Centers of Advanced Medicine
Center for Medical Entomology

Hirotaka Kanuka, *Professor and Director*
Kenji Ishiwata, *Professor*
Tatsuya Sakurai, *Assistant Professor*
Manabu Ote, *Assistant Professor*

General Summary

Arthropod vectors are organisms that play a role in the transmission of pathogens from humans or from animals to humans. Vectors tend to be blood-sucking insects that ingest the disease-causing organism with blood from an infected host and then inject the organism into a new host during their next blood-meal. A new strategy to control vectors should absolutely be developed and involved in integrated vector management, because such a strategy would be an extremely effective means of dealing with the problem while waiting for a vaccine or another effective dengue control strategy. In our center, based on our collaboration with institutions in endemic countries, such as Burkina Faso, Nigeria, and Taiwan, entomological studies promoting multilateral approaches have been performed to gather knowledge of the diagnosis, ethology, immunity, and epidemiology of vector species on effective vector control.

Research Activities

Vector control strategies utilizing the symbiotic bacteria Wolbachia

Symbiotic microorganisms prevail in huge varieties of insect species, supporting insect adaptation to diverse habitats based mainly on nutritious interactions. *Wolbachia* are the most prevalent endosymbiotic bacteria in invertebrates and are estimated to be infecting more than 60% of insect species. *Wolbachia* are transmitted vertically through host eggs and manipulate their hosts in a variety of ways: cytoplasmic incompatibility (CI), male killing, male-to-female transformation, and parthenogenesis. In particular, CI is the most prominent phenomenon induced by a variety of *Wolbachia* strains, causing embryonic lethality when infected males mate with uninfected females. Another fascinating feature of *Wolbachia* bacteria is their ability to induce positive-stranded RNA virus resistance in insect host cells. *Aedes* mosquitoes infected with *Wolbachia*, especially when introduced from other insects, have extremely lower levels of viruses after feeding on blood containing Zika, dengue, or yellow fever viruses and resultantly become incompetent to transmit them to mammalian hosts. Recently, promising practical approaches using *Wolbachia* have emerged to control *Aedes* populations in current or potential risk areas of dengue or Zika. Two distinct strategies have been adopted on the basis of the separate features implemented on *Aedes* mosquitoes by *Wolbachia* infection: male sterility by CI and virus blocking. Field releases of *Wolbachia*-infected male mosquitoes are expected to effectively reduce population size via CI. *Wolbachia*-infected females otherwise replace the current natural population with a virus-incompetent population after generations. Our research is now focusing on revealing molecular bases of the *Wolbachia*-host interactions and, concomitantly, the effect of *Wolbachia* on insect evolution and ecology. We have previously discovered that *Wolbachia* targets the RNA-protein complex processing body (P-body) in *Drosophila* female germline cells and controls the translation of host RNAs. We discovered that the same mechanism might be working against virus RNAs and thereby suppress virus replication. P-body proteins were recruited to virus replication sites and supported virus amplification. The *Wolbachia* factor Toxic manipulator of oogenesis (TomO) controlling the P-body targeted virus RNAs and suppressed virus replication. We hypothesize that the P-body proteins and the *Wolbachia* effector protein TomO are the common factors underpinning diverse *Wolbachia*-mediated phenomena by targeting different types of RNA.

Dissecting overwintering mechanism of Asian tiger mosquito, Aedes albopictus

Aedes albopictus is an *Aedes* species widely distributed from East Asia to India. In the tropic zone, the *A. albopictus* mosquito repeats its life cycle throughout the year. On the other hand, in temperate zones, such as Japan, when adult females of this species are exposed to short days and low temperatures in late autumn, they lay diapause eggs. In diapause eggs, development is paused in the stage of pharate first instar larvae. The arrest of hatching behavior is maintained until early summer, and the pharate larvae show strong resistance to coldness, drying, and starvation. To reveal the genetic mechanisms of preparation and maintenance of diapause in *A. albopictus*, we focused on 2 strains: a tropic strain and a temperate strain. We confirmed that a Kuala Lumpur strain and a Hiroshima strain showed different hatching rates when adult females were reared with short days and low temperatures. Furthermore, we compared temporal and comprehensive gene expression between eggs from the tropic strain and diapause eggs from the temperate strain using RNA sequencing to identify genes involved in the diapause mechanism. Our results have shown several candidate genes involved in environmental resistance and have also suggested the possibility that the neuropeptide gene Capability (*Capa*) induces hatching behavior. To elucidate the function of *Capa* in diapause, we produced deletion heterozygous mutants in *A. albopictus* using the CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 system) in both tropic strains and temperate strains. We are now attempting to establish *Capa* homozygous mutants to evaluate the function of Capa protein in the formation and maintenance of diapause eggs.

Publications

Badolo A, Sombié A, Pignatelli PM, Sanon A, Yaméogo F, Wangrawa DW, Sanon A, Kanuka H, McCall PJ, Weetman D. Insecticide resistance levels and mechanisms in *Aedes aegypti* populations in and around Ouagadougou, Burkina Faso. *PLoS Negl Trop Dis.* 2019 May 23; **13**(5): e0007439. doi: 10.1371/journal.pntd.0007439. PMID: 31120874; PMCID: PMC6550433.

Sato K, Ahsan MT, Ote M, Koganezawa M, Yamamoto D. Calmodulin-binding transcription factor shapes the male courtship song in *Drosophila*. *PLoS Genet.* 2019 Jul 25; **15**(7): e1008309. doi: 10.1371/journal.pgen.1008309. PMID: 31344027; PMCID: PMC6690551.

Ote M, Yamamoto D. Impact of *Wolbachia* infection on *Drosophila* female germline stem cells. *Curr Opin Insect Sci.* 2020 Feb; **37**: 8-15. doi: 10.1016/j.cois.2019.10.001. Epub 2019 Oct 17. PMID: 31726321.