Genetical relationships between malathion-resistance and fenthion-resistance in larvae of *Culex pipiens pallens* Coquillett¹⁾

By

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INTRODUCTION

The mode of inheritance of malathion-resistance has been shown to be monofactorial in *Culex pipiens fatigans* by Umino and Suzuki (1966), and in *C. p. pallens* by Tadano (1969) who demonstrated that the resistance factor was linked to chromosome 2 with a crossover unit of 40–43 % from *ru* (ruby eye).

A multiple-resistant colony of *C. p. pallens* was found in Hyogo Prefecture, Japan (Suzuki, 1968), which was cross-resistant to 6 organophosphorus insecticides including malathion and fenthion, and cross-tolerant to 4 other organophosphorus insecticides such as Dursban which had never been applied in the field. This study presents genetical relationships between malathion- and fenthion-resistance in a *C. p. pallens* colony which was derived from the multiple-resistant colony mentioned above.

MATERIALS AND METHODS

Two organophosphorus insecticides, malathion and fenthion, and the following two colonies were used in this study:

NMFR: a malathion- and fenthion-resistant colony without any visible mutant of *C. p. pallens* which was submitted to the larval selection with malathion for 10 generations and thereafter with fenthion for 5 further generations. The LC_{50} was 0.90 ppm for malathion and 0.17 ppm for fenthion.

ru (ruby eye): an autogenous colony of *C. pipiens* which is a chromosome-2 marker strain susceptible to both malathion and fenthion, the LC_{50} being 0.07 ppm for malathion and 0.0034 ppm for fenthion.

Crossing experiments were all made in cages (20×20×20 cm) at a temperature of $25 \pm 1^\circ\text{C}$ and the adults were fed on ca. 10 % sucrose solution, the female mosquitoes also on mice. Reciprocal crosses were made between the *ru* strain and NMFR strain, although from the cross of *ru* males and NMFR females sufficient numbers of offspring could not be obtained for the backcrossing due to cytoplasmic incompatibility, and then the F_1 hybrids from the cross between *ru* females and NMFR males were reciprocally backcrossed with the *ru* strain, using 100–150 individuals of each sex for one cross. All susceptibility tests were performed on larvae in the 3rd or 4th instar stage by the standard method for mosquito larvae (WHO Expert Committee on Insecticides, 1960) at a temperature of $23\text{--}24^\circ\text{C}$.

RESULTS

When the females of the *ru* strain were crossed with the males of the NMFR strain, of which larvae were resistant to both malathion and fenthion, the F_1 hybrids were intermediate in resistance to malathion and fenthion, though much closer to the resistant parent (Figs. 1 and 2, in Fig. 2 *ru* III, IV, F_1 III and IV are the dosage-mortality (d-m) lines for the third and fourth instar larvae of the *ru* strain and F_1 hybrids respectively). The d-m lines for the backcross offspring, which were produced by reciprocally crossing the F_1 hybrids with the *ru* strain, have inflexions at about

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Fig. 1

Fig. 2

50 % mortality level for malathion and fenthion, indicating that the backcross offspring segregated into the susceptible homozygotes and the heterozygotes in a 1:1 ratio. These facts prove that each of the resistance to malathion and to fenthion was monofactorial, and the factors for the resistance were incompletely dominant.

In order to find relationships between gene loci of the factors for malathion- and fenthion-resistance, the following procedure was performed:

1. The backcross offspring in the third instar, after separation of the larval eye colours into *ru* and wild type, were exposed to a discriminating dose of 0.0078 ppm fenthion for 24 hours (see Fig. 2) to remove the fenthion-susceptible phenotypes (*++* and *+MR*, in which *MR* represents a malathion-resistance factor) and none of the heterozygotes (*FR MR* and *FR +*, *FR* here represents a fenthion-resistance factor).
2. After the heterozygous survivors had been fed in deionized water for 24 hours, they were again exposed to a discriminat-

ing dose of 0.16 ppm malathion for another 24 hours, which was known to kill all the malathion-susceptible homozygotes (*FR+*) but to allow 91.8 % of the heterozygotes (*FR MR*) to survive.

After the above procedure, the data were adjusted to compensate for the discriminating dose of malathion killing 8.2 % of the heterozygotes (*FR MR*) and then summarized in Tables 1 and 2. The tables show that the

Table 1. Offspring of backcross *ru* ♀ × *F1*
(*ru* ♀ × *NMFR* ♂) ♂

Phenotypes				Phenotypes			
<i>ru</i>	<i>FR</i>	<i>MR</i>	338	+	<i>FR</i>	<i>MR</i>	717
<i>ru</i>	<i>FR</i>	+	147	+	<i>FR</i>	+	31
<i>ru</i>	+	<i>MR</i>	194	+	+	<i>MR</i>	34
<i>ru</i>	+	+	452	+	+	+	791
				Total=2704			

Recombination between *ru* and *FR*=47.8±1.0%

Recombination between *ru* and *MR*=50.0±0.9%

Recombination between *FR* and *MR*=14.9±0.7%

Table 2. Offspring of backcross *ru* ♂ × *F1*
(*ru* ♀ × *NMFR* ♂) ♀

Phenotypes				Phenotypes			
<i>ru</i>	<i>FR</i>	<i>MR</i>	1593	+	<i>FR</i>	<i>MR</i>	2362
<i>ru</i>	<i>FR</i>	+	394	+	<i>FR</i>	+	129
<i>ru</i>	+	<i>MR</i>	381	+	+	<i>MR</i>	107
<i>ru</i>	+	+	1525	+	+	+	1945
				Total=8436			

Recombination between *ru* and *FR*=48.2±0.5%

Recombination between *ru* and *MR*=48.2±0.5%

Recombination between *FR* and *MR*=12.1±0.1%

crossover unit between *ru* and *FR* was 47.8 % in the males and 48.2 % in the females, and the crossover unit between *ru* and *MR* was 50.0 % in the males and 48.2 % in the females, while the value for *FR-MR* was 14.9 % and 12.1 % in the males and females respectively. It is therefore concluded that a malathion-resistance factor is located at crossover distances of 48.2-50.0 % from *ru* and of 12.1-14.9 % from the fenthion-resistance factor which is linked to *ru* with a crossover value of 48 %.

DISCUSSION

Monofactorial inheritance has been found in malathion-resistance in *Culex p. fatigans* (Umino and Suzuki, 1966) and *C. p. pallens* (Tadano, 1969), and in fenthion-resistance in *C. p. pallens* (Suzuki and Matsunaga, personal communication); this study confirmed the

previous finding of the mode of inheritance of malathion-resistance and fenthion-resistance in the *C. p.* group.

The problem arose at the start of this experiment as to how closely the genes for resistance to malathion and fenthion are located in the multiple-resistant colony, and both the genes were found to be unexpectedly far linked with a crossover value of 12–15 %. This fact is interesting as compared with the housefly in which Nguy and Busvine (1960) assumed that the genes for malathion-resistance and parathion-resistance were either alleles or closely linked.

There was some difference between the values of crossover for *ru-MR* previously reported (40–43 %) (Tadano, 1969) and mentioned in this paper (48–50 %), though the colonies used in this study were originally the same as those employed in the previous study. Dorval, C. and Brown, A. W. A. (personal communication) found the crossover unit for *ru-MR* to be 42 %, whereas it was approximately 48 % in this study. These results indicate that both the resistance genes are located too far from the *ru* gene to calculate the precise loci. It is now really unfortunate for the genetics of this species that there is a basic shortage of genetic markers especially of chromosome 2 for analyzing in more detail the mode of inheritance of resistance to the organophosphorus compounds. Determination of gene order or map distances awaits further crossing experiments using more suitable visible markers.

SUMMARY

1. Monofactorial inheritance of resistance to malathion and fenthion was proved in the colony of *Culex p. pallens* which showed 13-fold resistance to malathion and 50-fold resistance to fenthion after 10 generations of the larval selection with malathion and 5 further generations with fenthion; the factors for malathion- and fenthion-resistance were incompletely dominant.

2. Genetical linkage relationships were investigated between malathion-resistance and fenthion-resistance in this colony; the results show that a malathion-resistance factor is located at crossover distances of 48–50 % from *ru* on chromosome 2, though of 40–43 % from *ru* in the previous study, and of 12–15 % from

the fenthion-resistance factor linked to *ru* with a 48 % crossover unit.

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摘 要

Culex pipiens pallens Coquillett の幼虫 における malathion 抵抗性と fenthion 抵抗性との遺伝的関係

兵庫県下で採集された *Culex pipiens pallens* の有機リン殺虫剤抵抗性のコロニーを malathion と fenthion で 5 代から 10 代淘汰したコロニーと殺虫剤感受性で第 2 染色体 marker である *ru* (ruby eye) コロニーとを用いて次の結果を得た。

1. *Culex p. pallens* の幼虫の malathion 抵抗性も fenthion 抵抗性も夫々、不完全優性の単一因子によって遺伝する。

2. これらの抵抗性因子は第 2 染色体上にあつて、*ru* (ruby eye) 因子から遠く離れているが、malathion 抵抗性因子と fenthion 抵抗性因子とは 12 % から 15 % (交叉価) ぐらい離れて存在すると結論された。

3. *ru* 因子、malathion 抵抗性因子及び fenthion 抵抗性因子の遺伝子配列順序は別のより適当な marker を用いて決定されなければならない。