CHAPTER II

LITERATURE REVIEW

2.1 Biology of *A. cerana*

Honey bees are highly eusocial insects. The social structure of a honey bee colony is based on a long-lived female, the queen, who is the mother of all members of the colony. Arising from a fertilized egg, the queen is heterozygote (diploid 2n=32). The queen also evidences a mechanism of controlling offspring by the "queen pheromone" (Wilde and Beetsma, 1982; Rinderer, 1986). About 95% of a queen's offspring are workers. The bee workers arise from fertilized eggs like queen but they never mate and never lay eggs so long as the queen is alive (Seeley, 1985). Whereas a fertilized egg develops into a female, an unfertilized egg becomes a male (drone) a hemizygote (haploid individual) (Rinderer *et al.*, 1986). The queen mates with seven to 17 drones and stores about 5 million sperms in the spermatheca during her sole mating period, which is a sufficient supply for egg laying about 3 years (Page and Erickson, 1986). The life cycle of honey bee colonies begins by the colony rearing a batch of queens in preparation for swarming. After the queen cell is capped, the mother queen with some workers leaves the colony to establish a new colony. In the parental nest, the first new queen daughteris is recognized and becomes the new queen (Wilde and Beetsma, 1982).

2.2 Systematic analysis

The "Eastern honey bee" was recognized by Ruttner (1988) after being first named as *Apis cerana* in 1793 by Fabricius from specimens found in China. The morphology and behavior of *A. cerana* is so similar to the best known, western honey bee or European honey bee, *A. mellifera* that was classified as an *A. mellifera* subspecies for a long time. (Buttel-Reepen, 1906 reviewed by Rinderer, 1986; Daly, 1991). It was recently found that they are closely related genetically.

The taxonomy of *A. cerana* has been recognized as follows (Borror *et al.*, 1976; Gojmerac, 1980);

Kingdom	Metazoa
Phylum	Arthropoda
Class	Insecta
Order	Hymenoptera
Super-family	Apoidea
Family	Apidae
Genus	*Apis*
Species	*Apis cerana* Fabricius, 1793

2.2.1 Morphometric analysis

The consideration of the diversity of living organisms are mostly characterised by the external appearance in the middle of the 18th century. Multivariate statistical analysis of morphometric characters, including distribution and behavior classify *A. cerana* populations into four morphological subspecies as recognized by Ruttner (1988) *A. cerana*

population ranges are shown in Figure 1, as the follows 1) a northern subspecies; *A. cerana cerana*, found throughout Afghanistan, Pakistan, North India, China and North Viet Nam, with highly different areas of distribution, 2) a southern subspecies; *A. cerana indica* distributed from South India, Sri Lanka, Bangladesh, Burma, Malaysia, Thailand, Indonesia and the Philippines. *A. cerana* from the Philippines has been identified as *A. cerana philipina* by discriminant analysis (Maa, 1953) 3) a himalaya; *A. cerana himalaya*, found in the Eastern South Asian mountains, from Nepal to Thailand (including the mountains in Thailand; Chiang-Mai) and probably South-Western China, 4) a japanese; *A. cerana japonica* distributed throughout Japan and Korea. Pang *et al.* (1989), clearly confirmed morphometric differences between *A. cerana* and *A. mellifera* by statistical analysis of morphometric data. The wide distribution throughout Thailand of *A. cerana* was studied by Limbipichai (1990) using multivariate statistical analysis (Canonical analysis) of morphometric characteristics, such as, proboscis, fore and hind wing, hind leg, third and sixth sternites, and second, third and fourth tergites etc. The research divided *A. cerana* populations into three distinctive groups as follows 1) Northern latitude bees (samples were collected from Chiang Rai-Phetchaburi), 2) Southern latitude bees (samples were collected from Chumphon-Songkhla) and 3) Samui Island bees. Using Clustering Analysis, *A. cerana* populations were divided into two distinctive groups, Northern latitude bees and Southern latitude bees. By Clustering analysis, the honey bee from Samui Island can be classified to the same group as the Southern latitude bees.

Figure 1 Dotted lines indicate the approximate ranges of *Apis cerana* subspecies as recognized by Ruttner (1988). A = *A. cerana cernana*; B = *A. c. indica*; C = *A. c. japonica*; D = *A. c. himalaya*. (Smith, 1991)

Morphometrics measures phenotype is the end result of the expression of genes. Furthermore, the features can be separated from the influence of the environment.

After Mendel's laws were rediscovered and began to be actively investigated at the begining of the 20th century, they help to understand the molecular basis of life and began to have a major impact in taxonomic studies.

2.2.2 Allozyme analysis

Biochemical variation can be used to separate populations. For example, allozymes are the variant protein products of genes produced by allelle differences at the same locus. Allozymes are from tissues of organisms and revealed after separation in an electrical field. The different proteins migrate different distances depending on their net charge. The protein bands can be visualized by histochemical stains specific for each enzyme. This technique of allozyme analysis has been used to study the variation within and among populations of honey bees for almost two decades (Daly, 1991). Sylvester (1982), Nunamaker *et al.* (1984) Page and Erickson (1985) and Sheppard and McPheron (1986) reported on distinct subspecies of Africanized bees and European bees by using partial difference in allozymes. Asian honey bees have also been compared for allozyme variation, such as Nunamaker *et al.* (1984) studied two allozymes; malate dehydrogenase (MDH) and esterase (EST); MDH showed *A. cerana* as distinct from either *A. dorsata* or *A. florea* (samples collected in Pakistan) and no intraspecific polymorphisms among them.

The studies of Tanabe and Tamaki (1985) and Sheppard and Berlocher (1989) indicated species specific differences between *A. cerana* and *A. mellifera*. Li *et al.* (1986) also studied EST enzymes in Asian honey bees from China showing a unique banding pattern for each of four species; *A. florea, A. dorsata, A. andreniformis* and *A. laboriosa.* Allozymes analysis is based on the immediate product of structural genes and is independent of environmental influences. In the social Hymenoptera, protein variation is limited (Graur, 1985) because of their sex-determination system (haploid males). Therefore, allozyme analysis cannot make certain identifications, especially the hybrids after several generations (Rinderer and Sylvester, 1981).

2.2.3 DNA analysis

The discovery of electrophoretic RFLP opened up a new field of DNA identification, established about 1980 by Wyman and White (Kirby, 1992). Recent techniques of DNA analysis allow directly to the examination of DNA primary structure. Within eukaryotic cells, most of the DNA is nuclear DNA and only extracellularly is found mitochondrial DNA. The products of DNA are controlled by regulatory and structural genes where genes consist of coding regions (exons; to form the functional mRNAs) and the excised, non-coding regions (intron) (Green, 1986). Eukaryotic genomes consist of non-protein coding regions of up to 100 times as much as coding. Many of them are repetitive (Kirby, 1992). The repetitive sequences have been classified into two groups based on their genomic arrangements, first; those in tandem repeats (refered to as satellite

DNA), second; those scattered throughout the genome (Jeffreys *et al.*, 1985). Honey bee showed little repetitive DNA only 3-5% of the genome (Crain *et al.*, 1976).

At the molecular level, the polymorphisms occur by single nucleotide base changes in the number of tandem repeats in a repetitive DNA sequences. The restriction endonuclease recognizes this site and cleaves into fragments. If the site is absent, different DNA fragments will be produced. DNA fragment lengths vary for several reasons; 1) the restriction sites are polymorphic, 2) insertion or deletion of nucleotides has occurred between restriction sites and 3) the number of tandem repeats varies (Kirby, 1992). The repetitive sequences have been used as DNA probes for polymorphism detection among closely related organisms (DNA-DNA hybridization method) by using the appropriate restriction endonuclease digested genomic DNA and separation through a gel by electrophoresis. A DNA fragment is amplified by cloning in a suitable bacterial host. The passenger DNA is used as a DNA probe by DNA end labeling. The basis of DNA-DNA hybridization is based on the complementation between single-strand DNAs forming into double-strand nucleotide molecules by sequence-specific base pairing.

The RFLP method is widely used to identify organisms. Such as members of the parasite causes disease; *Trypanosoma* (Majiwa *et al.*, 1986; Kukla *et al.*, 1987; Majiwa and Webster, 1987; Gibson *et al.*, 1988; Artama *et al.*, 1992; Mohamed *et al.*, 1993) and another vertebrates (Hoelzed and Amos, 1988; Jeffrey and Morton, 1987; Washio *et al.*, 1989) In Hymenoptera it indicated that the broods of solitary bee species raised in

single nests are mostly the offspring of one singly mated female (Planchetot, 1992). For the past few years, the publications on variation at the DNA level in honey bee has appeared (Smith, 1991). Initially reported by Hall (1986), the by establishment of random fragments of honey bee; *A. mellifera* nuclear DNA digested with *Pst*I and cloned into *E. coli* plasmid pBR322. The repetitive DNA probes of A. mellifera showed close relation among the European bees (*A. mellifera ligustica* and *A. m. carnica*) while the Africanized bees (*A. m. scutella*) are different. This result clearly comfirmed by Hall (1988). In addition, there are subsequent research on mitochondrial DNA (mtDNA) polymorphisms within and among *A. mellifera* subspecies (Moritz *et al.*, 1986; Smith, 1988; Smith and Brown, 1990; Smith *et al*; 1991). Especially the influence of "killer bee", Africanized bees in widely regions such as U.S.A., Yucatan peninsula and Argentina, this technique were identified their populations (Smith *et al.*, 1989; Hall and Muralidharn, 1989; Hall and Smith, 1991; Rinderer *et al.*, 1991 and Sheppard *et al.*, 1991). The mtDNA restriction mapping is verified and characterized morphology of the bees, *A. mellifera* in Kangaroo Island; South Australia (Oldroyd *et al.*, 1992; Sheppard and McPheron, 1993) and *A. cerana* in South Korea (Lee, 1993). Recently, the polymorphisms of mtDNA lineages on *A. cerana* indicated that populations on mainland (Northern and Southern Thailand, Penisular Malasia, Northern Borneo and Southen India) are the same as mtDNA genome. Whereas the populations on isolated Island such as Sulawesi (Indonesia), Luzon (Philippines) and the Andamans (India) are highly divergent mtDNA genome (Smith, 1993). Sylvester and Wongsiri (1993) used nuclear DNA

markers from European honey bees, *A. mellifera* to identified and characterized genetic veriation in Asian species of *Apis* (*A. cerana, A. dorsata, A. florea, A. andreniformis*) and suggested that they may be interspecies differences.