Thesis Title Identification of *Anopheles dirus* Isomorphic Species by Chemiluminescent DNA Probes.

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ABSTRACT

Anopheles dirus is a major vector of malaria in Thailand and Southeast Asia. It was shown by cytogenetic and morphological studies to be a complex of at least six isomorphic species, provisionally designated species A, B, C, D, E and F on the Southeast Asian mainland. The five species found in Thailand (A-D, and F) exhibit distinct geographic distributions, seasonal variation in relative abundance and different nocturnal biting cycles. These biological characteristics of this group of mosquitoes may have implication for understanding the epidemiology of malaria in Southeast Asia.

Identification of wild-caught female depends on rearing families from them and examination of larval chromosomes which is complicated, slow and laborious. Another identification method is using isozyme analysis which requires fresh or frozen stored specimens that is impractical for epidemiological studies.

Species specific fragments, pMU-A40.1#5, pMU-B5, pMU-C19.2 and pMU-D9 which had been constructed since 1986, could be used as DNA probes for identification of species A, B, C and D respectively. But the disadvantages of

the commonly used radio-isotopic DNA detection technique leads to its impractical use in the field conditions.

Chemiluminescent DNA detection technique is another option for identification of vector sibling species by nonradioactive means. In this study, DNA probes were amplified by PCR, and then directly labeled with hoseradish peroxidase (HRP). The signal generating technique was done by adding detection reagent, luminol, enhancer and H_2O_2 which are substrates for HRP. However, the normally squash-blot technique could not be used for this detection system because substances in mosquito tissue generated strong chemiluminescent signal with detection reagent. The signal from tissue could be eliminated by a phenol-chloroform extraction step before hybridization process.

In addition to direct labeling with HRP, the mosquito probes were labeled with fluorescein using both *Taq* DNA polymerase in PCR and terminal deoxynucleotidyl transferase. The fluorescein moieties were then detected by incubation with anti-fluorescein antibody-HRP conjugates. Both fluorescein labeling techniques have sensitivity comparable to direct labeling techniques; 1-5 ng purified DNA of *An. dirus* could be detected aftrer an exposure time of 30 minutes.

Field-caught specimens were collected from 4 localities in Thailand and identified. The results of identification of 182 mosquitoes were corresponding to cytogenetic and isozyme analysis studies. Moreover, 75 mosquitoes which were not identified by non DNA methods, were clearly detected by this chemiluminescent technique.