CHAPTER V

DISCUSSION

MtDNA is a potential marker to investigate genetic structure. Based on the fact that, the mtDNA of stingless bees are similar among nest mate. This is an advantage for sampling only one sample of bees from each colony. This mtDNA contains several regions such as 13 protein-encoding genes, two genes for rRNA subunits, and a non-coding region containing the origin of replication. Various regions of mtDNA on several species of the *Apis* has been studied.

The 16S rRNA gene in mtDNA has been widely used for studies of genetic variation at the intraspecific and interspecific level (Austin *et al.*, 2004; Costa *et al.*, 2003; Insuan, 2001; Nanork, 2001; Whitby *et al.*, 2004; Whitfield and Cameron, 1998).

From the complete mitochondrial genome sequence of *A. mellifera* (Crozier and Crozier, 1993), the expected amplification product of 16S rRNA gene was 739 bp. However, the amplified products in five species of *Trigona* (*T. collina*, *T. fuscobalteata*, *T. laeviceps*, *T. terminata*, and *T. thoracica*) were 550 bp which is different from the expected size of *A. mellifera* mtDNA sequence. The 16S rRNA gene in five species of *Trigona* is typically shorter than that of *A. mellifera*.

PCR products of five species of *Trigona* (16S rRNA gene) are slightly smaller than the corresponding products in *Plebeia* (Apidae: Meliponini), are considered a post Gondwanan group that originated in the region of

Southeastern Brazil (Camargo and Wittmann, 1989), and the differences, a total of about 250 bp, are being studied (Francisco *et al.*, 2001).

In the restriction enzyme screening step, after digested with a number of restriction enzymes; *Afl* II, *Ase* I, *Bam*H I, *Dra* I, *Eco*R V, *Hin*f I, *Hpy*188 III, *Mse* I, *Pac* I, *Rsa* I, *Sau*3A I, and *Ssp* I. There was no restriction site of four restriction enzymes; *Afl* II, *Bam*H I, *Eco*R V, and *Hin*f I in the PCR products of the 16S rRNA gene of mtDNA in five species of *Trigona*. Only two restriction enzymes; *Mse* I and *Sau*3A I could digest these PCR products but some DNA fragments are extremely short and could not be detected by agarose gel electrophoresis. Moreover, *Ase* I and *Rsa* I could digest the PCR products of 16S rRNA gene in some species of *Trigona*. Although PCR products of 16S rRNA gene in five species could be digested by *Pac* I but there was not different in some species. Therefore, *Dra* I, *Hpy*188 III, and *Ssp* I were selected to be employed for analysis of genetic variation in this study.

This is the first genetic diversity studied by using PCR-RFLP on stingless bees genus *Trigona* in Thailand. The selection of situate restriction enzymes and condition for this gene (16S rRNA) are new data base which should be useful and can be applied for further study.

5.1 Genetic variations of five *Trigona* species

5.1.1 MtDNA variation among *T. collina* populations

The cluster groups which produced from PCR-RFLP analysis with 3 restriction enzymes of 16S rRNA gene from 224 samples of *T. collina* revealed high level of variation among populations (10 haplotypes). The result showed highly significant genetic differences (*D*) between Southern [group (1)] and Northern to Central populations [group (2) and (3)] (D = 0.041682), indicated that restricted gene flow between them.

In Northern to Central populations could be divided into 2 groups [(2) and (3)]. However, genetic distances between two groups were very low (mean = 0.0247895), indicated that probably mtDNA of group (3) derived recent mutations of the ancestral mtDNA from group (2). DNA sequencing could be utilized to confirm this hypothesis.

In group (2) of *T. collina* consisted of Northern to Central groups and Southern (S 199-213) populations from Surat Thani Province, indicated that these Southern (S 199-213) populations more probability have been colonized by separated populations from group (2) than remaining groups. This suggestion was supported by genetic differentiation between Southern (S 199-213) populations and group (2) (D = 0.007897) which was lower than group (1) and (3), D = 0.014033 and 0.029238, respectively.

5.1.2 MtDNA variation among *T. fuscobalteata* populations

The cluster group produced from PCR-RFLP analysis with 3 restriction enzymes revealed two haplotypes which were clearly divided into 2 groups. However, low genetic distance among populations ($D = 0.009752$), indicated high level of gene flow between them appears to have homogenized this species. From this result showed low genetic variation in the 16S rRNA gene of mtDNA of *T. fuscobalteata* species in Thailand.

5.1.3 MtDNA variation among *T. laeviceps* populations

Genetic diversity of 16S rRNA gene within populations of *T. laeviceps* was relatively high. The distribution of 10 haplotypes demonstrated a clear two groups, they are (1) Central to Southern group (C, S and some parts of NE) and (2) Northern to Central group (C, E, N, and NE) (see Figure 4.28). Mean of genetic distance between these 2 groups was 0.02904. The genetic distance among populations ranged from 0.008727 to 0.049354, indicated high genetic variation within this species (Table 5).

The genetic distance between group (1) and group (2) ranged from 0.008727 to 0.049354. These values revealed clear genetic difference between Northern to Central region [group (2)] and Central (Kanchanaburi and Ratchaburi Province) to Southern region [group (1)]. However, there are samples from Northeast (NE 020-024) populations were divided into Central to Southern region [group (1)] and also Northeast (NE 020-024) showed high genetic distance with Northern to Central region [group (2)] ranged from 0.018384 to 0.041682, indicated that low gene flow between them and discontinuous distributions of haplotypes in this species were discovered (see Figure 4.28).

The most likely explanation for haplotype discontinuous distribution of Northeast (NE 020-024) is that it was established by a small founder population from Central to Southern region [group (1)]. Moreover, Southern (S 077-155) populations from Phatthalung and Songkhla Province that revealed same haplotypes with Northeast (NE 020-024) populations that support this hypothesis.

5.1.4 MtDNA variation among *T. terminata* populations

The two cluster group produced from PCR-RFLP analysis with 3 restriction enzymes revealed two haplotypes. High genetic distance value between two haplotypes groups, D = 0.0176, was detected, indicated that low gene flow level between two groups. From this result showed high genetic variation of 16S rRNA gene of *T. terminata* species in Thailand.

5.1.5 MtDNA variation among *T. thoracica* populations

When digested mtDNA at 16S rRNA gene with 3 restriction enzymes, the cluster groups constructed from PCR-RFLP analysis showed two haplotypes clusters from 6 populations. The genetic distance value within species is low, D = 0.007897, indicated that there is very high gene flow level but low genetic variation in this species.

5.2 Genetic variations of stingless bees in Thailand

Mitochondrial DNA variation among *Trigona* species in Thailand was carried out in 407 samples using PCR-RFLP analysis, by digestion with 3 restriction enzymes. The data of restriction fragments were transformed to 1-0 matrix for cluster analysis (Appendix III).

The cluster analysis adopted from this study divided *Trigona* species into five groups (Figure 4.23). They are (1) *T. terminata* (NE 009-021), (2) *T. laeviceps* and *T. terminata* (C 022-023, E 024-028, and N 001-008), (3) *T. thoracica*, (4) *T. collina*, and (5) *T. fuscobalteata* populations. From PCR-RFLP data of 3 restriction enzymes digestions (*Dra* I, *Hpy*188 III, and *Ssp* I) it could be separated different species of this genus, exception of *T. laeviceps* (Sam Roi Yot, Prachuapkirikhan; Waing Sa, Surat Thani; and Mueang, Phuket) and *T. terminata* (Mueang, and Chom Bueng, Ratchaburi; Khlung, Chanthaburi; Samoeng, Chiang Mai; Chiang Muan, Phayao; Long, Phrae; and Laplae, Uttaradit) samples which there were no haplotypes difference between species. However, using other genes and restriction enzymes may detect genetic difference between there two species.

This is first study of genetic diversity by using PCR-RFLP on stingless bees genus *Trigona* in Thailand. The results showed haplotypes difference of inter- and intraspecific levels. *T. collina* and *T. laeviceps* revealed high genetic differences within each species. However, external morphology studies were not found distinguished. From this result, indicated that cryptic species may be existed in two species. Many entomologists studied on external morphology of *Trigona* such as Sakagami 1978 and Dollin *et al.* 1997, had suggested that the probable existence of cryptic species in genus *Trigona*.

However, samples of *T. fuscobalteata*, *T. terminata*, and *T. thoracica* (18, 28, and 6 samples, respectively) were collected that are few specimens for genetic diversity study. In further study should be collect more locations in Thailand and including other countries.