Research Article

Genetic Study of Propoxur Resistance—A Carbamate Insecticide in the Malaria Mosquito, Anopheles stephensi Liston

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Anopheles stephensi Liston (Diptera: Culicidae) is the urban vector of malaria in the Indian subcontinent and several countries of the Middle East. The genetics of propoxur resistance (pr) in An. stephensi larvae was studied to determine its mode of inheritance. A diagnostic dose of 0.01 mg/L as recommended by WHO was used to establish homozygous resistant and susceptible strains. Reciprocal crosses between the resistant and susceptible strains showed an F1 generation of incomplete dominance. The progenies of backcrosses to susceptible parents were in 1 : 1 ratio of the same phenotypes as the parents and hybrids involved. The dosage mortality (d-m) lines were constructed for each one of the crosses, and the degree of dominance was calculated. It is concluded that propoxur resistance in An. stephensi larvae is due to monofactorial inheritance with incomplete dominance and is autosomal in nature.

1. Introduction

The acquisition of insecticide resistance has given one of the best opportunities to assess microevolution processes, partly because the nature of the selective agent is well identified and partly because, in response to this strong selection pressure, evolution is fast [1]. As resistance reflects changes occurring in genotypic architecture of natural populations, a full understanding of the evolution of this phenomenon requires an accurate knowledge of its genetic basis [2]. Resistance to insecticides has appeared in the major insect vectors from every genus [3]. Mosquitoes have developed resistance to all the major groups of insecticides, including biocides [4].

Anopheline mosquito species are obligatory vectors for human malaria, an infectious disease that affects hundreds of millions of people living in tropical and subtropical countries [5]. However, with the emergence of populations of Anopheles capable of withstanding lethal doses of insecticides, the efficacy of insecticide-based vector control tools is critically affected [6]. An. stephensi is one of the important carriers of urban malaria in the Indian subcontinent and roughly accounts for 15% of the total malaria incidence in India [7]. The said species has been selected as a model insect for research in our laboratory, since three decades. Considerable data on genetic and cytogenetic studies involving isolation of naturally occurring mutants and establishing Mendelian genetics, isolation of naturally occurring paracentric inversions by using polytene chromosomes, genetic cytological basis of insecticide resistance and the biochemical mechanism involved, isolation and establishment of chromosomal translocations; synthesis of refractory strains for malaria transmission, and so forth, have been generated. Such data shall be used in the synthesis of transgenic strains of the said species. These strains could be used in the genetic control programme of An. stephensi [7–11].

Present investigation was, therefore, undertaken with a view to study the laboratory development of propoxur-induced resistance, inheritance, and degree of dominance of propoxur resistance in An. stephensi.

1.1. Insecticide. Propoxur (Baygon) is a carbamate insecticide (2-isopropoxyphenyl methylcarbamate), with chemical formula C11H15NO3. It is approved and registered with the Central Insecticide Board and Registration Committee...
(CIBRC), Ministry of Agriculture, Government of India; World Health Organization (WHO); Environmental Protection Agency (EPA), USA for the use against household pest including mosquitoes. Like other carbamates, propoxur blocks the production and action of cholinesterase, paralyzing the nervous systems of insects causing a rapid "knockdown" effect. Propoxur is found to be effective in the area where mosquitoes were found to be resistant to organophosphate insecticides [12].

2. Materials and Methods

2.1. Mosquito Rearing. Thirty strains of An. stephensi derived from different geographical areas of India are currently maintained in our laboratory. Papareddypallya (PRP) strain from Bangalore, India was used to establish the propoxur resistant and susceptible strains, respectively. The adult mosquitoes were maintained at 25 ± 1°C and 75 ± 5% relative humidity under 16 hours photoperiods according to the procedure of Shetty [13]. The adults were fed on 10% sucrose in 8” × 8” × 8” iron cages covered with cotton net cloth. Females were provided with mice and pigeon as a source for blood meal. Plastic cup (3” diameter) containing clean water lined with filter paper was placed inside the cage for oviposition. The eggs were kept for 72 hours to ensure complete hatching. The hatched larvae were transferred to enamel tray and reared. Powdered mixture of fish feed and dog biscuits were given as larval diet.

2.2. Development of Propoxur-Resistant and Propoxur-Susceptible Strains. A WHO diagnostic dosage of 0.01 mg/L of propoxur was selected and resistance test was carried out according to the procedure of WHO [14, 15]. The remaining part of F1 progeny from both reciprocal crosses were used for larval bioassay, and the remaining part of F1 progeny from both reciprocal crosses were pooled and reared to adult stage for backcrossing to parental strains and intercrossing to produce the F2 progeny. The resulting backcross (F1 × S) and the F2 progeny were assessed in order to test the hypothesis of monogenic inheritance. The data was subjected to statistical analysis and mode of inheritance of insecticide resistance was carried out according to the procedure of Priester and Georgiou [16], and Mazzarri and Georgiou [17]. The null hypothesis of monogenic resistance was tested from mortality data of backcross progeny compared to theoretical expectations using the X^2 test. The degree of dominance (D) was determined using the formula of Stones [18], D = (2X2 – X1 – X3)/X1 – X3, where, X1, X2, and X3 are the logarithms of the LC50 (concentration for 50% lethality) values for resistant, F1 hybrid, and susceptible strains, respectively. The value of D varies from −1 to 1; D = 1 indicates complete dominance, 0 < D < 1 incomplete dominance, −1 < D < 0 incomplete recessivity, and D = −1 complete recessivity [18].

2.4. Larval Bioassay Test. Twenty-five late third instar larvae were transferred into a glass bottle containing the test concentration (249 mL of dechlorinated tap water + 1 mL stock concentration) each, with four replicates. Small amount of larval feed was added to avoid mortality due to starvation. Mortality was assessed after 24 hrs. Mortality data from bioassays were corrected by natural control mortality using Abbott’s formula [19]. LC50 and LC90 were calculated by log-dose probit analysis—LDP line [20].

3. Results and Discussion

The establishment of homozygous resistant (R) and susceptible (S) strains of An. stephensi for propoxur to a diagnostic dose of 0.01 mg/L is presented in Figure 1. Homozygous resistant and susceptible strains were synthesized by selective inbreeding for 17 and 6 generations, respectively. Exposure to diagnostic dose of propoxur exhibited gradual decrease in larval mortality with the progression of generations.

The data on various genetic crosses for susceptibility and resistance has been presented in Table 1. The crosses between the susceptible male to susceptible female (cross 1) and resistant male to resistant female (cross 2) showed the purity of gamete for susceptibility and resistance, respectively. The LC50 of the resistant strain was 91.68 times greater than the LC50 of the susceptible strain. The dose response of parental strains was characterized by straight lines when bioassayed with propoxur, indicating that susceptible and resistant strains were homogeneous for susceptibility and resistance to this insecticide, respectively (Figure 2). When a female of the propoxur resistant strain was crossed with the susceptible strain (reciprocal cross), the F1 hybrids showed 51.85% and 48.15% of resistance and susceptibility, respectively (cross 3). Similar result of 53.93% of resistance and 46.07% of susceptibility was recorded in F1 hybrids of propoxur-resistant males crossed with susceptible females (cross 4). Both the above-mentioned reciprocal crosses exhibit 1:1 ratio of susceptible and resistant response, which means, that the resulting F1 hybrids from the reciprocal crosses were intermediate in their
Development of resistance and susceptible strains (%)

F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 F13 F14 F15 F16 F17

Figure 1: Development of propoxur-resistant and propoxur-susceptible strains in each generation.

Table 1: Inheritance pattern of propoxur resistance in Anopheles stephensi Liston.

| S. No | Genetic crosses | No. of ♀’s tested | No. of larvae tested** | Resistant (♂) | Total | Resistant (♀) | Total | Susceptible (♂) | Total | Susceptible (♀) | Total | χ² |
|-------|-----------------|--------------------|-------------------------|---------------|-------|---------------|-------|-----------------|-------|-----------------|-------|-----|
|       | Parental        |                    |                         |               |       |               |       |                 |       |                 |       |     |
| 1     | S♂ × S♀        | 10                 | 978                     | —             | —     | —             | —     | 481             | 473   | 954             | 100   | —   |
| 2     | R♂ × R♀        | 10                 | 835                     | 378           | 432   | 810           | 100   | —               | —     | —               | —     | —   |
|       | F1 Generation   |                    |                         |               |       |               |       |                 |       |                 |       |     |
| 3     | S♂ × R♀        | 10                 | 648                     | 142           | 194   | 336           | 51.85 | 118             | 194   | 312             | 48.15 | 0.44*|
| 4     | R♂ × S♀        | 10                 | 725                     | 178           | 213   | 391           | 53.93 | 177             | 157   | 334             | 46.07 | 2.24*|
|       | Back Crosses    |                    |                         |               |       |               |       |                 |       |                 |       |     |
| 5     | S♂ × F1♀ [Cross 3] | 10               | 834                     | 240           | 195   | 435           | 52.16 | 189             | 210   | 399             | 47.84 | 0.78*|
| 6     | S♀ × F1♂ [Cross 3] | 10               | 723                     | 184           | 153   | 337           | 46.61 | 223             | 163   | 386             | 53.39 | 1.66*|
| 7     | S♂ × F1♀ [Cross 4] | 10               | 651                     | 160           | 194   | 354           | 54.38 | 146             | 151   | 297             | 45.62 | 2.50*|
| 8     | S♀ × F1♂ [Cross 4] | 10               | 782                     | 213           | 168   | 381           | 48.72 | 218             | 183   | 401             | 51.28 | 0.26*|
|       | F2 Generation   |                    |                         |               |       |               |       |                 |       |                 |       |     |
| 9     | F1♂ × F1♀      | 10                 | 912                     | 282           | 212   | 494           | 54.17 | 187             | 231   | 418             | 45.83 | 3.17*|
| 10    | F1♂ × F1♀      | 10                 | 898                     | 223           | 241   | 464           | 51.67 | 229             | 205   | 434             | 48.33 | 0.50*|

larval response to propoxur, the inheritance being slightly on the recessive side. The results of bioassay for reciprocal cross progenies also showed no significant differences (P < .05) between F1 generations (cross 3 and cross 4) (Table 1). Resistance was clearly observed in both sexes, this equality suggested that sex linkage of resistance did not occur and that the genetic basis of the propoxur resistance was autosomal. When the F1 hybrids were backcrossed with the susceptible parents, their progeny showed 52.16%, 46.61%, 54.38%, and 48.72% resistance and 47.84%, 53.39%, 45.62%, and 51.28% susceptibility, respectively (cross 5, 6, 7, 8). Progeny of backcrosses showed close fit to the 1:1 ratio of resistant and susceptible. The crosses 9 and 10 of F2 progeny showed 54.17% and 51.67% resistance and 45.83% and 48.33% susceptibility, respectively.

The position of F1 LD-P lines relative to the susceptible and resistant parental strains indicates the degree of dominance of the resistance. Since, the F1 LD-P line (Figure 2) is significantly more resistant than the intermediate but less resistant than the resistant parental strain, it indicates
incompletely dominant nature of the gene. Alternatively, the degree of dominance of the resistance response to propoxur (D) based on the LC_{50} data was found to be 0.50, indicating that the resistance was expressed as an incompletely dominant trait.

In the dose-response bioassay of the F2 or backcross offspring, monogenic control of resistance is indicated when a clear plateau of mortality is observed across a range of doses. When F1 (RS) heterozygous individuals are backcrossed to the susceptible parent, monogenic resistance will result in a dose response curve with a clear plateau between LD-P lines of the SS and RS individuals at the 50% mortality level, since 50% of the backcross offspring are RS heterozygotes that are unaffected over a range of insecticidal doses (Figure 2), indicating only a single gene was operational in conferring the resistance towards propoxur.

If the resistance is polygenic, recombination and independent assortment of R and S alleles of different loci will occur in F1 individuals. Backcrossing these individuals to susceptible individuals will result in offspring that include a number of genotypes that vary in their level of resistance. The slope of the LD-P line resulting from insecticide bioassay of these backcross individuals depends on a number of factors, in most cases a clear plateau will not be observed. If a number of unlinked genes contribute to resistance, the shape of the LD-P line should decrease compared to the lines for the susceptible and F1 individuals, and few backcross individuals will exhibit the level of resistance observed in the F1 [21].

Present study thus reveals that a single gene is responsible for propoxur resistance in An. stephensi. Insecticide resistance in mosquitoes has been studied among many vectors and to various insecticides. Georghiou [22, 23] demonstrated the genetic basis of propoxur resistance in Culex pipiens quinquefasciatus as polygenic in nature. Mechanism of propoxur resistance induced by laboratory selection was also studied in Anopheles albimanus [24]. The genetic basis of several insecticides resistance has been studied in An. stephensi in our laboratory. These include Malathion [25]; Fenthion and Methyl parathion [26]; Deltamethrin [27]; Fenitrothion [28]; Cypermethrin [29]; DDT [30]; Chlorpyrifos [31]; Temephos [10]; Neem and Bifenthrin [32]; Alphamethrin [33]. In Cx. Quinquefasciatus, inheritance mode for synthetic pyrethroid insecticide Deltamethrin and Cyfluthrin were found to be near dominant and autosomal [34], and in Ae. aegypti the inheritance mode was found to incompletely dominant and autosomal for two synthetic pyrethroid, namely, Alphamethrin and Malathion [35].

Understanding the resistance inheritance is also important for predicting the continuing and effective use of a chemical for a particular pest control [1]. The degree of dominance of resistance alleles plays a significant role in the expression and distribution of the resistant gene. Since resistance controlled by a single gene develops rapidly compared to that of resistance controlled by two or more genes [36], the data generated from the study could lead to a better understanding of the rate of resistance development by use of the information on the inheritance mode of the resistant gene involved.

4. Conclusions

The genetic basis of propoxur resistance in An. stephensi clearly showed that the gene pr is autosomal, monofactorial, and incompletely dominant. The propoxur-resistant gene pr established in the present investigation has several applications in conducting basic and applied genetic research. It can be used as an excellent genetic marker in An. stephensi. The insecticide-resistance gene can be used in synthesizing multiple markers, preparation of linkage maps, molecular mapping, and so forth. Such integrated strains could be used in studying the linkage relationship, to prepare the linkage map, chromosome linkage correlation, and in applied research including synthesis of refractory strains as well as genetic sexing strains (as a conditional lethal) for preferential elimination of females in the early developmental stages.

![Figure 2: Dosage-mortality relationships of propoxur-resistant and propoxur-susceptible strains of An. stephensi.](image_url)
Insecticide-resistant gene which is an autosomal (located in 2 or 3 chromosome) could be transferred to the male-determining chromosome via radiation-induced male-linked translocation. When the larvae from such line are exposed to the discriminating dose of insecticide all the females are killed, as the females do not have the resistant gene, and all the males will survive since they carry the resistant gene. Genetic sexing males thus obtained could be subjected for mating competitive ability with laboratory-maintained males and field-collected males in the presence of field-collected females in a large cage population. If the males (genetic sexing) compete higher than the normal males, pilot release experiments could be conducted in a selected area. Thus, the genetic sexing males could be used in the genetic control programme of any insect species through the sterile insect technique (SIT). Such genetic sexing strains could be used in the genetic control programme of any insect species through the sterile insect technique (SIT). Such genetic sexing strains could be used in the genetic control programme of any insect species through the sterile insect technique (SIT). Such genetic sexing strains could be used in the genetic control programme of any insect species through the sterile insect technique (SIT).

The expression of certain enzymes that are involved in two different types of resistance mechanisms, namely, metabolite resistance (esterases, phosphatases, dehydrogenases, etc.) and target site resistance (acetylcholine esterases) are very specific in their expression to each one of the insecticide resistant strains and their expression varied within the different life stages. Similarly, cytogenetic studies carried out in polytene chromosomes from ovarian nurse cells of various insecticide resistant strains are characterized by the presence of specific heterozygous inversion (s) which are absent in other insecticide resistant strain(s) and in natural populations thus far studied [40].

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