เนื้อหางานวิจัย

CHAPTER 1

INTRODUCTION

Anopheline mosquitoes are the exclusive vectors of human malaria. Salivary glands are of interest in the anopheline mosquitoes because transmission of malaria to vertebrate hosts depends on the ability of *Plasmodium* sporozoites to invade the salivary glands of female vector mosquitoes. The female salivary glands produce a wide array of secreted compounds that are delivered with the saliva and help blood-feeding by affecting the host hemostatic response. In addition, the saliva is the vehicle that carries pathogens and may also enhance or facilitate infectivity during the blood meal. Analyses of salivary gland proteins (sialomes) and/or genes (transcriptomes) of anopheline mosquitoes were performed in *Anopheles gambiae*, *Anopheles stephensi*, *Anopheles darlingi*, and *Anopheles funestus*. The sequences of the mosquito sialotranscriptomes were grouped in protein/gene families. Recently, some of secreted protein(s)/gene(s) are proposed to use as a molecular marker for assessing phylogeny among closely related species in *An. darlingi* complex, an important human malaria vector in Brazil, as has been demonstrated with triatomine bugs using the salivary heme proteins.

In Thailand, anthropophilic *Anopheles barbirostris/campestris* were recently incriminated as potentially natural vectors of *Plasmodium vivax* in the Aranyaprathet district of Sa Keao province. They were also considered as possible vectors playing an important role in increasing cases of *P. vivax* infection in this country. As morphologically indistinguishable, adult females of *Anopheles barbirostris* and *Anopheles campestris* lead to marked errors in identification between these two species in the study of malaria epidemiology and control. Saeung et al. (2007) investigated the anthropophilic *An. barbirostris/campestris* group strain from Chiang Mai province and revealed that besides the 4 forms of metaphase karyotypes of *An. barbirostris* [Form A: X_2, X_3, Y_1, B: X_1, X_2, X_3, Y_2, C: X_2, X_3, Y_3 and D: X_2, Y_4 (found only in Indonesia)] and the typical *An. campestris* (X, Y) strain from Ayutthaya province, there is a new karyotypic form (Form E: X_2,Y_5). The summation of branches of seta 2-VI of pupal skins 22.25 (18-30) is in the range of *An. campestris*, whereas its characteristics of metaphase karyotypes, particularly the

chromosome Y_5, is markedly different from *An. barbirostris* Form A, B, C, D, and the typical *An. campestris* strain. Thus, it is tentatively designated *An. campestris*-like Form E. The hybridization of sympatric *An. campestris*-like Form E with *An. barbirostris* Form B demonstrated strong reproductive isolations. In addition, the crossing experiments between two allopatric strains of *An. barbirostris* Form A (Chiang Mai and Phetchaburi) also indicated extensive reproductive isolations (Choochote et al. unpublished data). Choochote et al (personal communication) have investigated karyotypic variations of the *An. barbirostris* complex in the systematic direction and found that the *An. campestris*-like Form E is susceptible in a high level to *P. vivax* in laboratory but the *An. barbirostris* Form A is not a malaria vector. This result corresponds to the report of Apiwathnasorn et al (2002).

Despite their importance as malaria vectors, no protein/nucleotide sequence of the *An. barbirostris* complex salivary gland is available in the NCBI database. The comparative analysis of the proteomes and/or transcriptomes of the mosquito members in this complex that having distinct primary host [human (vector) or other animals (non-vector)], may supply better tools for determination of phylogeny of closely related species, population structure and speciation processes, and ultimately identify genes related to vectorial capacity and host preference. All of this information is likely to be useful for the improvement of existing and development of novel transmission-reduction malaria control strategies. Therefore, in this study, electrophoretic protein profiles of female salivary glands of each form and sibling species in the *An. barbirostris* complex from different regions in Thailand were analyzed using Sodium dodecyl sulphate polyacrylamide gel eletrophoresis (SDS-PAGE), Two-dimensional gel electrophoresis (2-DE), and nanoLC-MS.