CHAPTER 1

INTRODUCTION

1.1 RESEARCH RATIONALE

Foraging activity is very important for honeybees because they use this activity to find food sources, such as pollen and nectar from flowers (von Frisch, 1971). These food comprise of carbohydrate and protein (Seeley, 1985). This activity is only found in honeybee workers, known as foragers or foraging bees, the range for the onset of which has been recorded as low as 18.3 days (Sakami, 1953) and as high as 37.9 days of age (Winston and Ferguson, 1985). It is generally known that there are three castes in a honeybee colony, each caste having different activities. There is usually a single queen who lays the eggs in the colony and controls many colony activities by her production of pheromones, chemicals which strongly influence worker behaviour and physiology. There are several hundred or thousand drones present in the nest, their only known function is to mate with queens. The last caste is many thousands of workers which perform almost all of tasks in the colony, including brood rearing, construction; defense, foraging, thermoregulation, cleaning and many other tasks. In general younger workers tend to perform tasks within the colony and older workers do outside jobs like guarding and foraging (Seeley, 1985).

Five species of honeybees are found in Thailand, namely: *Apis andreniformis, A. cerana, A. dorsata, A. florea* (these four species are native to Thailand), and *A. mellifera*, the European honeybee which was introduced to Thailand for beekeeping (Oldroyd et al., 1992; Wongsiri et al., 1990, 1996[a]). According to my observations in the field, the foraging activities of the different honeybee species native to Thailand are quite different and also differ from the European honeybee, *A. mellifera*. It has also been reported that *A. mellifera* foragers use 2-heptanone to mark previously visited flowers to signal nectar depletion to other bees thus saving

time and energy (Engels et al., 1997; Guirfa, 1991; Hepburn and Radloff, 1996; Lensky, 1985; Maria and Leonard, 1985).

However, from my field observations, the four native species of the honeybees in Thailand do not appear to use pheromone-marking during foraging. Moreover, it has been established that they may revisit the same flower briefly after the first visit and they forage on the same flower simultaneously with several bees of their own species: *A. florea* as well as 2-3 bees of *A. cerana*, 1-2 bees of *A. dorsata* and 1-2 bees of *A. andreniformis* (See table1). Furthermore, it was found that more than one species foraged simultaneously in one flower, for example *A. florea* and *A. cerana* and sometime *A. cerana* foraged beside *A. andreniformis* (Oldroyd et al., 1992). These observations led to the hypothesis that it is likely that the four species endemic to Thailand do not use scent-marking during foraging.

Table 1. 1 The lapsing times (second) of the second bee revisited on the same flower after the first visit of honeybee foragers in Thailand.

No. of foragers	*A. andreni formis* (second)	*A. cerana* (second)	*A. dorsata* (second)	*A. florea* (second)	*A. mellifera* (second)
1	17	22	30	25	420
2	16	23	35	22	480
3	15	23	31	27	660
4	17	25	28	27	540
5	18	22	34	24	720
6	16	24	32	26	780
7	17	23	29	24	480
8	15	23	27	27	420
9	18	22	29	25	420
10	16	24	31	27	600
X ±SE	16.5±1.08	23.1±0.99	30.6±2.54	25.4±1.77	600±162.50

Fig. 1.1 *Apis florea* foragers forage on palm flowers.

Fig.1.2 *Apis dorsata* foragers forage on coconut flowers.

Because the foraging activity of the honeybees of each species is quite different, this research was designed to study the exocrine glands, which are thought to be related to this activity, especially the mandibular glands. *A. mellifera* is known to use this gland to produce pheromone and mark flowers during foraging (Free, 1987). It is also desirable to investigate the ultrastructure of these glands in the native Thai species of *Apis* and to compare them to *A. mellifera*, the imported species in Thailand. The other foraging activity among the native species are also different, for example in *A. dorsata*, which forages both during the day time and also after sunset, an activity never found in other species endemic to Thailand, but which has been recorded for African bees, *A. mellifera* (Hepburn and Radloff, 1998). Whether the ultrastructure of these glands are different and the native species in Thailand secrete 2-heptanone and/or other chemical compound with respect to foraging activity will be investigated in this research.

The second reason for undertaking this research is to establish whether the ultrastructure of the mandibular glands of *A. mellifera* differ among drone, queen and worker castes. Moreover, the mandibular gland pheromones have very different compositions and functions according to their castes. The queen's secretions act as both sexual pheromones (on the behavioural attraction of drones), and as inhibitory pheromones on worker ovaries. The main component of queen secretions is (E)-9-oxo-2-decenoic acid (9ODA) (Allan et al., 1987; Balerrma et al., 1996; Butler, Anderson and Holzer, 1964; Crewe and Hastings, 1976; Free, Ferguson and Simpson, 1988; Vallet et al., 1991), which is distributed in the colony by messenger workers (Seeley, 1979). Unlike the above, the mandibular gland pheromones of workers are the repellent substances that the foragers use to mark nectar depleted flowers, enabling other bees to recognize that a flower temporarily provides no forage. On the one hand, the mandibular gland of drones produce substances that attract others during mating behaviour and mark the flight path and drone congregation area during mating (Boch and Shearer, 1962; Blum et al., 1978; Engels et al., 1997; Ferguson and Free, 1979; Shearer and Boch, 1965).

So for these reasons, the mandibular glands in different castes of the honeybee have different functions in their colony and so they produce different pheromones.Likewise, foragers of the different species, *A. andreniformis*, *A. cerana*, *A. dorsata*, *A. florea* and *A. mellifera* are quite different in foraging activities. Therefore, they may have similar or different compositions in their mandibular gland pheromones. Furthermore, the ultrastructure of these glands may also differ.

The third reason for this investigation is that some researchers have shown that honeybees use a deterrent scent to mark recently visited and depleted flowers; however, the source of the pheromone is not confirmed (Balderrama et al.,1996) and it is suggested that possibly 2-heptanone, secreted by the mandibular gland, is the pheromone in question. This suggests that claims of marking-pheromones being secreted from mandibular glands are equivocal.

Since it is not clear whether honeybees can detect the nectar content and value of flowers until after probing, perhaps bees distinguish full from empty flowers visually, by intra-floral gradients of humidity, by the scent of nectar itself, and/or the possibility foragers of *A. mellifera* detect marking-pheromones thus avoiding empty flowers. In contrast, if the native species of the honeybee in Thailand do not use marking-pheromones, then it is possible that they detect the scent from flower odors and forage accordingly. This research will be investigated by studying whether foragers secrete marking-pheromones.

For this research, I have plane to study the mandibular glands of the workers of native honeybee species in Thailand and to determine whether they secrete 2-heptanone, or indeed, if the compositions is uniquely different from that of *A. mellifera.* Furthermore, the ultrastructure of the mandibular glands among these species may differ and this too will be investigated.

1.2 OBJECTIVES:

The main objectives of this research will be as follows:

1. To determine whether the ultrastructure of the mandibular glands among *A. andreniformis, A. cerana, A. dorsata, A. florea* and *A. mellifera* foragers are different in relation to their foraging activities.

2. To determine whether the mandibular glands of *A. andreniformis, A. cerana, A. dorsata, A. florea* and *A. mellifera* secrete 2-heptanone.

3. To determine whether the main components of the mandibular gland pheromones of *A. andreniformis, A. cerana, A. dorsata, A. florea* and *A. mellifera* foragers are different.

1.3 HYPOTHESIS

To accomplished the objectives of this research following hypothesis were tasted.

(i) Hypothesis I:

H0: The ultrastructure of the mandibular glands of the honeybee foragers in Thailand may differ.

H1: The ultrastructure of the mandibular glands of the honeybee foragers in Thailand may not differ.

(ii) Hypothesis H

H0: The mandibular glands of honeybee foragers of *A. andreniformis, A. cerana, A. dorsata* and *A. florea* do not secrete 2-heptanone. In *A. mellifera* may secrete 2-heptanone.

H1: The mandibular glands of honeybee foragers of *A. andreniformis, A. cerana, A. dorsata* and *A. florea* secrete 2-heptanone.

(iii) Hypothesis III

H0: The main compositions of mandibular gland pheromones of honeybee foragers in Thailand may be different.

H1: The main composition of mandibular gland pheromones of honeybee foragers inThailand may be not different.

1.4 BENEFITS OF STUDY:

Honeybees make their greatest contribution to the production of crops in that they facilitate cross-pollination. Recently, honeybees have been used to cross-pollinate fruit trees and field farm crops. Production has increased more than thirty times due to honeybee pollination. It is generally known that honeybees are social insects and that they exchange information using pheromones. From this research we may be able to use mandibular gland pheromones effectively to attract bees to a given location and also use this pheromone to control bee behaviour. For example, the repellent action can be use to prevent honeybees from foraging in farmlands during chemical spraying. Moreover, this research may provide more insights about the pheromones of each species in relation to foraging activity of honeybees and better define the important role of pollination by honeybees and better define the important role of pollination by increasing agricultural production in Thailand.