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Sequence characterization of cytochrome P450 CYP6P9 in pyrethroid resistant and susceptible *Anopheles funestus* (Diptera: Culicidae)

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ABSTRACT. *Anopheles funestus*, one of the main African malaria vectors, caused a major malaria outbreak in South Africa during 1999/2000, even though South Africa had an effective vector control program in place. The outbreak was due to pyrethroid resistant *An. funestus* invading KwaZulu/Natal. Increased activity of cytochrome P450 (monooxygenase) was responsible for the pyrethroid resistance in this species. A monooxygenase gene, CYP6P9, was highly overexpressed in the pyrethroid-resistant strain compared with a susceptible strain. Characterization of this gene as well as the redox partners involved in the catalytic cycle of P450s was investigated. The full length of the CYP6P9 sequence was isolated, sequenced and compared between the pyrethroid-resistant and -susceptible strains. Sequence identity between the two strains was 99.3%; the sequence differences were mainly outside of the conserved

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regions. The functional significance is still unknown, but it is feasible that these variations are associated with differences in insecticide metabolism. A second CYP6 gene (CYP6P13) was also isolated; it shared close similarities with CYP6P9. The putative redox partners, cytochrome b_5 (cyt b_5) and NADPH-cytochrome P450 reductase (CPR), were isolated from *An. funestus* (resistant strain) and showed high levels of sequence identity to other insect cyt b_5 and CPRs. Isolation of the coding sequences CYP6P9 and its cognate redox partners enables expression of functional recombinant protein for biochemical and structural analysis.

Key words: *Anopheles funestus*; CYP6P9; CYP6P13; Cytochrome b_5; NADPH-cytochrome P450 reductase

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