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学位論文要旨
Dissertation Summary

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論文名：マニラ首都圏におけるデング熱病動態とその制御に関する生態学的・生物学的研究

(Dissertation Title) **ECOLOGICAL AND BIOLOGICAL STUDIES OF DENGUE DISEASE DYNAMICS AND ITS CONTROL IN METROPOLITAN MANILA, PHILIPPINES**

Dengue fever is considered as a rapidly emerging arthropod-borne viral disease all over the world especially in the Philippines. The dynamics of the disease are affected through a comprehensive combination of factors that can be used appropriately in vector control measures. This dissertation assembles studies that focused on the (a) biological, (b) ecological aspect of dengue disease dynamics and (c) a potential vector control approach in Metropolitan Manila Philippines.

Dengue disease is transmitted by two vector mosquitoes namely; *Aedes aegypti* and *Ae. albopictus* wherein the former is considered to be the primary mosquito vector in Metropolitan Manila. Thus, this dissertation aims to investigate the biological aspects of *Ae. aegypti* by examining and describing its population structure and infer its dispersal in a fine-scale area. The study utilized two approaches: morphological (geometric morphometrics) and genetics (microsatellites). First, wings of 312 *Ae. Aegypti* adult individuals were examined and assessed for its wing geometry. Results of the analysis revealed population heterogeneity and structuring in *Ae. aegypti* populations for both sexes using Principal Component and Canonical Variate Analyses respectively. Isolation-by-distance and spatial autocorrelation detected significant spatial structure in male *Ae. aegypti* populations while female *Ae. aegypti* population structures are homogenized throughout the geographical area. From this approach, it can be inferred that dispersal of this mosquito vector is more widespread in females rather than in males, thus encouraging disease transmission. With this in mind, the use of genetics is applied in the study for a more in depth insight and understanding towards the genetic diversity, genetic structure and gene flow pattern of *Ae. aegypti.* 11 microsatellite markers were genotyped from 526 adult mosquitoes in 21 populations of Metropolitan Manila. Genetic diversity and diffentiation analyses revealed that all populations are nearly homogenous, suggesting active exchanges and sharing of allelles. However, a substantial number of genetic structuring (K=5) were observed, signifying genetic clustering in short distances (< 1km).

Ecological factors such as urbanization and climate greatly impact the dynamics of dengue disease. Changes from these factors are assumed to have an ecological effect towards the vector biology and ecology of *Ae. aegypti.* For this reason, many studies are investigating the role of these factors towards the temporal and spatial distribution or pattern of dengue. In this study, the main objective is to associate, model or predict temporal and spatial dengue incidence in Metropolitan Manila using selected ecological factors where it emphasizes the utilization of Machine Learning Methods (MLMs). First, the study compared MLMs (Random forest and Gradient Boosting) and conventional modelling techniques (General Additive modelling and Seasonal Autoregressive Integrated Moving Average) in predicting the temporal pattern of dengue incidence in Metropolitan Manila from 2009-2013 using metereological data such as flood, precipitation, temperature, southern oscillation index, relative humidity, wind speed and direction. The results revealed that the Random forest model showed the best predictive accuracy among all techniques. Moreover, relative humidity was shown to be the most important meteorological factor along with rainfall and temperature which can influence this temporal pattern. On the other hand, a comparative approach was also utilized in associating and modelling land use and flood factors to the spatial distribution of Dengue in Metropolitan Manila. The cumulative dengue incidence and the proportion of each land use type and flood were calculated in 660 villages/areas of the study area. Three (3) MLMs (Recursive Partitioning, Random Forest and Gradient Boosting) were utilized and compared to Generalized Linear Model (GLM). Results revealed that the Random forest model performed as the best MLM method. In addition, it inferred eight important environmental factors that are highly associated with the cumulative Dengue incidence of Metropolitan Manila which includes residential, non-residential areas and flood-prone areas. Overall, the results in both studies showcased the exceptional performance of MLMs as compared to conventional statistical techniques in associating, modelling or predicting dengue incidence from selected environmental factors.

Efforts to control and stop the transmission of dengue disease are focused to the primary mosquito vector, *Ae. aegypti*. Conventional approaches such as the use of insecticides proved to be ineffective due to the continuing rise of dengue cases. Thus, a novel and newer approach is being tested that show promising outcomes in vector and disease control. This approach is the utilization of intracellular bacterial endosymbiont, *Wolbachia*, where it alters the host's reproduction and limits the ability to transmit pathogens such as Dengue. There is currently a limited amount of studies in the Philippines that have explored and identified the diversity of *Wolbachia*, especially to medically important mosquitoes. In this study, the aim was to determine the presence and diversity of *Wolbachia* in two mosquito species, *Culex quinquefasciatus* and *Ae. aegypti*, predominately found in Metropolitan Manila. Adult *Cx. quinquefasciatus* mosquito populations (n=51) were subjected to *wsp* gene amplification assay. Five selected *wsp*-positive samples were then sequenced and further analyzed to infer their phylogenetic relationship with known *Wolbachia* strains. Results revealed that 59% of the *Cx. quinquefasciatus* mosquito populations were *wsp*-positive and observed to be widely distributed in the study area. Phylogenetic analysis inferred that sample sequences were *Wolbachia pipientis* isolated from known hosts, *Cx. pipiens* and *Cx. quinquefasciatus* belonging to supergroup B clade. On the hand, past literature had claimed that *Wolbachia* is not naturally found in *Ae. aegypti*. However, there are have been reports that recently reveals the presence of this endosymbiont in this mosquito vector. In the study, a total of 672 *Ae. aegpyti* adult mosquito samples were screened for *Wolbachia* infection using Wolbachia-specific 16S rDNA and the its surface protein (wsp) makers under optimized PCR conditions. Notably, 113 (16.8%) and 89 (13.2%) individual mosquito samples were determined to be Wolbachia infected using the *wsp* and 16S rDNA markers, respectively. The *Ae. aegypti wsp* sample sequences were similar or identical to five known Wolbachia strains belonging to supergroups A or B while majority of 16S rDNA sample sequences were similar to strains belonging to supergroup B. Overall, our findings illustrate that *Wolbachia* is present in Metropolitan Manila due to the observed prevalence in both mosquito species. However, the infection status in *Ae. aegypti* is an exceptional observation because it points out that the endosymbiont may appear common than previously recognized.