CHAPTER 5

DISCUSSIONS

5.1 The Ultrastructure of the Mandibular Gland of Honeybee Foragers.

The anatomy and general external morphology of mandibular gland of honeybee foragers of five species by using SEM are similar and correspond to result of Snodgrass and Lensky (Lensky et al., 1990; Snodgrass, 1935). However, the number of gland lobe are divided into 2 groups; one pair lobe which are found in *A. andreniformis*, *A. dorsata* and *A. florea* which are evidently old species with a number of highly specislized characteristics, and two pairs lobes which are found in *A. cerana* and *A. mellifera*. This suggests that the open-air nesting honeybee which are primitive *Apis* species show two pair lobes glands, whlie the cavity-nesting honeybees which are *A. cerana* and *A. mellifera*, these two species are closely related to each other. The present state of this knowledge from this research confirms the traditional concept of the genus *Apis* with *A. cerana* are closely related to *A. mellifera*. Moreover, These observations actually prove the relation among native open-air nesting (primitive) species (Ruttner, 1988). However, the main ten mandibular gland secretions are only 80% homology. Possibly, according to the geographical area that *A. cerana* apparent better adapt to hot climates (Dietz, 1992) and able to extend the foraging efficiency in abundant flora species area. This characteristic is another factor to be supported the phylogenetic relationship within the genus *Apis*, derived from differences in the amino acid sequence of mellitin, which was suggested by Kreil, 1975 (Kreil, 1975; Ruttner, 1988).

The ultramorphology of cell type I of these five honeybee species are similar to the structure of salivary gland of other animals. This suggests that probably, these cell types may secrete the homologous substances of salivary gland of other animals such as enzymes, which are used in the same functions. Consequently, the cell type II is very interesting because they show a well developed of smooth endoplasmic reticulum and mitochondria system, this structure show a classical of lipid secretory products. This appearance corresponds to the gland secretion which consist of fatty acid derivative, alkane and alkene. The other cell type (type III) is composed of various vesicles and enlarged mitochodria, these indicate that they are alcohol or acid producing cell or possibility they can produced saturated lipid. However, alcohol or acid producing cell is should be appropriate because the gas chromatogram data show that the main secretion of these glands are alcohol, acids, alkenes and alkanes.

5.2 Mandibular Gland of Honeybee Foragers and 2-Heptanone

The Gas chromatogram data of the mandibular gland pheromones reveal that 2-heptanone is undectable in *Apis florea* foragers while they show very low level in *A. andreniformis* (0.205 μg/bee) and *A. dorsata* (0.425 μg/bee). The higher levels are found in *A. cerana* (1.322 μg/bee). Unlike in *A. mellifera*, it shows very high level (7.076 μg/bee) when compare to other *Apis* species. These results support the foraging activities of them, by the way that the four native honeybee species in Thailand did not appear to use forage-marking pheromones then it also corresponds to their mandibular gland secretions, which show almost undetectable of 2-heptanone level. Although *A. cerana* which is shows higher level than other

native species this can explain that the low concentration of 2-heptanone was reported that it acts as attractant substance (Boch and Shearer, 1971; Kerr et al, 1974; Valett et al., 1991). Probably, low concentration of 2-heptanone in *A. cerana* acts as attractant pheromone, which is possibly to signal the other bee to revisit the high available remaining nectar flower.

In contrast, 2-heptanone in the mandibular gland pheromones of *A. mellifera* is very high level. The results of this analysis of 2-heptanone are comparable with these obtained by Crewe and Hatings in 1976 which was indicated that in productions of 2-heptanone was 16.9 μg/bee in *A. mellifera adonsanii* and 14.1 μg/bee in *A. mellifera capensis.* Furthermore, the amounts of 2-heptanone in the mandibular glands of *A. mellifera ligustica* foragers were 1.4 μl/bee by using GC-head space technique which was studied by Vallet and his co-workers (Crewe and Hastings, 1976; Vallett et al., 1991). This amount is higher than which is getting from this research (0.0621 μl/bee) about twenty times. The very high levels of 2-heptanone which are recovered by this research and supported by other researchs correspond to the foraging activity of this species because they appeared to use marking pheromones during foraging, then possibility the forager of this species uses 2-heptanone to mark the empty or nectar depleted flower after the first visited. This correspond to the study of Boch and Shearer, 1962; Engels et al., 1997; Free, 1979; Guirfa, 1991; Lensky et al., 1991; and Shearer and Boch, 1965. This confirms that *A. mellifera* forager do not inspect two time the same flower because they can recognize and response to the repellent scent, 2-heptanone left by the other individuals (Boch and Shearer, 1962; Engels et al., 1997; Free, 1979; Lensky et al., 1991 and Shearer and Boch, 1965). This thus suggests that the

repellent scent mark has the status of pheromones since it plays a role in the inter-individuals communications in foraging context (Guirfa, 1990). This enhance foraging efficiency is consistent with use of the repellent scent mark. The other support for this foraging activity is the reason that according to *A. mellifera* which has origin adaptation to distribute into cold climate regions. Possibly, this species was severely constrained by their advanced social organization because they have ability to thrive in cold temperature zone. For this reason, *A. mellifera* foragers have relatively little time to accumulate surplus resources because they must keep their thoracic temperature above 27°C (Seeley, 1985), that is the lower limit for steady flight (Heinrich, 1979). So it corresponds to their foraging activity which use marking pheromones to warn the other bees to avoid the empty flowers for saving time, energy and also keep their thoracic temperature. However, their foragers are remaining using marking pheromone whenever they were introduced into Thailand more than sixty years. Then the idea of economic defend ability are used to predict the levels of resource availability. If food source are very scarce (empty flower), the gain from excluding others may not be sufficient to play for the cost of territorial defense. Instead bees might avoid revisiting or move elsewhere to other flowers (Krebs and Davies, 1993).

5.3 The Main Compositions of Mandibular Gland Pheromones of Honeybee Foragers in Thailand.

The four native honeybee species of Thailand; *A. andreniformis*, *A. cerana*, *A. dorsata* and *A. florea* belonging to show greater chemicals similarity than the exotic species; *A. mellifera* (less closely related species). Indeed, the distinctive between

pheromones of taxonomically, *Apis* species appear to be based on the different proportions present of the same component. This appearance are similar to *Allied* species (Free, 1982). Furthermore, the numbers or variety of pheromones are different; they are 41, 41, 48, 27 and 18 chemicals of *A. andreniformis*, *A. cerana*, *A. dorsata*, *A. florea* and *A. mellifera*, respectively. These components include alcohol, acids, alkanes, alkenes and ketones (C7H14 - C21-H44). They has been reported as both attractant and repellent substances (Free, 1987), however; many component of mandibular gland pheromones of this research were also found in other insects; as follows, eicosanol which is the main component of mandibular gland of five species of honeybees, was also found in sting gland of *A. mellifera*. Moreover, it was suggested that it acts as alarm pheromone for guard bees, inhibit scenting and attractant foragers (Free, 1987). Heneicosane is also found in female sex pheromones of *Achaea janata* L. (Noctuidae; Lepidoptera) and rove beetle. Additionaly, it was also found in Dufour's gland of bumble bees (Krishnakumari et al., 1998). One of the ten main components of this gland is nonadecane which was also found in male sex pheromone of Lepidoptera and Dufour's gland of Euro-African Messor ant (Tengeo et al., 1991). 2-propyl-1-heptanol was also found in Koschevnikow gland of 4-days old queen of *A. mellifera* which acts as attractant pheromone. 1-octanol is acted as repellent for *Varroa* mite (Kraus, 1990). Dibutyl phthalate acts as insect repellence and also act as diminish stinging of guard bees. Limonene was found in alarm pheromone of burrowing bug (Krall et al., Kraus, 1990). Moreover, heptadecane was also found in Dufour's gland of myrmicine ant, *Messer capensis* (Brand and Mpuru, 1993). Eicosane was found in sex pheromones of *Acro lepiopsis* (Lepidoptera) (Thibunt, 1994).

According to the mandibular gland pheromones of five species of honeybee foragers in Thailand show great similar, 80% homology of ten main components. In contrast, the numbers of compounds in the mixture of mandibular gland pheromones of each honeybee species are largely dissimilar chemical compositions, possibility they may lead to the different odour in each species. Indeed, possibility they may lead to the unique specific odour of each species. Then thus suggests that the chemical composition has even helped to clarify the taxonomic status of the different species (Free, 1987).

The idea of **economic defendability** (territorial behaviour should be favoured by selection whenever the benefits are greater than the costs (Krebs and Devies, 1993) is also used to predict the level of resource availability of honeybee foragers. These can explain that whenever a bee discovers a new rich food source, she promptly recruits nest-mates to it and to helps ensure that her colony's forager force stay focused on the richest available patches by both the mechanical function (waggle dance) and chemical communication (pheromones) (Seeley, 1985; Wilson, 1974; Lensky et al. , 1990). The other bees follow these dances to learn the distance, direction of patch. Furthermore, odour of flowers can translate this information into a flight to the specific flowers (Seeley, 1985; Wilson, 1974; Lensky et al. , 1990).

From the field observation were found that four native honeybee species in Thailand did not appear to use marking pheromones during foraging this appearance may cause of their mandibular gland secrete rare of 2-heptanone and is lacking in *A. florea* forager. Moreover, the main compositions of these gland are

composed of largely attractant pheromones, especially 1-eicosanol. This corresponds to their foraging activities which can explain that after the first bee of four native species foraged flower and it could be detect the status of nectar source in that flower. If the rich nectar source are remained available in flowers, it may release the mandibular gland pheromones which is composed of largely attractant pheromones on flower disc to signal the other bee to revisit on the same flower. This suggests that possibility the honeybee foragers of four native species use attractant pheromone during foraging instead of repellence pheromones. This activity is very different from *A. mellifera* activity. Probably, the floral species in Thailand may be visually distinguished on basis of their color information or their odours. These are supported by the report of Abrahamsom, in 1989 that a plant must provide attractant factor (colour and odour), That signal its locations and suggested to pollinators that a visit will be rewarding by nectar which is an aqueous solution of sugars and it often contains small amount of other substance, such as amono acid, protein, alkaloids, phenolics, antioxidants, lipids and vitamins (Barker and Barker, 1983). If a plant do not secrete or provide attractants it may cause the honeybee foragers to avoid revisiting. Because the forager can discriminate between rewarding and non-rewarding flowers without using marking pheromones. So it seems likely that only attractant pheromone that the four native species honeybee foragers use during foraging. This recruitment is very useful for bee to save time and energy for finding the direction of new patch of food source and mark the rich nectar remaining flower. This thus the forager uses less energy than without using chemical communication.

Consequently, According to the present of high level of 2-heptanone in the mandibular gland in *A. mellifera* foragers it lead to posses of repellent property of this gland which affect foraging activity (Butler, 1966; Simpson, 1966). This property is also observed in the field of this study. This indicates that the mandibular gland pheromones of this species might play the different role in foraging activity, this correspond to its foraging activity which is appeared to use marking pheromone during foraging. Furthermore, the entire compounds of these glands of each *Apis* species are unique, probably their odours are different and specific for their own species. Possibility the meaning of pheromone of this gland may play the important role in other behaviour of bees. This was also suggested by Free that many pheromones components release different behaviour patterns and so have different functions or different combinations of functions (Free, 1987).

Additionally, the relative proportions of the different components of a pheromones must vary at different distances from the same of secretions according to their volatility (Free, 1987). The behvioral responses of bees and other pollinators to each of the characterized components of these gland should be investigate, it may give us further information of their pheromonal functions.

In addition, the mandibular gland secretions may play the other important role such as chemical using to modified wax from abdominal gland of bee (Hepburn, 1986); moistening of olfactory membranes, colony defense by the use of an alarm pheromone,low concentration of 2-heptanone (Boch and Shearer, 1962; Heburn, 1986; Wilson, 1974).

From this research I have some suggestions that economically, the most important potential use of synthetic honeybee pheromones is to increase crop pollinations. It should be possible to use 1-eicosanol and other attractant substances to attract bees to the particular crops needing pollination. Perhaps some mixture of mandibular gland pheromones of honeybee foragers could be more effective. On the other hand, if insecticide is applied to crop while it is still in flowers, the repellent pheromones such as 2-heptanone or other repellent substances might be appropriate to apply in crop, it should be possible to repel foragers from it. Additionally, from the suggestions of Free in 1987, many of pheromone components release different behaviour patterns and so have different functions or different combination of functions. Moreover, the relative proportions of the different components of a pheromones must vary at different distances from the source of secretions according to their volatility. It is essential to test mixtures of the different components of the same pheromones as well as the individual components (Free, 1987).

Table 5.1 Comparison of the main components of mandibular gland pheromones of honeybee foragers in Thailand.

No.	*A. andreniformis*	*A. cerana*	*A. dorsata*	*A. florea*	*A. mellifera*
1	+	+	+	+	+
2	+	+	+	+	
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8					
9	+		+		+
10	+	+	+	+	+
11		+	+	+	+
12		+			
13				+	
14	*	*	*		+

1= Eicosanol
2=1-butanol-3-mthyl-acetate
3= Dibutyl phthalate
4= Nonadecane
5=2-hexyl-1-decanol
6= Heneicosanol
7= Eicosane
8=1-octanol
9=2-propyl-1-heptanol
10= 2-butyl-1-octanol
11= Heneicosane
12= heptadecane
13= Limonene
14= 2-heptanone
*= undectable level

Table 5.2 Summary of known functions of the honeybee forager mandibular gland pheromone components.

Pheromones	Repel foragers	Attracts foragers	Diminish stinging	Release stinging	Repel at hive entrance	Release alerting
1-butanol-3-methyl-acetate				+ (4,6)		+ (4,6)
2-butyl-1-octanol	+ (4)					+ (4)
Dibutyl phthalate		+ (4)	+ (2,)			+ (4)
Eicosane		+ (4)				
Eicosanol		+ (2,4)				+ (2,4)
Heneicosane		+ (4)				
Heneicosanol		+ (4)				
1-heptanol		+ (4)				+ (1,2,3)
2-heptanone	+ (1,2,3,4)				+ (4)	+ (1,2,3,4)
2-hexyl-1-decanol		+ (4)				
Limonene		+ (4,7)				+
Nonadecane		+ (4,7)				
1-octanol	+ (4,5)					+ (4,5)
2-propyl-1-heptanol		+ (4,5)				+ (4)

Identified by 1= Sherer and Boch, 1965
2= Free, 1982,1983,1987
3= Blum, 1984
4= This research
5= Kraus, 1990
6= Hepburn et al., 1994
7= Engel et al., 1996