

Sodium Channel Mutations Associated with Pyrethroid Resistance in *Anopheles sinensis* Wiedemann (Diptera: culicidae) and Resistance Detection by PASA-based Genotyping Method

Hyunwoo Kim, Won-Ja Lee¹, E-Hyun Shin¹ and Si Hyeock Lee*

Entomology Program, School of Agricultural Biotechnology,

Seoul National University, Seoul, Korea. *E-mail: shlee22@snu.ac.kr

¹Department of Medical Zoology, National Institute of Health, Seoul, Korea.

Anopheles sinensis is a major vector mosquito of malaria, which is the most important vector-borne disease in Korea. Intensive use of pyrethroids for the control of agricultural and medical arthropod pests was quickly followed by development of resistance in a variety of arthropod species including *An. sinensis*. Target site insensitivity mechanism, named knockdown resistance (*kdr*), is one of the major mechanisms of pyrethroid resistance and caused by highly conserved mutations of voltage-sensitive sodium channel gene such as Leu1014Phe mutation. To determine the presence or absence of the mutations associated with *kdr* in *An. sinensis*, we PCR-amplified and sequenced IIS5-6 fragment of the sodium channel gene that encompasses the most widely known mutation sites from a permethrin-resistant field population. Sequencing results revealed that three genotypes [TTG(Leu), TTT(Phe), and TGT(Cys)] were mixedly present at the same position of the house fly Leu1014Phe mutation, indicating that the population is heterogeneous in terms of *kdr* trait. In addition, the susceptible TTG-genotype was different from the most common CTT-genotype. To determine the correlation between resistance phenotype and the three sodium channel genotypes, permethrin resistance levels of four regional populations of *An. sinensis* were assessed by immersion method and compared with each population's sodium channel sequence. The relatively susceptible Ansan (LC50=1.77) and Paju (LC50=1.29) strains exhibited high frequency of TTG (Leu) genotype as expected. In contrast, moderately (Junju, LC50=14.9) and highly (Gurye, LC50=51.3) resistant strains showed high frequencies of TTT (Phe) and TTT(Phe)/TGT(Cys), respectively, implying that both TTT and TGT genotypes are likely associated with pyrethroid resistance in *An. sinensis*. The Leu to Cys-type mutation was first found as a putative *kdr*-related mutation but its function remains to be elucidated. To facilitate the detection of resistance-associated sodium channel mutations in *An. sinensis*, we have developed a PASA (PCR amplification of specific allele)-based genotyping protocol and discussed its applicability in a large scale resistance monitoring and management.