CHAPTER 1

General introduction and thesis objective

The most important activity of honey bees is their pollination of natural vegetation. In addition, the products of honey bees such as wax, honey and royal jelly are benefits.Alltree species in tropical forests are insect-pollinated and that usually means bee-pollinated (Bawa, 1990). Wild bees may now become even more important as pollinators than in the past, because of the dramatic decrease in feral honey bee populations. Giant Asian honey bee, *Apis dorsata* and dwarf Asian honey bee, *Apis florea* are Thai native honey bees. They play important roles in the rain forest ecology and provide the income tolocal people in Thailand for their wild honey and their products. Nowadays, the populations of these wild bees have been seriously reduced by pathogens and also human activities for example destruction of natural habitats, nesting sites, and overuse of insecticides.

Insects display a wide range in degree of dependence on gut bacteria for basic function, while scientists have reached deeper into the field of studying the indigenous bacterial communities within the digestive tract of honey bees (Moran et al., 2012; Engel and Moran, 2013). Then, this study focused on bacteria community structure in the intestinal tract of *A. dorsata* and *A. florea* specially midgut since it is the primary site of digestion and absorption. Also, most bee pathogens have infection or attachment sites in the bee midgut in their pathogenicity for example *Paenibacillus larvae*. Bacteria in insect guts have been shown to provide nutrition, protection from parasites and pathogens, modulation of immune response, and communication (Engel and Moran, 2013). Several insects have a large number of bacteria in their guts, but most insects have few microbial species. Previous reports suggested that bacterial community structure was different by insects, life stages and geographic locations (Disayathanoowat et al., 2012; Ahn et al., 2012).

Studies in intestinal honey bees by using culture-dependent and various culture-independent based methods have focused on the European honey bee, *Apis mellifera* (*A. mellifera*) and a cavity-nesting Asian honey bee, *Apis cerana* (*A. cerana*) which are farmed on industrial scale. However, most of the information that is available on the composition of the insect gut microbiota derives from extensive analysis of 16S rRNA sequence amplification. A variety of non-culture based methods have been used to examine microbial composition of domesticated honey bee gut for example 16S rRNA library, T-RFLP and pyrosequencing (Jeyaprakash et al., 2003; Cox-Foster et al., 2007; Martinson et al, 2011; Disayathanoowat et al., 2012; Moran, et al., 2012; Ahn et al., 2012). In our study, we used a polymerase chain reaction coupled with denaturing gradient gel electrophoresis (PCR-DGGE) and 16S rRNA gene sequencing methods to examine the diversity and distribution of bacterial flora in midguts of *A. dorsata* and *A. florea*.

Chapter 2 reviews basic background knowledge of bacteria associated with honey bees and its environment highlighting pathogenic group, as well as non-pathogenic group and explains the general roles of midgut microflora in insect and honey bee.

Chapter 3describes the cultured-independent studies of bacterial dynamic in midgut of the giant Asian honey bee (*A. dorsata*) during developmental stages by denaturing gradient gel electrophoresis (DGGE) and 16S rRNA sequencing.

Chapter 4describes the studies of bacteria in midgut of the dwarf Asian honey bee (*A. florea*) larvae bynested PCR, denaturing gradient gel electrophoresis (DGGE) and 16S rRNA sequencing.

Chapter 5 describes the diurnal flight activity of *A. dorsata* living in tropical region. This research is the first to document foraging patterns of *A. dorsata* in Northern Thailand.

Chapter 6 is the general conclusion of these studies.

Research objectives

1. To investigate the bacterial community dynamic in the midgut of *A. dorsata* during developmental stages and compare the bacterial diversity in different coloniesas well as different geographical locations by denaturing gradient gel electrophoresis(DGGE) and 16S rRNA sequencing

2. To investigate the bacterial community in the midgut of *A. florea* larvae and compare the bacterial diversity in different coloniesas well as different geographical locations by denaturing gradient gel electrophoresis(DGGE) and 16S rRNA sequencing

3. To investigate the diurnal flight activity of *A. dorsata* living in Northern Thailand