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CYTOCHROME P450 GENES: MOLECULAR CLONING AND OVEREXPRESSION IN A PYRETHROID-RESISTANT STRAIN OF *ANOPHELES MINIMUS* MOSQUITO

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ABSTRACT. We previously determined that physiological resistance in a laboratory-selected pyrethroid-resistant *Anopheles minimus* species A Theobald mosquito is associated with increased detoxification via a P450-mediated mechanism. A CYP6 gene, *CYP6AA3,* was subsequently cloned and found overexpressed in 2 resistant mosquito generations (F_{13} and F_{19}). We report herein the cloning of *CYP6P7* and *CYP6P8* genes with full coding sequences from the same *An. minimus* mosquito colony strain. *CYP6P7* and *CYP6P8* encode proteins, each with 509 amino acids. *CYP6P7* had the closest (81%) amino acid identity with *Anopheles gambiae CYP6P2. CYP6P8* genes had 79% identity with *An. gambiae CYP6P1.* Using semiquantitative reverse transcription-polymerase chain reaction analysis, the mRNA expression level of *CYP6P7* presented ~2- and 4-fold increases in F_{19} and F_{25} deltamethrin-resistant populations, respectively, compared with the parent susceptible strain. *CYP6P8* mRNA expression levels were not significantly different between the 3 filial generations. The overexpression of *CYP6AA3* mRNA was greater than that of *CYP6P7* in F_{19} and F_{25} resistant populations. The relative increase of both *CYP6AA3* and *CYP6P7* mRNA was correlated with increased resistance to deltamethrin in *An. minimus.*

KEY WORDS Cytochrome P450 monooxygenases, deltamethrin, DDT, *Anopheles minimus,* Thailand

INTRODUCTION

Cytochrome P450 monooxygenases (P450, CYP) represent a superfamily of enzymes that can metabolize a diverse range of complex endogenous and exogenous compounds, including many insecticides. In mosquitoes, multiple CYP family 6 (CYP6) and family 4 (CYP4) genes have also been isolated. For instance, multiple CYP6 genes and CYP4 genes have been reported in *Anopheles gambiae* (Ranson et al. 2002b, Nikou et al. 2003), and 17 CYP4 genes have been identified in *Anopheles albimanus* (Scott et al. 1994).

Insect CYPs are important enzymes that appear to help regulate insect growth and development, tolerance to plant toxins, and insecticide resistance (Hodgson and Kulkarni 1983, Scott et al. 1998, Feyereisen 1999). With the wide diversity of known CYPs, the identification of specific forms associated with insecticide resistance can often be difficult. In insects, monooxygenase-mediated pyrethroid resistance is associated with overexpression of one or more cytochrome P450 genes in the CYP families 4, 6, and 12 (Feyereisen 1999). For example, in pyrethroid-resistant *Musca domestica,* increased *CYP6D1* transcript and protein are the underlying cause of pyrethroid resistance, and there is no evidence for gene duplication (Tomita et al. 1995, Liu and Scott 1998, Kasai and Scott 2000). Overexpression of other CYPs associated with insecticide resistance have been identified, including *Cyp6a8, Cyp12d1, Cyp6g1* in dichlorodiphenyltrichloroethane (DDT)-selected strains of *Drosophila melanogaster* (Brandt et al. 2002, Le Goff et al. 2003). *CYP6Z1* gene was mapped within the quantitative trait loci associated with resistance to permethrin in *An. gambiae* (Ranson et al. 2002a, Nikou et al. 2003).

Daborn et al. (2001, 2002) mapped the DDT-resistant (DDT-R) locus in *D. melanogaster* and identified the *Cyp6g1* gene within this region. This gene was shown to be overexpressed in 28 DDT-resistant *D. melanogaster* strains (Daborn et al. 2002), thus strongly implicating it in the enhanced detoxification of DDT and a wide range of insecticide classes, including organophosphates, neonicotinoids, and benzoylphenylureas. The *Cyp12d1* has shown cross-resistance to DDT, neonicotinoids, and malathion, while *Cyp6g8* provides cross-resistance to both DDT and neonicotinoids (Le Goff et al. 2003).

Anopheles minimus is an important malaria vector and species complex in the Mekong region of Southeast Asia, including Thailand, Laos, Cambodia, and Vietnam (Subbarao 1998). Pyrethroid resistance was recently discovered in a population of *An. minimus* from northern Thailand (Annual Malaria Report 2000), making it imperative that this mosquito species receive more attention. We previously established a deltamethrin-resistant *An. minimus* colony strain by laboratory-based selection pressure against deltamethrin (Chareonviriyaphap et al. 2002). Within 13 sequentially selected generations, this strain conferred resistance to deltamethrin with a >25-fold increase in the 50% lethal dose compared with the parent colony. Moreover, during the course of deltamethrin selection, the resistant colony also presented cross-resistance to DDT (Chareonviriyaphap et al. 2002). Biochem-

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