

viral replicon particles, which express the green fluorescent protein gene in cells, indicate the operation of tissue conduits, possibly involving tracheae and visceral muscles, that facilitate virus movement through the basal lamina. Ultrastructural studies of the midgut reveal evidence for possible complete penetration of the basal lamina by tracheal cells and regions of modified basal lamina associated with visceral muscle. The modified basal lamina closely resembles proventricular matrix material known to allow virus passage.

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FIELD VALIDATION OF *Aedes aegypti* (DIPTERA: CULICIDAE) AGE ESTIMATION BY ANALYSIS OF CUTICULAR HYDROCARBONS

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In previous studies, we developed linear regression models to age-grade female *Aedes aegypti* L. reared and maintained under controlled laboratory conditions. The models were based on temporal differences between two cuticular hydrocarbons, pentacosane (C₂₅H₅₂) and nonacosane (C₂₉H₆₀), which were extracted from *Ae. aegypti* legs and analyzed by gas-liquid chromatography. These initial models predicted adult female age up to 165 DD (12-15 calendar d at 28°C). The age of older mosquitoes, however, could not be accurately predicted. In this study, our original regression models were tested using age data obtained from mosquitoes maintained in a field laboratory and those that were marked, released, and recaptured in northwestern Thailand. Our field data led to the development of two new regression models: one for the cool-dry season (February-March) and one for the rainy season (July-August). Both models resulted in better estimates of age than the original model and thus improved our ability to predict the age of *Ae. aegypti* to 15 calendar d. Females older than 15 d can be identified as such, but their exact age cannot yet be estimated. The new models will be useful for epidemiological studies and evaluating the impact of *Ae. aegypti* control interventions for disease prevention.

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GEOGRAPHIC INFORMATION SYSTEM AS EPIDEMIOLOGICAL TOOL IN SURVEILLANCE OF DENGUE VIRUS-INFECTED Aedes MOSQUITOES

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A Geographic Information System (GIS) was used as analysis tool to study the spatial distribution of dengue virus-infected *Aedes* mosquitos in Thailand. Global Positioning System (GPS) instruments were used to map villages involved in dengue epidemiological studies in Ratchaburi Province, Thailand. Differentially processed GPS data, with a spatial resolution of approximately 1 meter, were incorporated into a GIS for analysis and mapping. Databases associated with a village GIS included village number, *Aedes aegypti* populations, and test results. Epidemiological surveillance for dengue infection through the detection of the dengue

virus type(s) infecting *Aedes* mosquitos during epidemic periods constitutes a reliable sentinel system for dengue outbreaks. Various techniques were applied including: enzyme linked immunosorbent assay (ELISA), indirect immunofluorescent assay (IFA), and reverse transcriptase - polymerase chain reaction (RT-PCR) assay for the virologic surveillance of the type-specific detection of dengue viruses in artificially infected and in field-caught adult *Aedes* mosquitos. In laboratory experiments, all assays showed sufficient sensitivity to detect one virus infected mosquito and the rapid RT-PCR clearly showed serotype-specificity with very high detection sensitivity. In the field study conducted from April to September 2000, female adult *Aedes* mosquitos were collected from selected dengue-sensitive areas in Chom Bung district, Ratchaburi Province and assayed by ELISA, IFA and RT-PCR with 18.3% (44/240), 28.98% (20/69) and 15% (3/20) positive for dengue virus, respectively. Geographic distribution of the virus-infected *Aedes* mosquitos and household locations were demonstrated by the GPS and the GIS. The development of disease mapping data coupled with RT-PCR laboratory-based surveillance of dengue virus infection can successfully serve as epidemiologic tools in an early warning system for dengue hemorrhagic fever (DHF) epidemics.

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GEOGRAPHICAL DISTRIBUTION OF *ANOPHELES MINIMUS* SPECIES A AND C IN WESTERN THAILAND

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Elucidating vector distribution based on accurate species identification is important in understanding the nature of species complex in order to achieve target in vector control. Morphologically *Anopheles minimus* s.l. is difficult to be distinguished from both its species complex and its closely related species. A Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique developed for species identification was applied in this study in comparison with morphological identification. Both methods were used to determine the distribution of *An. minimus* species A and C. The investigation on the breeding habitats was performed in the malarious area of western Thailand. Larval samples of mosquitoes collected from 25 bodies of water along stream margin from five districts (Sangklaburi, Thongphaphum, Sisawat, Muang and Saiyok) of Kanchanaburi Province were reared into adult for species identification. Adult samples of the species complex were also collected from human and cow baits in the vicinity of their larval breeding habitats. Morphologically identified individual mosquito was subjected to PCR-RFLP using primers corresponding to internal transcribed spacer 2 (ITS2) ribonuclear DNA (rDNA). The result shows that species C is predominant only within the distance of 30-40 kilometers in Saiyok, whereas species A predominates in the remaining four districts. Differences of species distribution of *An. minimus* in these areas will be analyzed and discussed.

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