MONITORING RESISTANCE GENE FREQUENCIES IN MALAYSIAN *CULEX QUINQUEFASCIATUS* SAY ADULTS USING RAPID NON-SPECIFIC ESTERASE ENZYME MICROASSAYS

HL Lee and T Tadano

Division of Medical Entomology, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia

Abstract. The ability to identify the occurrence of different resistance genotypes in field populations of mosquito is considered important for the purpose of optimising chemical control operations. The recent development of rapid microassays of enzymes responsible for resistance has provided a means for rapidly assessing the genetic background of target mosquito populations. This concept is the topic of investigation in this study. Non-specific esterase activity, which is responsible for the resistance to organophosphates in Malaysian *Culex quinquefasciatus* Say adults, was determined in 3 field populations from Kuala Lumpur City using rapid enzyme assay. The optical density results were used to estimate the genotypic frequencies of the populations. Subsequently, time-dependent changes in the various frequencies were determined. Such techniques allowed rapid assessment of resistance genotypes for decision-making and its possible use in insect control merits further investigation.

INTRODUCTION

Mosquito resistance to chemical insecticides widely used to control them is a major global problem today. Such resistance when widespread may adversely hamper vector control programs, rendering them highly ineffective as a tool for control. The detection and determination of insecticide resistance should therefore be incorporated as an important component in control programs. Towards this end, standard resistance test kits were produced by the World Health Organization (WHO, 1981). These tests, though are easy to use especially with the inclusion of diagnostic dosages, are often time-consuming, requiring a large number of mosquitos and limited number of insecticides or impregnated papers for testings. The recent development of rapid biochemical microassays of enzymes responsible for resistance have greatly facilitated detection of insecticide resistance. However, as most researchers in this important area are mainly concerned to assess the susceptibility status of mosquitos at a particular point of time, the changes in resistance gene frequency in field populations are often overlooked. Accurate determination of resistance gene frequency is useful in optimising outcome of vector control operations when, for example, fogging should be conducted at a time when the resistance gene frequency in the target population is low. In the past, the continuous monitoring of gene frequencies using the WHO test kit was often difficult, but the development of rapid biochemical microassays has provided a convenient tool for such studies. This paper reports for the first time an attempt to characterize changes in resistance gene frequencies in field populations of Malaysian *Culex quinquefasciatus* Say adults using such tests. Resistance to organophosphates in this mosquito was attributed to elevated levels of non-specific esterase (Lee, 1990; Lee *et al,* 1992).

MATERIALS AND METHODS

Adult mosquito collection

Adult *Cx. quinquefasciatus* collected from 3 localities in Kuala Lumpur, Malaysia were used for this study. These localities are Puchong, Taman Seputeh and Taman Segar in the vicinity of Kuala Lumpur City. Female mosquitos were collected weekly using bare-leg catch techniques between May and June 1993. Captured mosquitos were fed on mice blood and allowed to lay eggs in a bowl of water 4 days post-feeding. Egg rafts were hatched in a tray of tap water containing ground monkey pellets. Pupae were collected into a bowl and introduced into a cage. Adult female mosquitos that emerged (F1 generation) were used for the WHO bioassay test and microassay of

non-specific esterases. A malathion-selected strain (MAL_{F3}) of *Cx. quinquefasciatus* was also tested to ascertain its resistance status for comparative studies. This strain originated from Kuala Lumpur City and was selected by using 5% malathion paper for 3 generations.

Bioassay

Diagnostic tests on the resistance status of MAL_{F3} to malathion were conducted by exposing the adults to malathion as designed by the WHO test (1981). In this method, 25 blood-fed, less than 7 days old adult female mosquitos were exposed to papers impregnated with 5% malathion inside an exposure tube for 1 hour. The insecticide-impregnated paper was obtained from the WHO test kit. After the exposure time, the mosquitos were transferred into clean holding tubes and sugar solution in cotton pad was provided. Adult mortality was recorded after a 24 hour holding time and the results were pooled. Each bioassay experiment comprised 4 replicates and controls.

Microassay of non-specific esterase

A substrate solution was first prepared by mixing 0.5 ml α-naphthyl acetate in acetone (6 g/l) with 50 ml phosphate buffer (0.02M; pH 7.0). A coupling reagent consisting of 150 mg of Fast Blue B salt in 15 ml water and 35 ml aqueous sodium dodecyl sulphate was also prepared. Individual adults were similarly homogenized in 0.5 ml buffer using a glass rod and centrifuged. With a micropipette, 50 µl of the homogenate was transferred to a well in a microplate. Fifty µl of the substrate solution was then pipetted into each well and left for 60 second. The coupling reagent (50 µl) was then added. A deep purple color would develop which turned to blue after standing for 10 minutes. The reaction was stopped by the addition of 50 µl 10% acetic acid into each well. The intensity of the final color, indicative of esterase activity, was scanned by an immunoassay reader at 450 nm in order to determine the colour intensity quantitatively. Optical density readings were pooled and analysed by using computer programs. For each population, at least 80 mosquitos were assayed for esterase activity at different time intervals.

RESULTS AND DISCUSSION

The diagnostic test of the laboratory-selected strain MAL_{F3} indicated a mortality of 43%. It is assumed that the field populations have been selected similarly and resistance was dependent on a single locus with the resistant and susceptible alleles. Hence the frequency of susceptible gene (p) = 0.24 and frequency of resistance gene (q) = 0.76. The mortality would also indicate that 43% of the optical density readings were indicative of susceptibility to malathion. Based on these findings, examination of the OD values showed that susceptible mosquito would exhibit values of < 0.25. Thus it is possible to estimate the occurrence of each genotype from the OD values. Assuming Hardy-Weinburg equilibrium, the genotypic frequencies of susceptible homozygotes, heterozygotes and resistance homozygotes were 0.24^2 (=0.058), $2 \times 0.24 \times 0.76$ (=0.36) and 0.76^2 (=0.58), respectively. Variations in the genotypic frequencies of *Cx. quinquefasciatus* adults from different localitis are shown in Figs 1-3. As expected, changes in gene frequencies were detected in all populations under study. In the field populations, the gene frequencies varied and appeared to be time-dependent.

Presently, the management of insecticide resistance depends on several preventive and remedial measures such as rotational use of chemicals, use of synergists, etc. However, these measures though effective at times, may be difficult to implement. Therefore judicious and careful use of an insecticide would be more cost-effective and less stressful on the environment. Such a method, however if dependent on knowledge of gene frequencies of the target populations. Variations in resistance and susceptible gene frequencies, of course, can be determined by using the WHO diagnostic kits. But several constraints of the kit may hinder continuous long-

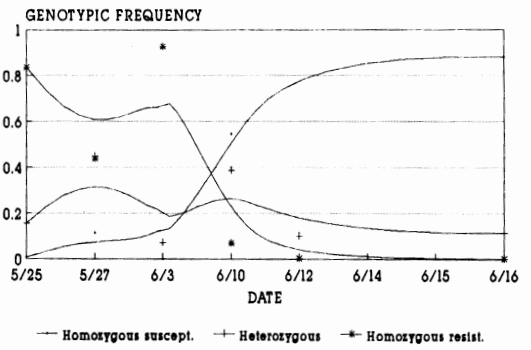

Fig 1—Time-dependent variation in genotypic frequencies of *Culex quinquefasciatus* (Puchong).

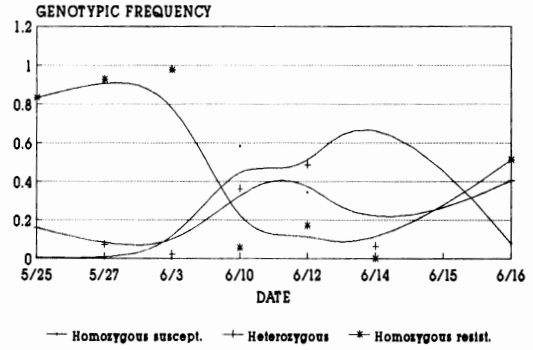

Fig 2—Time-dependent variation in genotypic frequencies of *Culex quinquefasciatus* (Tmn Seputeh).

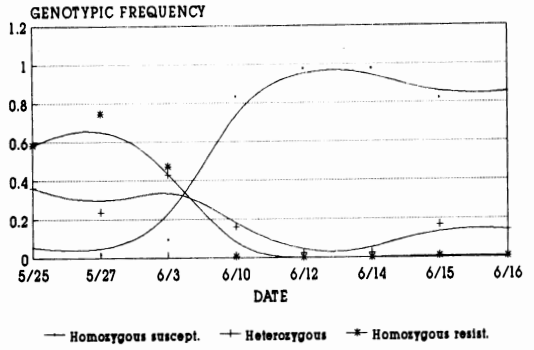

Fig 3—Time-dependent variation in genotypic frequencies of *Cules quinquefasciatus* (Tmn Segar).

term monitoring of variations in gene frequencies. With the advent of rapid resistance enzyme microassay, these changes can be rapidly determined and such knowledge can be immediately applied in control operations to effect a favorable outcome, for obviously insecticide should not be applied at a time when the susceptible gene frequency is low in order to conserve the susceptible homozygotes in the population (Denholm and Rowland, 1992). In the presence of high resistance frequency, probably a new and effective chemical can be used or other non-chemical control agents such as microbial control agents (*Bacillus thuringiensis* H-14 and *B. sphaericus*) can be considered. Hopefully with these manipulations, the resistant population can be eliminated or further reduced.

As the history of insecticide application (*eg* fogging) in the 3 study areas was not available, the effect of chemical intervention on changes in gene frequencies could not be ascertained. Similarly, the immigration and emigration pattern is not known. These factors may influence the changes in gene frequencies of these populations. However, though this concept of "genetic management" of insecticide resistance by employing rapid enzyme microassays has yet to be tested in the field, its applicability and suitability in enhancing control operations merits further investigation.

ACKNOWLEDGEMENTS

The authors wish to thank Dato' Dr M Jegathesan, Director, Institute for Medical Research, Kuala Lumpur for permission to publish. This investigation receives partial financial support from a Malaysian Government Research and Development Grant ID No. IMR-92-8.

REFERENCES

Denholm I, Rowland MW. Tactics for managing pesticide resistance in arthropods : Theory and Practice. *Ann Rev Entomol* 1992; 37: 91-112.

Lee HL. A rapid and simple biochemical method for the detection of insecticide resistance due to elevated esterase activity in *Culex quinquefasciatus. Trop Biomed* 1990; 7 : 21-8.

Lee HL, Abiombola O, Inder Singh K. Determination of insecticide susceptibility enzyme microassays. *Southeast Asian J Trop Med Public Health* 1992; 23 : 458-63.

World Health Organisation. Instructions for determining the susceptibility or resistance of mosquito adults to insecticides. WHO/VBC/81.805. 1981.