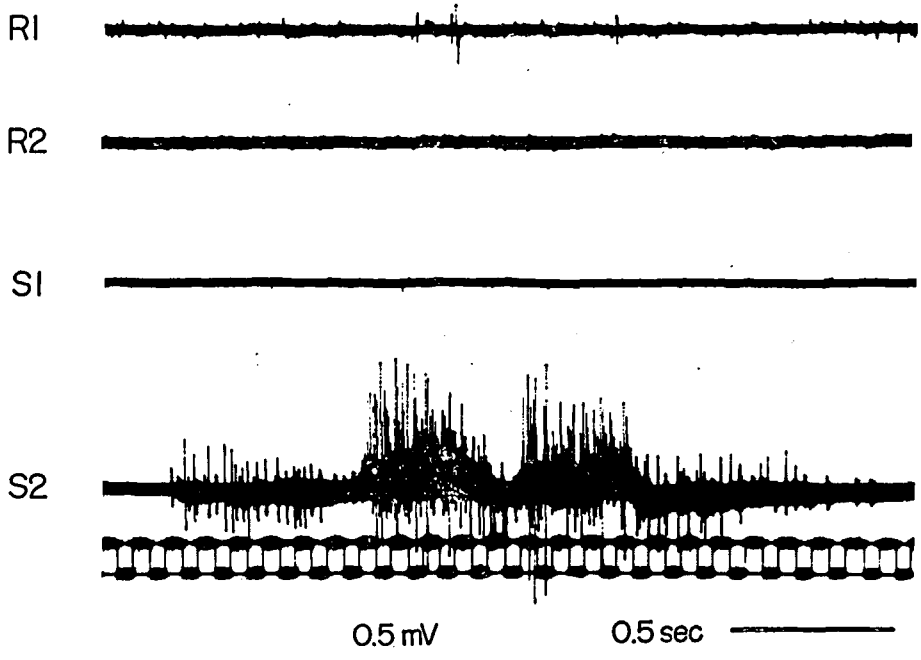

Fig. 1. Discharges of impulses in the femur muscle causes by direct application of 2.8×10^{-5}M DDT to the exposed thoracic ganglia in DDT-resistant (R *bwb*; *ocra*; *ar*; *ac*) strain and in susceptible Lab strain of houseflies. R: resistant strain. S: susceptible strain. 1: before application of DDT. 2: 9 minutes after DDT.

suggested by the finding that DDT can be detoxified in the nerve tissue as well.[13] The present study has been undertaken in order to establish the relationship between major resistance genes and low nerve sensitivity in DDT-resistant strains of houseflies.

Materials and Methods

The housefly strains used and the genetic techniques employed were essentially the same as those described previously[21,23]. The measurement of nerve sensitivity to the insecticide was based on the observation of action potentials from the femur muscles before and after application of a drop of saline containing the insecticide[26,27] onto the exposed thoracic ganglia that control the muscles. Purified *p, p'*-DDT stimulated the ganglia to discharge impulses which in turn excited the muscles (Fig. 1). The frequency of discharges was measured by means of an electronic spike counter. An appropriate discrimination level was set and only spikes exceeding this level were counted. After exposure of the ganglia, 15 minutes were allowed for the housefly preparation to reach the steady-state nerve activity. Two control counts were made, 5 minutes apart: the insecticide in saline was then applied to the ganglia, and test counts were made 5, 10 and 15 minutes after application. When the frequency of discharges per minute in any of the three test counts exceeded the mean control frequency by 1,000 or more, the housefly was taken as positive or "responded". The level of nerve sensitivity was expressed as the percentage of the "responded" flies in a population tested.

Results and Discussions

Nerve sensitivity of DDT-resistant strains was markedly different from that of susceptible strains. A cross was then carried out between females of a multichromosomally marked resistant strain, R (*bwb* ; *ocra* ; *ar* ; *ac*), and males of a wild-type, susceptible laboratory strain, Lab. The dosage-nerve sensitivity relationship of the F_1 hybrids (Fig. 2) indicates an incompletely recessive genetical characteristic of reduced nerve sensitivity to DDT.

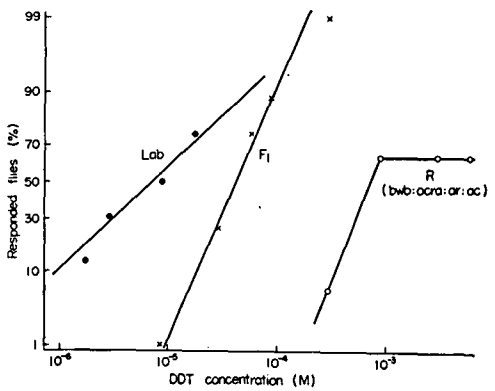

Fig. 2. Dosage-nerve sensitivity relationships to DDT in susceptible Lab strain (•), in resistant marker strain(◦), and in the F_1 hybrid(×). Nerve sensitivity is expressed as the percentage of the "responded" flies in a population tested.

Table 1. Nerve response of the housefly to 1.7×10^{-4}M DDT in different genetic make-ups of chromosomes from the following backcross:
R(*bwb* ; *ocra* ; *ar* ; *ac*) ♀ ×F_1{R(*bwb* ; *ocra* ; *ar* ; *ac*) ♀ ×Lab♂}♂

Phenotype (2;3;5;6)	Exp. 1				Exp. 2				Pooled survivals in arc-sine
	No. of flies		Rate of responded		No. of flies		Rate of responded		
	Tested	Responded	%	Arc-sine	Tested	Responded	%	Arc-sine	
+ ; + ; + ; +	16	14	87.5	69.30	24	18	75.0	60.00	129.30
+ ; + ; *ar* ; *ac*	16	13	81.2	64.30	24	14	58.3	49.78	114.08
+ ;*ocra*; + ; *ac*	16	14	87.5	69.30	24	19	79.1	62.80	132.10
+ ;*ocra*; *ar* ; +	16	14	87.5	69.30	24	11	45.8	42.59	111.89
bwb; + ; + ; *ac*	16	3	18.7	25.62	24	3	12.5	20.70	46.32
bwb; + ; *ar* ; +	16	4	25.0	30.00	24	2	8.3	16.74	46.74
bwb;*ocra*; + ; +	16	6	37.5	37.76	24	4	16.6	24.04	61.80
bwb;*ocra*; *ar* ; *ac*	15	2	13.3	21.39	24	3	12.5	20.70	42.09
Total	127	—	—	386.97	192	—	—	297.35	684.32

Table 2. Factorial analysis of the effect of recessive autosomal genes on lower nerve sensitivity to DDT.

Source of variations	Effect	Sum of squares	Degree of freedom	Mean square	F
Total	—	6264.25	15	—	—
Chromosome	—	5543.38	7	791.91	25.32**
2	145.21	5271.49	1	5271.49	168.58**
3	−5.72	8.18	1	8.18	0.26
5	27.36	187.14	1	187.14	5.98*
6	7.52	14.14	1	14.14	0.45
2-3 / 5-6	5.11	6.53	1	6.53	0.21
2-5 / 3-6	8.07	16.28	1	16.28	0.52
2-6 / 3-5	−12.56	39.44	1	39.44	1.26
Duplication	—	501.97	1	501.97	16.05**
Error	—	218.90	7	31.27	—

* Significant at 5 per cent level. ** Significant at 1 per cent level.

Similar results were also obtained by another cross using different resistant or susceptible strains (*i.e. JIR*♀ ×*ro* ; *ext* ; *cm* ; *acv*♂).

In order to analyze the recessive genetic factor (s) for nerve insensitivity, males of the F_1 hybrid of this cross (heterozygous for both nerve insensitivity and DDT-resistance) were backcrossed to females of the marked resistant strain, R (*bwb* ; *ocra* ; *ar* ; *ac*). Since each autosome except the 4th chromosome is labeled with a visible mutant marker, it can be determined to which linkage group the recessive nerve insensitivity character belongs. Although this type of backcross yielded 16 phenotypes of the segregants, only 8 out of the 16 phenotypes were examined for their nerve sensitivity because these data were sufficient to submit to statistical analysis. Both males and females were used in a 1:1 sex ratio and the data obtained were combined together. Table 1 shows the relationship between the different genetic make-up in the backcross progeny and the percentage of flies that responded to 1.7×10^{-4} M DDT. The nerve of the *bwb*-type flies possessing the homozygous resistance gene (*r*/*r*) on the 2nd chromosome is less sensitive to DDT than that of the corresponding wild-type flies (*r*/+). To confirm this point, the percentage of "responded" flies was transformed into the arc-sine unit, and then the homozygous effect of each chromosomal factor on inheritance of low nerve sensitivity was calculated by partial factorial analysis. The results of such statistical analyses clearly indicate that the 2nd chromosomal factor is responsible for low nerve sensitivity to DDT, whereas the 5th chromosomal factor contributes only to a small extent, if any, to it (Table 2).

Nerve sensitivity to an insecticide may generally be associated with at least three factors: 1) penetration of the insecticide through the nerve sheath and Schwann cell layers; 2) detoxification of the insecticide in the nerve; and 3) sensitivity of the nerve membrane (excitable membrane) to the insecticide. Although the housefly nerve has an ability to detoxify DDT[13], the present experiments demonstrate that the enhanced detoxification of DDT is not the major but an additional cause of low nerve sensitivity in the housefly because the former is controlled by the 5th chromosomal resistance gene[15,24]. Therefore, low nerve sensitivity is mainly ascribed either to reduced penetration of DDT through the nerve sheath and Schwann cell layers or to low sensitivity of the nerve membrane itself to DDT, or to both.

Preliminary similar experiments with gamma-BHC indicated that the major genetic factor for low nerve sensitivity to BHC could be associated neither with the 2nd nor with the 5th linkage group. Therefore, low nerve sensitivity to DDT may not

be the cause of "non-specific" cross resistance or vigor tolerance to BHC.

Summary

Low nerve sensitivity to DDT as one of the major resistance mechanisms in the housefly is genetically controlled by an incompletely recessive gene pair on the 2nd chromosome. Local detoxification of DDT in the nerve system is not the major cause of low nerve sensitivity because dehydrochlorination of DDT is controlled by a different resistance gene on the 5th chromosome.

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