Contribution of Recessive Factor on the Third Chromosome to Pyrethroid-Resistance in Houseflies, *Musca* *domestica* L. (Diptera: Muscidae)

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We investigated the contribution of the recessive factor on the third chromosome to the pyrethroid-resistance in 228_{e2b} strain and in Miyakonojo colony of houseflies using "Bx²" gene. In the 228_{e2b} strain, the resistance to permethrin and fenvalerate is associated with the recessive factor on the third chromosome. The contribution of the recessive factor on the third chromosome in the Miyakonojo colony, however, was undetectable. Furthermore, the resistance factor on the third chromosome in the 228_{e2b} strain influenced not only the mortality but also the knockdown time. The genetic method used in this study is considered very simple and useful to learn the importance of the recessive resistance factor on the third chromosome.

INTRODUCTION

It is known that the 228_{e2b} strain of the housefly from Denmark has a major recessive resistance factor associated with nerve insensitivity for permethrin on the third chromosome, and that no other factors are found (AHN et al., 1986 a, b). We found that factors conferring resistance in the Miyakonojo colony with selection by permethrin existed on autosomes 2, 3 and 5 (TAKADA et al., 1988).

In this study, we compared the contribution of the recessive factor on the third chromosome to pyrethroid-resistance in 228_{e2b} strain and in Miyakonojo colony without selection using the dominance marker "Bx²" on the third chromosome.

MATERIALS AND METHODS

Houseflies. The following strains and colony of houseflies (*Musca domestica* L.) were used. The miyakonojo colony was collected in Miyakonojo, Miyazaki Prefecture, Japan, in 1983 (HAYASHI et al., in preparation), and showed moderate resistance to both pyrethroids and organophosphates. The 228_{e2b} strain obtained from Dr. J. KEIDING is a strain resistant to pyrethroids (KEIDING, 1976). The susceptible Bx² strain has one dominant marker on the third chromosome. The wing is snipped from various directions and its phenotype is shown in Fig. 1. The homozygote of the gene is lethal. Viability of the heterozygote is about 50 percent that of the wild type (HIRO-

Fig. 1. The phenotype of Bx^2 strain of houseflies. In Bx^2 type, the wing is snipped from several directions. Homozygotic lethal character.

Fig. 2. Scheme of crosses used to investigate the contribution of the recessive resistance factor on the third chromosome.

YOSHI, 1977). A susceptible CSMA strain was obtained from Dr. O. F. BODENSTEIN, USDA in 1974.

Chemicals. The insecticides used and their purities are as follows: permethrin : 3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (91.8%), fenvalerate : (*RS*)- α -cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate (94.2%), tetramethrin : 3,4,5,6-tetrahydrophthalimidomethyl (1*RS*)-*cis,trans*-chrysanthemate (94.2%), fenitrothion : O,O-dimethyl O-*p*-nitro-*m*-tolyl phosphorothioate (96.7%).

Toxicity test. Topical application method: The appropriate dosages of test chemicals in 0.5 μ l of acetone were applied topically to the thoracic dorsa of four-day old female adult flies. Acetone was applied to control flies. Each assay was replicated twice, using 30 flies per replicate. Treated flies were kept at $26 \pm 1^\circ\text{C}$ and mortalities were determined 24 hr after treatment.

Oil spray method: After 20 female adult flies four-day old were released into a glass chamber (0.34 m³), 0.7 ml of predetermined concentrations of a test chemical in oil formulation was sprayed into the chamber with a spray gun at a pressure of 0.8 kg/cm². Records of knockdown were made at various intervals up to 15 min and thereafter all the insects tested were collected and transferred into a recovery container to observe the mortality after 24 hr.

Analysis of recessive resistant factor on the third chromosome. By the backcross method using F₁ males, the recessive resistant factor for an insecticide was investigated on the third chromosome in houseflies. The males of Bx^2 strain ($+/Bx^2$) were crossed to the females of the resistant strain or colony in mass, and then F₁ males ($+/Bx^2$) were backcrossed to the females of the resistant strain or colony in mass (Fig. 2). In order to obtain virgin females, adults were isolated within 4 hr of emergence.

Degree of dominance. The degree of dominance (D) of resistance in hybrids between the susceptible and resistant houseflies was calculated by the method of STONE (1968) and GEORGHIOU (1969). $D = (2X_2 - X_1 - X_3) / (X_1 - X_3)$, where X_1 , X_2 and X_3 represent the LD₅₀ values of the resistant, F₁ hybrid and susceptible houseflies respectively.

RESULTS

LD₅₀ values of resistant strains as well as of the susceptible CSMA strain for three insecticides are given in Table 1. The 228_{e2b} strain possesses a high level of resistance

Table 1. Susceptibility of 228_{e2b} strain and Miyakonojo colony of the housefly to three insecticides

Insecticide	LD ₅₀ (μg/female fly)		
	CSMA	228 _{e2b}	Miyakonojo
Permethrin	0.021	4.4 (210) ^a	0.27 (13)
Fenvalerate	0.028	20 (710)	0.40 (14)
Fenitrothion	0.064	1.1 (17)	2.0 (31)

$$^a \text{ Resistance factor} = \frac{\text{LD}_{50} \text{ value of resistance fly}}{\text{LD}_{50} \text{ value of CSMA strain}}$$

Table 2. Susceptibility of 228_{e2b} strain and Miyakonojo colony of housefly to pyrethroids (1% oil spray method)

Insecticide	KT ₅₀ (min)		Mortality (%) ^a	
	228 _{e2b}	Miyakonojo	228 _{e2b}	Miyakonojo
Permethrin	> 15	5.9	15.0	100
Fenvalerate	13	7.2	22.5	92.5
Tetramethrin	10	1.6	0	7.5

^a Mortality after 24 hr for all houseflies tested.

Table 3. Susceptibility of *Bx*² strain of housefly to insecticides

Insecticide	Genotype of III chromosome	LD ₅₀ (μg/female fly) (95% C.L.)
Permethrin	+ / +	0.018 (0.016–0.020)
	+ / <i>Bx</i> ²	0.021 (0.018–0.025)
Fenvalerate	+ / +	0.037 (0.031–0.043)
	+ / <i>Bx</i> ²	0.041 (0.032–0.047)
Fenitrothion	+ / +	0.058 (0.047–0.065)
	+ / <i>Bx</i> ²	0.059 (—) ^a

^a Not determined.

to the two pyrethroids and a moderate level of resistance to fenitrothion. On the other hand, the Miyakonojo colony shows only a moderate level of resistance to these insecticides. KT₅₀ values and mortalities of the 228_{e2b} strain and Miyakonojo colony with the three pyrethroids by 1% oil spray method are given in Table 2. In this method as well as in the topical application method, 228_{e2b} strain shows a lower level of susceptibility to pyrethroids than Miyakonojo colony in both KT₅₀ value and mortality.

Susceptibility of two genotypes in *Bx*² strain of the housefly to pyrethroids was investigated by 0.1% oil spray method and topical application method (Tables 3 and 4). From these results, no difference in susceptibility between the two genotypes to the insecticides tested was observed.

Table 5 shows the susceptibility of F₁ progeny and *D* value to permethrin. Here, it is clear that 228_{e2b} strain is quite different from Miyakonojo colony in *D* value.

Table 4. Susceptibility of Bx^2 strain of housefly to pyrethroids (0.1% oil spray method)

Insecticide	Genotype of III chromosome	KT ₅₀ (min)	Mortality (%) ^a
Permethrin	+/+	8.9	95.0
	+/ Bx^2	8.4	90.0
Tetramethrin	+/+	1.6	2.5
	+/ Bx^2	1.3	7.5

^a Mortality after 24 hr for all houseflies tested.Table 5. Susceptibility of F₁ progeny of housefly (resistant strain or colony ♀ × Bx^2 strain ♂) and *D* value to permethrin

Insecticide	Genotype of III chromosome	LD ₅₀ (μg/♀) ^a		<i>D</i> value	
		228 _{e2b}	Miyakonojo	228 _{e2b}	Miyakonojo
Permethrin	+/+	0.089	0.17	-0.97	0.21
		(0.079-0.096)	(0.14-0.19)		
	+/ Bx^2	0.091	0.18	-0.97	0.28
		(0.080-0.11)	(-) ^b		

^a Figures in parentheses mean 95% confidence limit.^b Not determined.Table 6. Susceptibility of backcrossed progeny of housefly (resistant strain or colony ♀ × F₁ progeny +/ Bx^2 ♂) to insecticides

Insecticide	Genotype of III chromosome	LD ₅₀ (μg/female fly) ^a	
		228 _{e2b}	Miyakonojo
Permethrin	+/+	2.5 (2.0-3.1)	0.14 (0.12-0.17)
	+/ Bx^2	0.14 (0.13-0.16)	0.13 (0.12-0.15)
Fenvalerate	+/+	12 (9.2-19)	0.32 (0.27-0.38)
	+/ Bx^2	0.59 (0.49-0.73)	0.33 (0.23-0.58)
Fenitrothion	+/+	0.51 (0.41-0.62)	1.6 (1.4-1.9)
	+/ Bx^2	0.59 (0.43-0.69)	1.6 (1.4-1.8)

^a Figures in parentheses mean 95% confidence limit.

D value in each phenotype of the two resistant houseflies was identical in susceptibility to permethrin. Thus, the presence of polygene was not recognized. Table 6 shows the LD₅₀ values of backcrossed progeny (resistant strain or colony × F₁ progeny). In the 228_{e2b} strain, a remarkable difference between F₂ progeny of the two genotypes in the LD₅₀ values of permethrin and fenvalerate were recognized, while no difference was observed for fenitrothion. In the Miyakonojo colony, however, no difference in LD₅₀ values between the two genotypes was observed for the three insecticides tested. The KT₅₀ values and the mortalities of backcrossed progeny showed a similar tendency

Table 7. Susceptibility of backcrossed progeny of housefly (resistant strain or colony ♀ × F₁ progeny +/Bx² ♂) to pyrethroids by 1% oil spray method

Insecticide	Genotype of III chromosome	KT ₅₀ (min)		Mortality (%) ^a	
		228 _{e2b}	Miyakonojo	228 _{e2b}	Miyakonojo
Permethrin	+/+	>15	5.1	50	100
	+/Bx ²	5.0	4.0	100	100
Fenvalerate	+/+	>15	7.1	30	100
	+/Bx ²	4.4	4.0	93	100
Tetramethrin	+/+	>15	1.2	2.6	10
	+/Bx ²	1.3	0.89	10	12

^a Mortality after 24 hr for all houseflies tested.

to the results obtained by topical application method (Table 7).

DISCUSSION

The 228_{e2b} strain possessed a high level of resistance to pyrethroids in both knock-down and lethal activities (Tables 1 and 2). It has been reported that this strain has a major recessive resistance factor associated with nerve insensitivity for permethrin on the third chromosome, and that no other resistant factors are found (AHN et al., 1986 a, b). The Miyakonojo colony, however, showed a moderate level of resistance to pyrethroids and fenitrothion, and the factors conferring resistance to permethrin in this colony with selection by permethrin were found on autosomes 2, 3 and 5 (TAKADA et al., 1988).

In this paper, we investigated the contribution of the recessive factor on the third chromosome for the resistance in 228_{e2b} strain and Miyakonojo colony without selection using the dominance marker "Bx²" on the third chromosome, because this gene was independent of the susceptibility to insecticides in both knockdown and lethal activities (Tables 3 and 4). In the 228_{e2b} strain, the recessive factor on the third chromosome influenced the resistance to permethrin and fenvalerate (Table 6). In contrast, the contribution of the recessive factor on the third chromosome in the Miyakonojo colony without selection was undetectable. Therefore, it is clear that the two strains differ the contribution of the recessive factor to insecticide-resistance on the third chromosome. The difference in this experiment and in linkage group analysis (TAKADA et al., 1988) is thought to be the result of the change of frequency of the recessive resistance factor on the third chromosome by selection with permethrin. Furthermore, no significant differences in the LD₅₀ values of Bx² genotypes of backcrossed progeny to permethrin and fenvalerate were observed between 228_{e2b} strain and Miyakonojo colony. It is therefore presumed that the two resistant houseflies used in this study would show approximately the same susceptibilities for pyrethroids, respectively, if the recessive resistant factor on the third chromosome were removed.

As shown in Table 7, it is apparent that the resistance factor on the third chromosome in 228_{e2b} strain influences not only the mortality but also the knockdown time. In the two genotypes in the Miyakonojo colony, there was no difference either in lethal

activity or knockdown activity.

The genetic method used in this study is considered very simple and useful to determine the importance of the recessive resistance factor on the third chromosome in a population of houseflies.

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