CHAPTER I

INTRODUCTION

Honey bees are especially important everywhere, since a majority of agricultural cultivation and crop plants require insect pollination. The value of crops pollinated by honey bees has been reported to be \$3.3 billion worth of agricultural food production in California alone and almost \$19 billion in the U.S.A. (Levin and Weller, 1989). Commercial honey products also have a high economic value in industrial beekeeping. Pollen, a product derived from flowers (collected by honey bees), is an essential food source that has a high protein, mineral and vitamin content etc. (Parkhill, 1982). Commercial bee pollen is also important in Chile, China, Israel, Mexico, Taiwan and Viet Nam (Svensson, 1991).

Thailand is a unique place of honey bee diversity. Five species of honey bees are found, including the Western honey bee, *Apis mellifera*, an "imported species" and the best known species, and the Eastern honey bee, *A. cerana* one of the native species of Thailand. Their nests are in sheltered cavities with multiple-paralled combes. The giant or rock honey bee, *A. dorsata*, the dwarf honey bee, *A. florea*, and the small dwarf honey bee, *A. andreniformis*, are open-nesting species with a single exposed comb (Ruttner, 1988; Tingek *et al.*, 1988).

Apis cerana, one of the best known honey bees (Wongsiri *et al.*, 1990), is widely distributed over the different geographical areas of Thailand. Samui Island is not only known as a place for beekeeping with domestic hives but it also has a long history in coconut plantations, orchards or houses (Nakamura *et al.*, 1991). Until now little information has been audilable especially on the variation between many ecotypes (Crane, 1993). As Oldroyd *et al.* (1992) claimed "without knowledge of diversity, it is impossible to manage populations in order to preserve their diversity". Therefore, more informations give the potential of genetical improvement. Beekeeping with *A. cerana* in Thailand still belongs to "household culture" and gives valuable economic benefits to the beekeeper. In contrast, the introduction of European honey bees; *A. mellifera*, recently succeeded as an industry in the early 1970's (Wongsiri *et al.*, 1987) and modern beekeeping methods were also applied to beekeeping in China, giving an economic value higher than traditional methods (Wongsiri *et al.*, 1986). *A. cerana* has several advantageous traits over *A. mellifera* such as, greater resistance to endemic diseases and parasites and suitable adaptation to climatic conditions. For these reasons, the basis biology of *A. cerana* should be studied further for strain improvement.

A. cerana has been classified into four subspecies by Ruttner (1986; 1988) as, 1) *A. cerana cerana*, 2) *A. cerana indica*, 3) *A. cerana himalaya*, and 4) *A. cerana japonica*. In Thailand, Limbipichai (1990) divided *A. cerana* on the basis of multivariate statistical analysis of morphometric characteristics into 3 distinctive groups, 1) Northern latitude bees (samples collected from Chiang Rai-Petchburi), 2) Southern latitude bees (samples collected from Chumphon-Songkhla) and 3) Samui island bees. Using a combination of earlier taxonomy and new statistical characterization of diversity gives much information on geographic variation, but these methods not only require about 5 hours for one person to process a sample of ten bees and high quality equipment such as a high quality stereomicroscope plus computer, digitizer and computer program, etc., but also require a skilled person (Daly, 1991). Measurements are also influenced by the environment. For example, 1) the season of development, 2) the temperature of the surroundings during the pupal stage, 3) the size of the cell, 4) feeding by nurse bees of different age and 5) individual of the colony. The environment has a pronounced effect on the body size of worker bees (Alpatov, 1929 reviewed by Daly, 1991; Rinderer, 1986). Thus, measuring geographic variation requires much more effort.

Advance in genetic studies of the underlying genes, particularly the novel molecular techniques, has supported the old classification methods (Oldroyd, 1992). Various samples of different base pair composition can be detected by restriction endonuclease enzymes (Smith, 1988). One example is the restriction endonuclease patterns (restriction patterns); digestion of DNA samples at specific base sequences reduces the DNA molecules to fragments which are then separated by electrophoresis through a gel matrix. The position of each DNA fragments is then compared. Restriction pattern have been used to classify a member of organisms, such as the genus *Aspergillus* (Kozlowski and Stepien, 1982), *Streptomyces* spp. (Tanskul, 1991) etc. But no one reporting on honey bees, especially in Thailand, has used this technology. Another classification technique is restriction fragment length polymorphisms (RFLP); the generated-DNA fragments (denatured into single strands) are transfered by capillary action from electrophoresis gel onto a solid support (nylon membrane). A specific DNA fragment (DNA probe) is labeled and hybridized with the DNA on the membrane. The fragments binding with the DNA probe are visualized by a detection system, i.e. X-ray film exposure. The result are compared among different organisms. This technique has already been reported on the research of Africanized bee problem in the U.S.A. For example, genetic differences between Africanized bees and European bees were identified by using nuclear DNA (Hall, 1986; 1988; 1990), and mitochondrial DNA (mtDNA) (Severson *et al.*, 1988; Smith, 1988, Hall and Muralidharan, 1989; and Hall and Smith, 1991). Recently, Crozier and Crozier (1993) reported the complete sequence of mtDNA of *A. mellifera*. Mitochondrial DNA was used to study gene flow between African and European-derived honey bee populations in Argentina (Sheppard *et al.*, 1991), and compared to the morphology of Kangaroo island bees (Oldroyd *et al.*, 1992). Nuclear DNA probes have also been used to study the genetic variation of Asian honey bees (Sylvester and Wongsiri, 1993).

Therefore, the aim of this thesis is to used DNA to study the diversity of *A. cerana* from different regions of Thailand by using restriction patterns and RFLPs. The results will provide information on the biology and geographic variation of *A. cerana* in Thailand and provide a basis for future selection and breeding for strains improvement.