Biochemical detection of pyrethroid resistance mechanisms in *Anopheles minimus* in Thailand

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ABSTRACT: Enzyme-based metabolic mechanisms of insecticide resistance were investigated, comparing a deltamethrin-susceptible parent stock and resistant colonies of *Anopheles minimus* species A using biochemical assays. The control parent colony was determined susceptible to the diagnostic lethal concentration of deltamethrin (0.05%), whereas the 6 resistant test populations at selected 4, 8, 12, 14, 16, and 18 filial generations (F_4, F_8, F_{12}, F_{14}, F_{16}, and F_{18}) demonstrated varying levels of tolerance/resistance to deltamethrin. Expression of levels of non-specific esterases, monooxygenases, and glutathione S-transferases (GSTs) were measured. Results indicated that monooxygenase activity was consistently elevated in resistant-selected test populations compared to the parent colony and increased as resistance intensified from F_8 to F_{18}. There was a 5-fold increase in monooxygenase in the F_{18} generation compared to the parental stock. Fluctuations in alpha and beta-esterase activity, measured by hydrolysis of alpha and beta-naphthylpropionate, provided no conclusive evidence of an association with pyrethroid resistance in this mosquito species. GSTs were not elevated in the 6 resistant test populations. Based on our results, it appears likely that the development of physiological resistance to deltamethrin in laboratory, resistant-selected generations of *An. minimus* is primarily associated with increased detoxification by over-expression of monooxygenases. The oxidases are the major contributors to pyrethroid resistance and the importance of *kdr* has yet to be convincingly determined. This finding represents the first report from Thailand of this metabolic mechanism of resistance in anophelines. ***Journal of Vector Ecology* 28(1): 108-116. 2003.**

Keyword Index: Pyrethroids, deltamethrin, resistance, esterases, monooxygenases, glutathione s-transferases, *Anopheles minimus*, Thailand.

INTRODUCTION

Over half of the world's population resides in malarial areas, resulting in an estimated 2 to 3 million deaths annually from the disease (WHO 1996). The burden of malaria is increasing, in part, because of drug and insecticide resistance and complex social and rapid environmental changes that have intensified in the last several decades (Greenwood and Mutabingwa 2002), as well as a general breakdown of organized effective malaria control activities. In general, most countries in Southeast Asia where malaria is endemic are experiencing increased malaria problems resulting from sociological and ecological changes stemming from poorly controlled population movement and extensive exploitation of natural environments. In Thailand, malaria remains one of the most important infectious diseases affecting rural populations with over 100,000 cases reported annually during each of the last 10 years (Chareonviriyaphap et al. 2000). Recent medical surveillance indicates that malaria has expanded in the country and continues to be a serious concern along the undeveloped frontier borders with eastern Myanmar and western Cambodia (Annual Malaria Reports 1995-2001).

The prevention of malaria transmission in Thailand has relied mainly on accurate diagnosis, prompt effective