

(第3号様式)(Form No. 3)

学位論文要旨
Dissertation Summary

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論文名: 節足動物の集団遺伝学的アプローチとマイクロバイーム

(Dissertation Title) POPULATION GENETIC APPROACH IN ARTHROPOD VECTORS AND ITS MICROBIOME

Vector-borne diseases (VBDs) are a complex interaction of the vector-host-pathogen in an environment. VBDs are transmitted by arthropod vectors such as mosquitoes and ticks that parasitize humans and animals. The most frequently occurring arthropod vectors are mosquitoes and ticks, the main vectors of the top 10 most common VBDs distributed worldwide.

In this study, we focused on two top VBDs: mosquito-borne diseases such as dengue and tick-borne diseases such as Japanese spotted fever. Mosquito borne-diseases such as dengue fever caused by the bite of female *Aedes albopictus* or *Aedes aegypti* and the tick-borne diseases commonly caused by Ixodid ticks *Ixodes ovatus* and *Haemaphysalis flava* are a public health concern in endemic countries. The main goal of this study is to understand how the interactions between the vector-host-pathogen in an environment can influence disease transmission. Due to the low quality and quantity of the *Aedes sp.* RNA, the pathogen detection of dengue virus were not performed. This dissertation focused on the (a) population genetic structure of (Chapter I) dengue vector *Ae. aegypti* as influenced by the environment (e.g roads) and (Chapter II) Ixodid ticks *I. ovatus* and *H. flava* and its relationship with the host mobility and the vector's (b) microbiome, (Chapter III) *Wolbachia* in *Ae. albopictus* and *Ae. aegypti* that can be used as a potential dengue vector control strategy, and the (Chapter IV) ticks' pathogen *Rickettsia* causing tick-borne disease. Population genetic studies on these arthropod vectors can provide information about the basic biology of the arthropod vectors through the estimation of their movement and their potential to spread the pathogens. Meanwhile, studies on the vector microbiome are also essential in understanding the biology of the pathogen causing

disease and discovering potential biocontrol agents against VBDs.

Dengue is transmitted mainly by *Aedes aegypti* and is endemic in tropical countries such as the Philippines. The movement of the arthropod vector, *Ae. aegypti* can be influenced by the environment, such as human-made structures (e.g., roads). The population genetic structure of *Ae. aegypti* can be affected by roads that can act as a barrier to mosquito movement. Thus, in this dissertation, the genetic structure and gene flow of the dengue vector, *Ae. aegypti* as influenced by a primary road in Manila, the Philippines, was investigated. Mosquitoes were collected along a 2000-meter-long stretch and 24-meters-wide in a very-fine spatial scale of España Boulevard (EB road). Since roads can act as a barrier to mosquito movement, the *Ae. aegypti* populations separated by EB road will differ due to the limited gene flow. In this dissertation, a total of 376 adults and larval *Ae. aegypti* collected during the rainy season from June to September 2017 in 21 sites across the North and South of EB road were analyzed. A total of 211 and 165 individuals comprised the North (N1-N11) and South (S1-S10), respectively. The AMOVA F_{ST} (0.0321 at $p < 0.05$) revealed significant genetic differentiation across the North and South of EB roads. Our constructed UPGMA dendrogram displayed four genetic groups that revealed the clear separation across the North and South sites of the EB road. The Bayesian cluster analysis revealed four genetic clusters at $K=4$, indicating that each mosquito has no distinct genetic cluster showing genetic admixture. Overall, our results suggest that human-made structures such as roads are potential barriers to mosquito movement, thus limiting its gene flow across the road. We can use this information to implement an effective dengue mosquito control program on a very fine-spatial scale.

Tick-borne diseases are a public health concern in Japan, commonly caused by arthropod vectors such as *Ixodes ovatus* and *Haemaphysalis flava* ticks, the most frequently occurring ticks in Japan. For this reason, in this dissertation the population genetic structure and genetic divergence of two Ixodid ticks, *I. ovatus* and *H. flava* as influenced by the ticks' host mobility was investigated. It can be expected that there will be contrasting genetic structures between *I. ovatus* and *H. flava* because of the low mobility of small rodent hosts of immature *I. ovatus*. In contrast, immature *H. flava* has highly mobile avian-mediated dispersal. We analyzed 307 adult *I. ovatus* and 220 *H. flava* collected from 17 locations across Niigata Prefecture, Japan. We found significant genetic variation among populations from the pairwise F_{ST} and analysis of molecular variance (AMOVA) analyses using the *I. ovatus* *cox1* sequences. For *H. flava*, both the *cox1* and the 16S rRNA markers revealed non-significant genetic variation among sites. The haplotype network and Bayesian tree from the *cox1* *I. ovatus* revealed three genetic groups. Most haplotypes from group 2 were from low altitudinal sites. The results also revealed cryptic species and reference sequences from China were added to the phylogenetic analysis. Overall, the results support the hypothesis and suggest that the host mobility of the ticks during the immature stage influences its genetic structure. This information can be utilized to understand better the interaction between the ticks and their host in the field, giving insights into the transmission of tick-borne diseases and designing effective tick control program.

Vector-borne disease control programs usually focus on eradicating or controlling the arthropod vector. In dengue-endemic countries such as the Philippines, they utilize traditional methods such as fogging and insecticides to control the dengue vector, *Ae. aegypti*. However, another novel approach is using biocontrol agents for mosquito control, such as *Wolbachia*. It is a bacterial endosymbiont of arthropods such as *Ae. albopictus* and

was recently detected in *Ae. aegypti*. The use of *Wolbachia* can alter the host's reproduction, thus limiting its ability to transmit the dengue virus. In this study, we aim to provide additional support for the presence of *Wolbachia* in field-collected *Ae. albopictus* and *Ae. aegypti* by detection using *Wolbachia*-specific markers, *wsp*, and 16S. A total of 12 adult *Ae. albopictus* and 359 *Ae. aegypti* were collected and were also used in the previous population genetic study mentioned. Larval *Ae. aegypti* (n=509) from 17 water-holding containers from 11 households were also analyzed for the presence of *Wolbachia*. Using these samples, polymerase chain reaction (PCR) using the *wsp* and 16S markers was analyzed, and after PCR amplification, *Wolbachia* positive samples were sequenced. The results revealed three out of the 359 (0.84%) adult *Ae. aegypti* and 12 out of 12 (100%) adult *Ae. albopictus* as *Wolbachia* positive. Six *Wolbachia*-positive *Ae. albopictus* were found using the *wsp* marker. On the other hand, a total of three *Ae. aegypti* and 10 *Ae. albopictus* were *Wolbachia* positive using the 16S marker. All larvae were *Wolbachia* negative. In summary, results showed that *Wolbachia* supergroups A and B are currently infecting the natural populations of *Ae. albopictus* and *Ae. aegypti* in Manila, Philippines

Tick-borne diseases such as Japanese spotted fever caused by *Rickettsia* sp. pathogen are reported in Niigata Prefecture, Japan. The Ixodid ticks, *Ixodes ovatus*, and *Haemaphysalis flava*, the causative agent of the Japanese spotted fever, are widely distributed in Niigata. In this study, we described the population genetic structure and gene flow in *I. ovatus* and *H. flava*, in relation to its *Rickettsia* infection rate along an altitudinal gradient. We hypothesized that a heterogeneous *Rickettsia* infection rate would be observed in a highly genetically divergent *I. ovatus* due to the limited gene flow compared to the homogenized population genetic structure in *H. flava*. A total of 347 adult *I. ovatus* and 245 *H. flava* were tested for the presence of *Rickettsia* using the primers: 17kDA, *gltA*, *rOmpA*, and *rOmpB* and for population genetic structure using the mitochondrial *cox1* marker. The *Rickettsia* infection rate is 46/347 (13.26%) in *I. ovatus* and 15/243 (6.17%) in *H. flava*. For *I. ovatus*, the global F_{ST} value revealed significant genetic differentiation among populations, while *H. flava* showed non-significant genetic differentiation. The *cox1* *I. ovatus* cluster dendrogram showed 2 cluster groups while the haplotype network and phylogenetic tree showed three genetic groups. The 2 cluster groups and the three genetic groups found in *I. ovatus* showed a significant difference in their *Rickettsia* infection rate and mean altitude per group. Meanwhile, no significant differences were found in the mean altitude and *Rickettsia* infection rate in *H. flava*. The results imply that the low gene flow in *I. ovatus* populations along an altitudinal gradient have caused spatially heterogeneous *Rickettsia* infection rates. This information is essential in understanding the relationship between the tick vector and its pathogen, *Rickettsia*, as influenced by environmental factors such as altitude.

In conclusion, this dissertation successfully contributed to population genetic studies of medically-important arthropod vectors, dengue mosquito *Ae. aegypti* and Ixodid ticks, *I. ovatus* and *H. flava* and the role of its microbiome as potential vector control agent and as a pathogen causing bacteria. In summary, gene flow pattern among arthropod vector (e.g. mosquitoes and ticks) populations can be influenced by environmental factors (e.g. roads and altitude) and the hosts affecting the pathogen transmission. These studies will significantly benefit the scientific community and the medical legislators and policymakers involved in designing and planning effective arthropod vector control programs to reduce the transmission of vector-borne diseases in

endemic areas.