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# Linkage Group Analysis of Profenofos Resistance in the Housefly (Diptera: Muscidae)

# Profenofos에 대한 집파리(*Musca domestica* L.) 抵抗性 遺傳子의 連鎖群 解析

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**ABSTRACT** The profenofos-resistant P-Pro strain of house fly (*Musca domestica* L.) was derived from the pyraclofos-resistant strain by selecting with profenofos for 7 generations. The resistance was shown to be incompletely dominant by the reciprocal crosses between the resistant and susceptible strains. Linkage group analysis for the dominant factor responsible for this resistance was carried out by the $F_1$ male-backcross method, using susceptible multi-chromosomal marker strain. The major factors for profenofos resistance were located on the second and the fifth chromosome and the other chromosomes had a little effect on the development of this resistance. The male determining factor (M) was linked to the third chromosome in this strain.

**KEY WORDS** **Housefly,** *Musca domestica*, Profenofos, Resistance, Inheritance, Linkage group analysis, Male determining factor

**초 록** 유기인계 살충제인 pyraclofos에 抵抗性인 집파리를 profenofos로 7세대 도태시킨 P-Pro 계통을 이용하여 profenofos 저항성의 유전학적인 분석을 행하였다. Profenofos저항성은 불완전우성으로 나타났기 때문에 可視突然變異로 각 염색체를 標識한 감수성 *ac;ar;bwb;ye;snp* 계통을 이용하여 $F_1$ male-backcross에 의한 連鎖群 分析을 한 결과, 이 계통의 profenofos 저항성의 우성유전자는 각 염색체상에 고루 존재하지만 제 2염색체와 제 5염색체 상의 유전자에 크게 지배받으며, 雄性決定因子 M은 제 3염색체와 관련이 있는 것으로 나타났다.

**검색어** 집파리, Profenofos, 저항성, 유전양식, 連鎖群 解析, 雄性決定因子

## INTRODUCTION

The history of resistance to insecticides in the Danish housefly shows that the appearance and development of resistance are inevitable and stepwise biological process (Keiding 1977). Profenofos, a promising insecticide for the control of various insect pests, is no exception. At least 6 insect species were known as the resistance to this chemical until 1995 in spite of its short-term use. For example, field populations of the diamondback moth collected from cabbage in Shimane and Wakayama showed 77- and 164-fold resistance to profenofos, respectively (Hama 1986), and many other insect pests including tobacco whitefly, green peach aphid, and tobacco budworm also developed resistance to this chemical (Dittrich et al. 1990, Elzen et al. 1992, Herron & Rophail 1994). The evolution of resistance can be influenced by many factors, one of which is genetical (Georghiou & Taylor 1977a,b, Taylor & Georghiou 1979, Wood & Bishop 1981). In spite of its practical importance in insect pest control, the genetics of profenofos resistance in insects has not been fully investigated.

In the laboratory study described herein, we have done linkage group analysis of profenofos resistance in

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the profenofos-selected strain of housefly.

## MATERIALS AND METHODS

### Housefly strains

Two strains of *Musca domestica* L. were used in this study. The strain resistant to profenofos was derived by selecting the pyraclofos-resistant, P strain with profenofos for 7 successive generations at a selection pressure of 30-90% mortality for the next generations. The P strain had high resistance ratio of 640 to pyraclofos and had cross resistance to profenofos with resistance ratio of 98 (Lee 1995). Hereafter, this profenefos-resistant R strain was referred to as P-Pro. Selections by topical application were performed on female and male adult flies which were isolated within 12 hr after emergence. Flies which survived 48 hr after treatment were kept at 25±1°C, 65±2% relative humidity (RH), and a photopheriod of 16 : 8 (L : D) hr to produce the offsprings. A susceptible *ac;ar;bwb;ye;snp* strain (S) has the first, second, third, fourth, and fifith chromosomes marked with recessive visible mutant markers, ali-curve, aristapedia, brown body, yellow eyes, and *snp*, respectively.

### Chemical

Profenofos (*O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate) of 95% [AI] was supplied by Ciba-Geigy Ltd (Japan).

### Toxicity test

The appropriate dosages of test chemical in 0.5 $\mu l$ of acetone were topically applied to the dorsal thorax of 5-day-old female and male adult flies. Control flies were received acetone. Each assay was replicated three times, using 20 flies per replicate. Treated flies were held at the same conditions as above, and mortalities were determined at 48 hr after treatment. Data from all bioassays were corrected for control mortality using Abbott's (1925) formula. $LD_{50}$ values and slopes were estimated by probit analysis (SAS 1986). A resistance ratio (RR) was calculated according to the formula, RR =$LD_{50}$ value of the R strain/$LD_{50}$ value of the S strain.

### Degree of dominance

The degrees of dominance (D) of resistance in hybrids between the S and R flies were calculated by the method of Stone (1968) and Georghiou (1969) as follows, $D=(2X_2-X_1-X_3)/(X_1-X_3)$, where $X_1$, $X_2$ and $X_3$ represent the log of $LD_{50}$ values of R, $F_1$ hybrid and S flies, respectively.

### Linkage group analysis

The crossing design and consequent statistics for linkage group analysis for the dominant factor of profenofos resistance in the P-Pro strain were based on the $F_1$ male-backcross method (Tsukamoto 1964) as follows: (*ac;ar;bwb;ye;snp*)♀ × $F_1${(*ac;ar;bwb;ye;snp*)♀ × P-Pro ♂}♂. The progenies resulting from this backcross were separated into 32 phenotypes, and were topically treated with 0.2 $\mu g$ profenofos solution per fly to discriminate their susceptibility. To determine the linkage group of the resistance factor, the percentage survival of each phenotype was transformed into arcsine unit and subjected to factorial analysis as previously described (Tsukamoto, 1964). This male-backcross experiment was replicated twice.

## RESULTS AND DISCUSSION

### Toxicity test

Results of toxicity tests with profenofos on susceptible, $F_1$(R♀ × S♂), $F_1$(S♀ × R♂), and resistant fe-

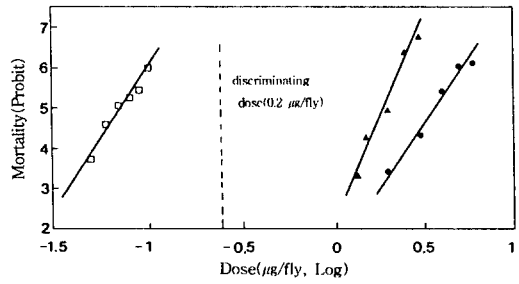


**Fig. 1.** Log dosage-probit mortality regression lines for female adults of profenofos-resistant P-Pro strain (●—●), susceptible *ac;ar;bwb;ye;snp* strain (□—□), and their $F_1$ (P-Pro ♀ × *ac;ar;bwb;ye;snp* ♂) progeny (▲—▲).

male and male houseflies are given in Fig. 1 and 2, respectively. $LD_{50}$ values, slope of ld-P lines, resistance ratios and degrees of dominance are summarized in Table 1. In tests with male flies, $LD_{50}$ values of the two $F_1$ hybrids to profenofos are greatly larger than those of the S flies. Resistance ratios of the S, $F_1$(R♀×S♂), $F_1$(S♀×R♂), and R flies were 1:15:11:41 for profenofos. Dominance values (D) in each pair of reciprocal crosses were nearly identical in susceptibility; 0.31 for $F_1$(S♀×R♂) and 0.45 for $F_1$(R♀×S♂). These results indicate that resistance to profenofos is incompletely dominant and not related to either sex-linkage or cytoplasmic factor. Similar results were also reported in the housefly resistant to fenitrothion (Shono & Tsukamoto 1983). In tests with female flies, resistance ratios of the S, $F_1$(R♀×S♂) and R flies were 1:26:50. Dominance value in $F_1$ hybrid were 0.67, indicating that profenofos resistance was inherited by incompletely dominant factor(s) in this strain.

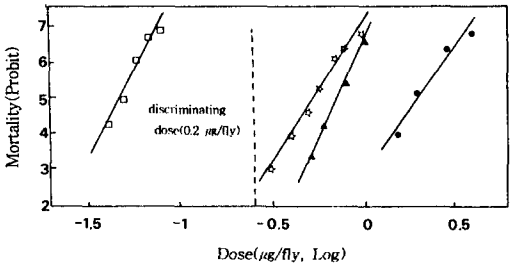


**Fig. 2.** Log dosage-probit mortality regression lines for male adults of profenofos-resistant P-Pro strain (●—●), susceptible *ac;ar;bwb;ye;snp* strain (□—□), and their $F_1$ (P-Pro ♀×*ac;ar;bwb;ye;snp* ♂, ▲—▲; *ac;ar;bwb;ye;snp* ♀×P-Pro ♂ ☆—☆) progenies.

**Linkage group analysis**

For determination of the linkage group for the dominant factor in profenofos resistance, the $F_1$-male backcross method (Tsukamoto, 1964) was used. The dosage of 0.2 μg/fly was used to discriminate susceptible and resistant flies (Fig. 1 and 2). Survival rates of each phenotype of the backcross progenies are shown in Table 2. The backcross progenies by the application of profenofos were clearly divided into two groups: *ar* and non-*ar*. Flies possessing the *ar* character were killed more than those possessing the non-*ar* character. Survival rates of *ar* phenotype progenies are below 50% in almost all phenotypic flies. This type of response is more clear in the flies possessing the *snp* character: the survial rates of *ar* flies among *snp* flies are below 7.03%. Therefore, some of the major factors for dominant resistance is considered to be located on the second chromosome. Similar results were also observed with *snp* and *ac* phenotype progenies, indicating that the major resistant factors are on the first and fifth chromosomes, too. The results of statistical analysis summarized in Table 3 further confirmed that the second and the fifth chromosomal effects were significant among those of each autosome, and the other chromosome had a little effect on the development of this resistance.

**Table 1. $LD_{50}$ values, slopes of ld-p lines, resistance ratios (RR) and degrees of dominance (D) for susceptible, $F_1$ hybrids and resistant houseflies**

| Strain | n | Slope±SE | $LD_{50}$, ug/fly (95% CL) | RR | D |
|---|---|---|---|---|---|
| Female | | | | | |
| P-Pro | 300 | 6.16±0.56 | 3.664 (3.438-3.944) | 50.2 | - |
| $F_1$(P-Pro♀×*ac;ar;bwb;ye;snp*♂) | 225 | 9.20±0.89 | 1.919 (1.821-2.021) | 26.3 | 0.67 |
| *ac;ar;bwb;ye;snp* | 360 | 6.58±0.67 | 0.073 (0.069-0.076) | 1.0 | - |
| Male | | | | | |
| P-Pro | 180 | 7.00±0.98 | 2.018 (1.881-2.182) | 41.2 | - |
| $F_1$(*ac;ar;bwb;ye;snp*♀×P-Pro♂) | 315 | 7.84±0.40 | 0.555 (0.528-0.584) | 11.3 | 0.31 |
| $F_1$(P-Pro♀×*ac;ar;bwb;ye;snp*♂) | 165 | 10.44±0.27 | 0.729 (0.691-0.769) | 14.9 | 0.45 |
| *ac;ar;bwb;ye;snp* | 300 | 9.68±0.88 | 0.049 (0.047-0.051) | 1.0 | - |

**Table 2. Relation between phenotypic expression of visible markers and profenofos resistance in backcross progeny from cross [*ac;ar;bwb;ye;snp* ♀ × $F_1$ (*ac;ar;bwb;ye;snp*♀ × **P-Pro** ♂) ♂]**

| Phenotype | | | | | No. of flies | | Survival rate | |
|---|---|---|---|---|---|---|---|---|
| (1; | 2; | 3; | 4; | 5) | Tested | Alive | % | θ |
| +; | +; | +; | +; | + | 337 | 303 | 89.91 | 71.46 |
| *ac*; | +; | +; | +; | + | 149 | 113 | 75.84 | 63.84 |
| +; | *ar*; | +; | +; | + | 309 | 67 | 21.68 | 28.08 |
| *ac*; | *ar*; | +; | +; | + | 199 | 21 | 10.55 | 16.00 |
| +; | +; | *bwb*; | +; | + | 285 | 276 | 96.84 | 79.48 |
| *ac*; | +; | *bwb*; | +; | + | 209 | 184 | 88.04 | 71.17 |
| +; | *ar*; | *bwb*; | +; | + | 223 | 127 | 56.95 | 49.47 |
| *ac*; | *ar*; | *bwb*; | +; | + | 115 | 32 | 27.93 | 31.04 |
| +; | +; | +; | *ye*; | + | 334 | 305 | 91.32 | 72.93 |
| *ac*; | +; | +; | *ye*; | + | 167 | 134 | 80.24 | 62.22 |
| +; | *ar*; | +; | *ye*; | + | 213 | 19 | 8.92 | 17.43 |
| *ac*; | *ar*; | +; | *ye*; | + | 151 | 11 | 7.28 | 15.81 |
| +; | +; | *bwb*; | *ye*; | + | 31 | 123 | 93.89 | 76.31 |
| *ac*; | +; | *bwb*; | *ye*; | + | 110 | 89 | 80.91 | 66.69 |
| +; | *ar*; | *bwb*; | *ye*; | + | 77 | 35 | 45.45 | 46.25 |
| *ac*; | *ar*; | *bwb*; | *ye*; | + | 39 | 4 | 10.26 | 24.05 |
| +; | +; | +; | +; | *snp* | 230 | 150 | 65.22 | 53.89 |
| *ac*; | +; | +; | +; | *snp* | 150 | 30 | 20.00 | 27.42 |
| +; | *ar*; | +; | +; | *snp* | 245 | 2 | 0.82 | 5.29 |
| *ac*; | *ar*; | +; | +; | *snp* | 181 | 2 | 1.10 | 4.36 |
| +; | +; | *bwb*; | +; | *snp* | 162 | 123 | 75.93 | 57.79 |
| *ac*; | +; | *bwb*; | +; | *snp* | 201 | 77 | 38.31 | 37.76 |
| +; | *ar*; | *bwb*; | +; | *snp* | 128 | 9 | 7.03 | 14.94 |
| *ac*; | *ar*; | *bwb*; | +; | *snp* | 71 | 4 | 5.63 | 11.10 |
| +; | +; | +; | *ye*; | *snp* | 211 | 7 | 3.32 | 11.14 |
| *ac*; | +; | +; | *ye*; | *snp* | 200 | 7 | 3.50 | 10.60 |
| +; | *ar*; | +; | *ye*; | *snp* | 162 | 2 | 1.27 | 5.05 |
| *ac*; | *ar*; | +; | *ye*; | *snp* | 97 | 0 | 0.00 | 0.00 |
| +; | +; | *bwb*; | *ye*; | *snp* | 166 | 76 | 45.78 | 43.83 |
| *ac*; | +; | *bwb*; | *ye*; | *snp* | 115 | 13 | 11.30 | 19.55 |
| +; | *ar*; | *bwb*; | *ye*; | *snp* | 88 | 2 | 2.27 | 9.58 |
| *ac*; | *ar*; | *bwb*; | *ye*; | *snp* | 45 | 0 | 0.00 | 0.00 |
| | | | | | 5,496 | 2,347 | | 1,104.5 |

**Table 3. Factorial analysis of resistance effects using *ac;ar;bwb;ye;snp* (1; 2; 3; 4; 5) multichromosomal mutant strain**

| Chromosome | Effect | d.f. | Mean square | F value |
|---|---|---|---|---|
| Total | 1104.5 | 63 | 699 | |
| Phenotype | | 31 | 1361 | 33.02*** |
| 1 | 181.3 | 1 | 2055 | 49.84*** |
| 2 | 547.6 | 1 | 18744 | 454.72*** |
| 12 | 33.9 | 1 | 72 | 1.74 |
| 3 | -173.5 | 1 | 1881 | 45.63*** |
| 13 | -51.2 | 1 | 164 | 3.98 |
| 23 | 15.3 | 1 | 15 | 0.36 |
| 123 | 17.5 | 1 | 19 | 0.46 |
| 4 | 141.7 | 1 | 1254 | 30.42*** |
| 14 | 14.1 | 1 | 12 | 0.30 |
| 24 | 57.4 | 1 | 206 | 5.00* |
| 124 | 20.5 | 1 | 26 | 0.63 |
| 34 | 8.7 | 1 | 5 | 0.11 |
| 134 | 44.3 | 1 | 122 | 2.97 |
| 234 | 31.1 | 1 | 60 | 1.47 |
| 1234 | 12.6 | 1 | 10 | 0.24 |
| 5 | 479.9 | 1 | 14395 | 349.22*** |
| 15 | -0.1 | 1 | 0 | 0.00 |
| 25 | 124.3 | 1 | 966 | 23.44*** |
| 125 | -70.0 | 1 | 306 | 7.43* |
| 35 | -19.9 | 1 | 25 | 0.60 |
| 135 | -1.8 | 1 | 0 | 0.00 |
| 235 | 85.3 | 1 | 454 | 11.03** |
| 1235 | 37.2 | 1 | 86 | 2.10 |
| 45 | -84.0 | 1 | 440 | 10.69** |
| 145 | -0.95 | 1 | 6 | 0.14 |
| 245 | -83.9 | 1 | 440 | 10.68** |
| 1245 | -42.6 | 1 | 113 | 2.75 |
| 345 | -22.4 | 1 | 31 | 0.76 |
| 1345 | -19.4 | 1 | 23 | 0.57 |
| 2345 | -47.4 | 1 | 140 | 3.40 |
| 12345 | -44.6 | 1 | 124 | 3.01 |
| Error | | 31 | 41 | |

*, **, *** ; Significant at 5, 1, 0.1 % level.

It has been well established that there are at least four genes responsible for OP resistrtance in the housefly, *P450, g, a, Ox-5*, carboxyesterase and *AChe-R* on the second chromosome, *pen (tin)* and *kdr* on the third chromosome, and *Ox-5* on the fifth chromosome (Plapp, 1976; Tsukamoto, 1969). In our study, multiple factors are involved in profenofos resistance in the housefly. This is very interesting, because P-Pro strain was originated from pyraclofos-resistant strain (P strain) which the dominant resistance factors for pyraclofos are located on the second, third, fourth and fifth chromosomes, and the interaction of the second and fifth. The only difference of resistance factor between P strain and P-Pro strain is on the first chromosome. These results indicate that selection with profenofos might cause the first chromosomal effect in the resistance, although this case is very rare.

The male determining system of P-Pro strain was

**Table 4. Sex ratio of *bwb* and non-*bwb* houseflies in backcross progeny from the cross (*ac;ar;bwb;ye;snp*) ♀ × $F_1$(*ac;ar;bwb;ye;snp* ♀ × P-Pro ♂) ♂**

| Phenotype | No. of flies | | % male |
|---|---|---|---|
| | Female | Male | |
| *bwb* | 1,341 | 108 | 7.5 |
| non-*bwb* | 0 | 2,354 | 100 |

also analyzed by the crossing method used by Tsukamoto *et al.* (1980). In the backcross progenies, 92.5% of the houseflies possessing visible character *bwb* were female and all of the non-*bwb* houseflies were males without exception (Table 4). This indicates that the male determining factor M is located on the 3rd chromosome in this strain, i.e. the sex determining system of the P-Pro strain was the $III^M$ type. It has been reported that the male determining factor M in the fenitrothion-resistant strain of the houseflies is located on the third chromosome (Shono & Tsukamoto 1983).

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