Location of a gene conferring resistance to knockdown by permethrin and bioresmethrin in adults of the BKPM3 strain of *Aedes aegypti*

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**Abstract**

A genetic analysis of resistance to knockdown by permethrin and bioresmethrin in adults of the homozygous resistant strain of *Aedes aegypti*, BKPM3, was made. A major resistance gene $R^{py}$ was located approximately 13 cross-over units from the linkage group III marker, $b_{lt}$ (black tarsus).

**Introduction**

The establishment of a homogeneous strongly permethrin-resistant strain of *Aedes aegypti*, BKPM3, has been reported in a previous study (Malcolm & Wood, 1982). This strain was derived by mass selection and single-family sib selection from the Bangkok field-collected strain BKK, which had low resistance to several pyrethroids as well as high resistance to DDT (Chadwick *et al.*, 1977).

This paper describes an analysis of the mode of inheritance of resistance to two synthetic pyrethroids, permethrin and bioresmethrin, in the BKPM3 strain. A major resistance gene was located with respect to visible mutant markers.

**Materials and methods**

*Chemicals*

- **Permethrin:** (3-Phenoxybenzyl)-3-(2,2 dichlorovinyl)-2,2-dimethyl) cyclopropane carboxylate, cis/ trans ratio 25%:75%.

- **Bioresmethrin:** 5-Benzyl-3-furymethyl (+) trans chrysanthemate.

- **Silicone fluid:** DC556 Dow Corning Ltd.

- **Insecticides** were provided by Wellcome Research Laboratories (Berkhamsted).

**Strains of Aedes aegypti L.**

- **New 64:** Triple marked strain having the markers $r_{e}$ (red eye) on linkage group I, $s$ (spotless abdomen) on group II and $b_{lt}$ (black tarsus) on group III. Synthesized from various sources. Susceptible to insecticides. Supplied by Prof. G. B. Craig, Notre Dame University, U.S.A. in 1976.

- **Normal Susceptible (NS):** Unmarked, insecticide-susceptible strain. Originated from West Africa over 50 years ago. Supplied in 1962 by Prof. J. R. Busvine, London School of Hygiene and Tropical Medicine.

- **BKPM3:** Unmarked, homogeneous for high resistance to permethrin. Prepared from a strain supplied by Dr. P. R. Chadwick, Wellcome Research Laboratories (Berkhamsted) (see Malcolm & Wood, 1982).

Rearing procedures were those used by Wood (1976). Prior to all crosses the sexes were separated at the pupal stage and placed in $30 \times 30 \times 30$ cm cages. Sex was checked again over the period of...
Table 1. Results of adult insecticide tests.

<table>
<thead>
<tr>
<th>Strain</th>
<th>KC50 mg/m²</th>
<th>S.E. mg/m²</th>
<th>Variance of log KC50</th>
<th>KC50 mg/m²</th>
<th>S.E. mg/m²</th>
<th>Variance of log KC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Susceptible</td>
<td>24.75</td>
<td>±0.57</td>
<td>9.98 x 10⁻²</td>
<td>20</td>
<td>±0.38</td>
<td>7.44 x 10⁻⁵</td>
</tr>
<tr>
<td>New 64</td>
<td>14</td>
<td>±0.36</td>
<td>1.3 x 10⁻⁴</td>
<td>11.5</td>
<td>±0.23</td>
<td>7.8 x 10⁻⁵</td>
</tr>
<tr>
<td>BKPM3</td>
<td>785</td>
<td>±11.12</td>
<td>3.8 x 10⁻⁵</td>
<td>720</td>
<td>±5.3</td>
<td>1.39 x 10⁻⁵</td>
</tr>
<tr>
<td>(BKPM3 x New 64)F₁</td>
<td>70.5</td>
<td>±1.39</td>
<td>7.3 x 10⁻⁵</td>
<td>90</td>
<td>±0.95</td>
<td>2.13 x 10⁻⁵</td>
</tr>
<tr>
<td>(New 64 x BKPM3)F₁</td>
<td>73</td>
<td>±1.48</td>
<td>7.8 x 10⁻⁵</td>
<td>88</td>
<td>±0.87</td>
<td>1.86 x 10⁻⁵</td>
</tr>
</tbody>
</table>

Emergence, after which the crosses were made by transferring 400 of each sex to 45 x 45 x 30 cm cages.

Insecticide testing was carried out in WHO adult test kits (WHO, 1970); 25 unmated, unfed females were exposed in each cylinder, replicated 4 times at each concentration. A 60-minute exposure period was used in each test. Knockdown was scored at the end of the exposure period.

Preparation of insecticide-impregnated papers was carried out following procedures described by Chadwick et al. (1977). Both rearing and testing were carried out under conditions of constant humidity 75 ± 5% RH and temperature 28 ± 1 °C.

Results and discussion

Genetic analysis

Table 1 lists KC₅₀ (the concentration producing 50% knockdown) values obtained from tests with permethrin and bioresmethrin. New 64 was found to be even more susceptible than the NS strain, most probably due to differences in vigour between the two strains which had been evident in rearing. Permethrin and bioresmethrin gave similar KC₅₀ values indicating that the two compounds have similar toxicity to Ae. aegypti, which has been observed previously (Chadwick et al., 1977). Resistance levels to permethrin and bioresmethrin were also similar in the resistant strain BKPM3 as they had been in the original parental strain BKK (Chadwick et al., 1977). Thus selection with permethrin increased resistance to bioresmethrin to the same extent. F₁ progeny from reciprocal crosses between New 64 and BKPM3 (3/64 and 64/3) gave virtually identical responses in tests with both permethrin and bioresmethrin (Table 1), indicating no differences in inheritance of resistance from males and females, and thus no cytoplasmic influences on resistance.

The degree of dominance (D) was calculated for each set of results using the formula suggested by Falconer (1960) as outlined by Stone (1968). These are presented in Table 2 along with the variance and estimates of 't' for the null hypotheses D = 0 and D₃/₆₄ - D₆₄/₃ = 0 (Misra, 1968).

For bioresmethrin, the 't' value obtained indicated that the degree of dominance was not significantly different from 0, i.e. exactly intermediate between completely recessive and completely dominant, whereas the permethrin results gave a value which was significantly different from 0. However, if instead of the purely quantitative expression of dominance suggested by Stone (1968), the terminology and five categories of dominance suggested by Georghiou (1969) are adopted, then both the bioresmethrin and permethrin data would be considered to indicate 'intermediate' dominance (D = 0) as the latter is more different from the next closest category 'incompletely recessive' (D = -0.5).

Table 2. Results of determination of degree of dominance.

<table>
<thead>
<tr>
<th>Strain</th>
<th>D</th>
<th>V₁(D)</th>
<th>t</th>
<th>t</th>
<th>D = 0</th>
<th>t</th>
<th>D₁₆₄ = D₆₄/₃</th>
<th>D</th>
<th>V₁(D)</th>
<th>t</th>
<th>t</th>
<th>D = 0</th>
<th>t</th>
<th>D₁₆₄ = D₆₄/₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>BKPM3</td>
<td>-0.197</td>
<td>1.07 x 10⁻⁴</td>
<td>-19.04</td>
<td></td>
<td>-0.00534</td>
<td></td>
<td>5.60 x 10⁻⁵</td>
<td>-0.072</td>
<td>-0.739</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(New 64 x BKPM3)F₁</td>
<td>-0.179</td>
<td>1.13 x 10⁻⁴</td>
<td>-16.84</td>
<td></td>
<td>-0.016142</td>
<td></td>
<td>5.18 x 10⁻⁵</td>
<td>-2.24</td>
<td>-0.739</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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