CHAPTER 2

LITERATURE REVIEWS

2.1 TAXONOMY OF HONEYBEES IN THAILAND

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Oder: Hymenoptera

Family: Apidae

Genus: *Apis*

Species: *Apis andreniformis*

Apis cerana

Apis dorsarta

Apis florea

Apis mellifera

2.2 HONEYBEES OF THAILAND

The genus *Apis* is apparently tropical in origin most likely India and Southeast Asia (Otis, 1991). This genus of bees is native to Asia, Africa and Europe including such continental islands as Japan, Taiwan, the Phillippines and most of the Indonesian archipelago (Seeley, 1985). Honeybee also thrive in North America, South America and Australia, but only since European introduced them at various times during the seventeenth to nineteenth centuries (Seeley, 1985). Today there are more than eight living species of *Apis* in the world. Nevertheless, the genus *Apis* in Thailand are classified into five species; the European honeybee, *Apis mellifera* Linnaeus,1758, which was introduced various time from Europe, Australia, USA and Taiwan, and the four indigenous species of the honeybees in Thailand, *A. andreniformis* Smith, 1858 (the smallest honeybee); *A. cerana* Fabricius, 1793 (Asiatic honeybee); *A. dorsata* Fabricius, 1793 (the giant honeybee); and *A. florea,* Fabricius 1787 (dwarf honeybee) (Wongsiri et al., 1990,

1996[a]). The ecology of these bees and their relevance to beekeeping are outlined below:

(i). The Asiatic Honeybee (*Apis cerana* Fabricius, 1793)

This species is expected to become most important beekeeping resource in Asia among the other native bees, more than *A. mellifera* colonies are kept in China and Vietnam. At least four subspecies of *A. cerana* have been identified (Ruttner, 1988). Its nest structure is similar to that of *A. mellifera* in that it is made by a multiple-comb nest in which the combs hang vertically in the nest cavity. Because *A. cerana* has not been domesticated to the same extent as *A. mellifera*, it holds some problems for apiculture such as high swarming and absconding rates, its parasitic and more limited honey storage ability in traditional beekeeing. However, they have strong resistance to *Varroa jacobsoni* and predaceous *Vespa* (Kerr et al., 1974). The Asiatic honeybee closely resembles *A. mellifera* morphologically and in nesting behaviour. These two species of bees are closely related to each other, but are clearly separated species (Ruttner, 1988; Wongsiri et al., 2000).

(ii). The European Honeybee (*Apis mellifera* Linnaeus, 1758)

The European honeybee, the most widely known *Apis* species is the western hive bee. Its native distribution extends from Europe as far North as the Southern Norway and into all of Africa except its great desert areas. This species builds multiple combs nest that consists of several parallel vertical comb. This species is found in diverse climatic regions ranging from cold temperate to tropical zones. They produce valuable apicultural products and sustain commercial beekeeping in Thailand and other parts of Asia (Crane, 1990; Ruttner, 1988; Seeley, 1985; Wongsiri et al., 2000).

(iii). The Small Dwarf Honeybee (*Apis andreniformis* Smith, 1858)

This species was recognized as a separated species from *A. florea* and a new record found in Thailand by Wongsiri et al in 1990. They were first rediscovered in South China in the same habitat as *A. florea* and named as *Microapis andreniformis* (Wongsiri et al., 1996[a]). The observation that, in Thailand, this species is reproductively isolated from *A. florea* supported the recognition of this bee as an independent species. However, it was only a few years ago that two separate dwarf

honeybee species were clearly differentiated. In body size of *A. andreniformis* are smaller than *A. florea*. *A. andreniformis* nest is a single comb (Wongsiri et al., 1990, 1996[a], 2000).

(iv). The Dwarf Honeybee (*Apis florea* Fabricius, 1787)

This small bee builds a single comb in small trees of the Asia tropics. They are small in body size and have an ability to survive in very hot, dry climates. The single comb nest contains cells of four sizes. The large storage cells for honeybee are very deep and constructed in such a manner that the comb bulges out on either side and at the top. The small worker cells (2.7-3.1mm) are located below the honey storage cells. Corresponding to the decreasing body size of the bee from North to South, the diameter of worker brood cells shows a similar geographic variability. Although the considerably larger drone cells (4.2-4.8 mm) are mostly found in the lower part of the comb, the pear shaped queen cells, which are the largest of all cells are located near the bottom (Ruttner, 1988). Moreover, this species applies a sticky resin-like substance to branches to support its comb and which prevents ants and other insects from invading its nest (Wongsiri et al., 1990, 1996[a], 2000).

(v). The Giant Honeybee (*Apis dorsata* Fabricius, 1793)

This is the large-sized bee species that is found in Thailand and Southeast Asia. The nest is made as a single comb about 1-2 meters long, approximately 0.5m high on thick branches 20 to 40 cm in diameter on the upper parts of large trees from 30 to 60 m high. Other preferred nesting site is beneath overhanging rocks, on cliffs or in cavities of large buildings (Lindauer, 1961). In general, several nests are formed aggregately. Some populations of this species of honeybee are very aggressive (Wongsiri et al., 1990, 1996[b]). It is found throughout India and Southeast Asia including Palawan, Borneo, and the string of islands of Indonesia from Sumatra to Timor and Estward to the Kai Islands (Otis, 1991). The queen, workers and drones of this species are all produced in cells which are similar in size and shape (Richards, 1953). The average cell diameter measurement of this species is 5.42-6.35 mm. (Dietz, 1992; Graham, 1992)

From the evolution of *Apis* species are classified into two groups based on the various nest types: (i) the single comb, open-air nesting bees comprising *A. andreniformis, A. dorsata* and *A. florea,* which are restricted to the Asia tropics and subtropics. The other group which has multiple-combs and cavity nesting which includes *A. cerana* and *A. mellifera.* This latter group is able to colonize cold climate zones without losing the ability to complete in tropical climate. It seems likely that this rough division of the genus based on nesting behaviour make sense also because of the ecological implications (Seeley, 1985; Wongsiri et al., 1991, 2000; Otis, 1990)

It has been shown that essential physiological differences exist between the cavity nesting species, *A. cerana* and *A. mellifera* compared to *A. andreniformis, A. florea* and *A. dorsata* (Ruttner, 1988). The former fly faster and have a high thoracic temperature than the latter. For these reasons *A. mellifera* which forages flower in cold climate, has relatively little time to accumulate surplus resources use pheromones to mark nectar depleted flowers and thus signal other bees to avoid them. Given totally different climate circumstances, the native species of the honeybees in Thailand may not use pheromones to mark flowers during foraging.

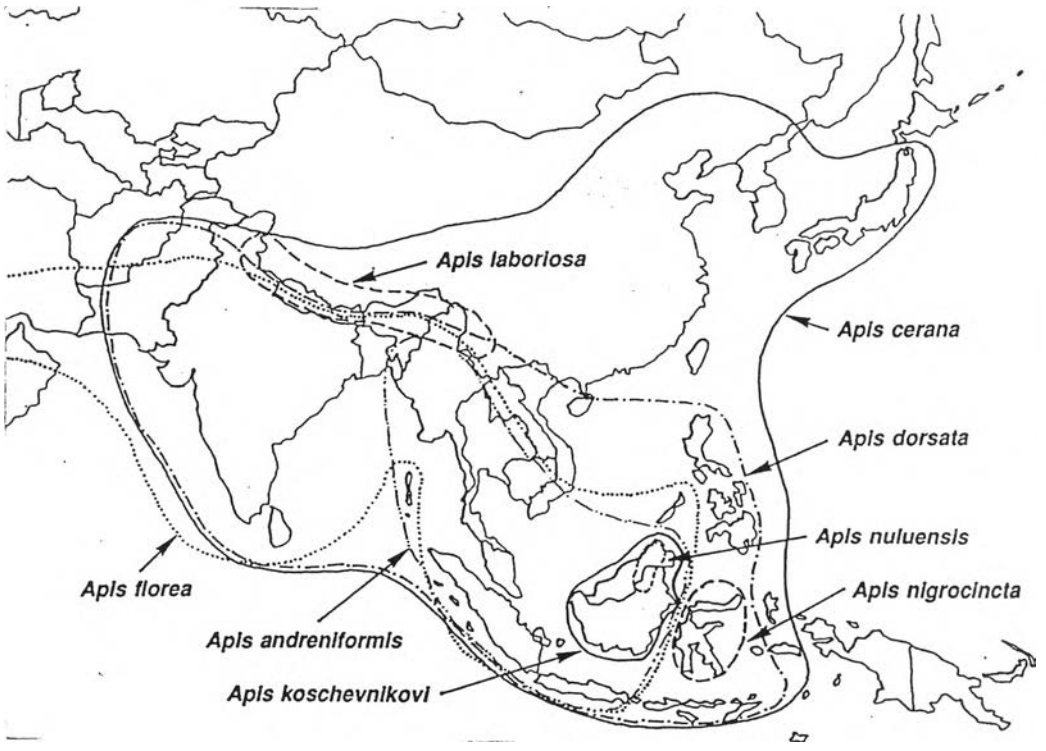


Figure 2.1 Distribution areas of bees in Asia (Yoshida, 1999).

2.3 BIOLOGY OF HONEYBEES

2.3.1 Caste Development and Differentiation:

There are three types or castes in a honeybee colony, the female queen and workers, and the male drones. There is generally one queen, who lays eggs in the colony and controls many activities by pheromones. There are a few thousand drones which have only one task, mating with queens. Workers perform almost all of the works in the colony, including brood rearing, construction, defense, thermoregulation, cleaning, foraging and many other tasks. In general younger workers tend to perform within the colony tasks are called house bees and the older workers do outside jobs like foraging are called field bees (Graham, 1992; Seeley, 1985; Winston, 1992; Winston and Ferguson, 1985).

All of the honeybee castes pass through the same four stages during their development: egg, larva, pupa and adult. An egg laid by the queen has a potential to develop into any one of the three castes and its developmental direction is determined by both genetic and nutritional factors. Usually unfertilized eggs develop into drones while fertilized eggs can develop either into workers and queen; female differentiation depends on the type of food that developing larva is fed (Graham, 1992; Winston, 1992). The timing of bee development depends on the caste of the developing individuals well as environmental and genetic factors. All three castes have egg stages which average 3 days, the remainder of development shows different timing. For example, in *A. mellifera,* the mean duration of uncapped larval period is about 4.5 days for queens, 5.5 days for workers and 6.3 days for drones. The total development times average 16, 21 and 24 days for queen, workers and drones, respectively (Graham, 1992; Seeley, 1985; Winston, 1992). In the case of workers, there are four clearly defined age-related polyethism among them. The first is a cell cleaner, comprise bees of age 0-2 days of age. The second consist of workers 2-11 days old and is a brood nest group. Workers in the third group, age 11-20 days constitute a food storage group, where tasks occurs in the peripheral, food storage region of the nest. Finally, the fourth group contains only foragers, bees which are generally 20 or more days old which work outside the nest (Seeley, 1985).

Temporal polyethism in honeybees is closely linked to glandular development and resorption, particularly for the brood food and wax glands. The hypopharyngeal, mandibular and wax glands begin to enlarge shortly after a young worker emerges, reaching and maintaining their maximum size at 5 to 15 days of age (Ribbands, 1953). Pheromone producing glands show a similar, age based relationship with task; alarm pheromone production is low in younger bees and rises as workers age (Boch and Shearer, 1962). The most important functions which have been attributed to the secretions from worker mandibular glands are as follows; production of larval food by nurse bees; worker colony defense by the use of alarm pheromones by guardian bees; 2-heptanone marking of visited flowers as empty flowers by foragers which volatile substances and repelling other bees (Wilson, 1974; Ferguson and Free, 1979).

2.3.2 Foragers and Foraging Activities

Foraging activity of the honeybee foragers is a social enterprise. This activity is very importance for finding and exploiting rich sources of nectar and pollen by the group of workers known as **foragers**. Even through honeybees forage on many different species of flowers at any particular time, the size of foraging area is quite variable, depending upon the number and density of available flowers, their nectar and pollen content and the amount of competition provided by the other foragers or other insects. The rate at which bees visit flowers and the time they spend on each flower, depend on the amount of nectar and pollen present. This varies with the type of flower, stage of development, climate conditions and the degree of competition from other foraging insects. It was reported that trips for pollen collection were considerably shorter in time than that for those of nectar collection. Actual time will vary according to many factors e.g. weather conditions, distance of the food sources from the hives (Gary, 1992; Graham et al., 1991; Seeley, 1985).

Many reports showed that foraging bees use a marking-pheromones during foraging, 2-heptanone which is secreted from mandibular glands of *A. mellifera* workers. They used this chemical to mark the nectar-depleted flowers to warn the other bees to avoid revisiting the empty flowers thus saving time and energy (Butler, 1940; Giurfa, 1991; Reith et al., 1986; Simpson, 1966).

2.4 MANDIBULAR GLANDS AND THEIR PHEROMONES

2.4.1 Biology of Mandibular Gland

The mandibular glands of honeybees are classified as pheromone-producing exocrine gland whose products can be secreted to the exterior or the outside of bees. All compounds are produced in the glands that secrete to the exterior are called *semiochemicals*, this term includes the pheromones which are utilized for communication between individuals of the same species or interspecific communication (Blum, 1992; Graham, 1992). The mandibular glands of honeybees are paired, large saclike structure located in the lateral part of the head capsule. The ventral part is closely related with the proximal part of the mandibles where the secretory opening is located and which serves to drain the glandular secretions toward a groove (Snodgrass, 1925). They were first described as " olfactory mucous glands" and that their secretion served to keep moist certain sense organs on the epipharynx. In addition, this organ had olfactory function because the secretion had a effective smell (Ferguson and Free, 1979; Ribbands, 1953).

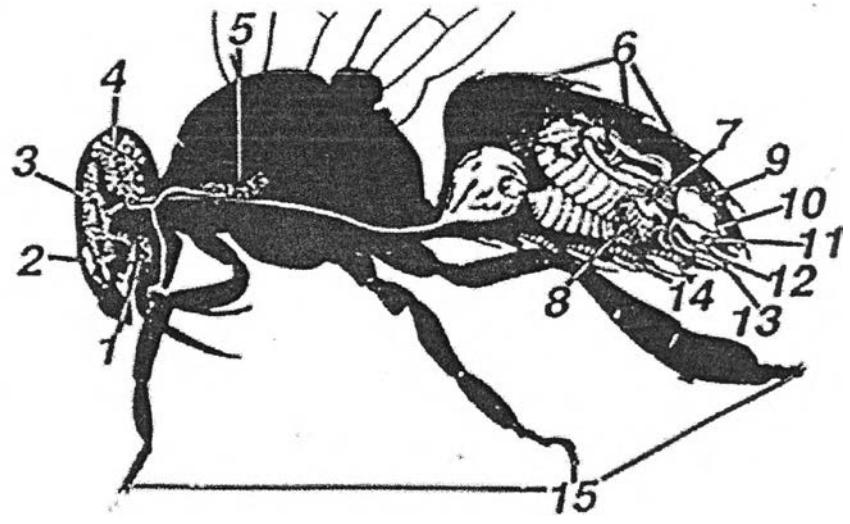


Fig. 2.2 The exocrine glands of the honeybee worker (Gary, 1992).1, Postgenal gland; 2, Mandibular gland; 3, Hypopharyngeal gland; 4, Cephalic gland; 5, Thoracic labial gland; 6, Tergal gland; 7, Reserviour of poison gland; 8, Poison gland; 9, Nasonov gland; 10, Rectum; 11, Kochevnikov gland; 12, Dufour's gland; 13, Sting shaft with setose membrane; 14, Wax gland; 15, Tarsal gland.

Moreover, it was shown that the mandibular glands had an odourous secretion analogous to that of the anal gland secretions of certain ants and usually function as alarm pheromone (Blum, 1969). *A. mellifera* workers and queens have a similar anatomy and general structure of the mandibular glands (Butler, Anderson and Holzer, 1964; Maria and Leonard, 1985; Moritz and Crewe, 1991; Plettner et al., 1993; Snodgrass, 1925).

They are well developed in workers, very large in queens and in contrast very small in drones (Fig2.2). Furthermore, they do not vary in size with age or division of labour (Butler, Anderson and Holzer, 1964; Maria and Leonard, 1985; Moritz and Crewe, 1991; Plettner et al., 1993; Snodgrass, 1925). Each gland consists of an aggregate of secretory cells and a reservoir with its associated efferent duct. Each of the secretory cells possesses ductules which open into the apical portion of the reservoir. The efferent duct terminates in a slit-like pore located at the base of the mandible. The opening and closing of the duct is accomplished by chitinous plates which are in turn control by apidomes (Butler , 1975).

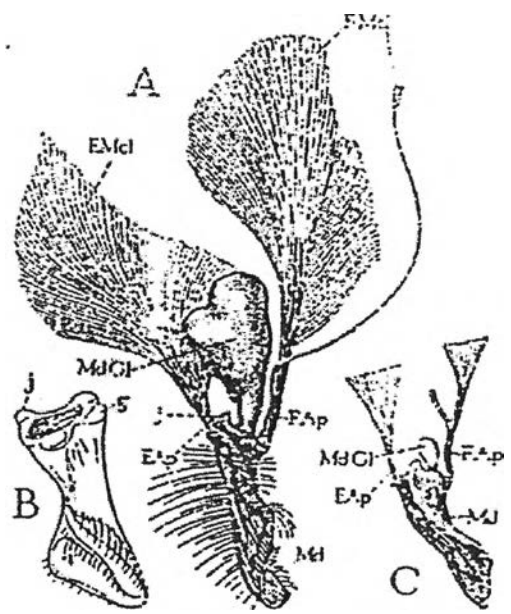


Figure 2.3 The mandible and mandibular gland of worker and drone of honeybees , *Apis mellifera* (Snodgrass, 1925).A, EAp and FAp, apodeme; Emcl; extensor muscle; FMcl, flexor muscle; MdGl, mandibular gland. B, g, posterior anticular surface; j, anterior anticular surface. C, Anterior view of right mandible and mandibular gland of drone.

Moreover, histological and ultrastructural study was investigated by Lensky and his co-workers in 1985, the results of which showed that the structure of the tiny mandibular gland varied according to age of *A. mellifera*. Its secretory activity in 0-3 days old drones was evident from the abundant rough endoplasmic reticulum. After seven days th gland was fully developed. Five days later the gland was no longer active and showed an autolytic process: the product was stored in the gland lumen for further emission during drone mating flight (Lensky et al., 1985).

Ultrastructure of mandibular glands were also studied by Vallet and his co-workers in 1991 in *A. mellifera* workers. Their result showed that the glandular unit or secretory units of these glands consist of two kinds of cells. First is *a cell with a canal* (*duct cell*), which opens in the axial glandular cavity. The wall of the duct is made only of epicuticle. The other is a *large polyploid glandular cell* with an apical secretory reserviour (Diameter 0.9μm). Moreover, they also reported that the glandular unit of the mandibular gland were provided into three kinds of cells.

(i) *Epithelial cells*: the cytoplasm of these cells contain only mitochondria, short cisternae of rough endoplasmic reticulum, but cytoskeleton were well developed

(ii) *The duct cells*: (diameter 25μm). They reduced cytoplasmic mass contained only scarce mitochondria. The lenticular nucleus.

(iii) *Glandular cells* or *secretory cells*: The giant spherical or ovoid polyploid nucleus with a sineus outline is located in the basal part. It contained numerous chromatin clumps stuck to the inner membrane of the nuclear envelope.

The mitochodria are major components of these cells; however, less detail about study in ultrastructure of native species of honeybees in Thailand. Then, this research has plane to investigate this group and compare them to the imported species, *A. mellifera* (Vallet et al, 1991).

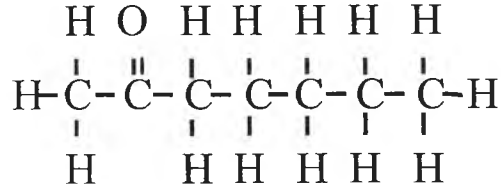
2.4.2 Mandibular Gland Pheromones

Pheromones are chemical compounds secrete to the exterior and function as messengers between individuals of the same species. These chemical or signaling agents carry information to receiver individuals and their response to the chemical message is considered to be favorable to the emitter. A pheromone may consist of a single chemical compound or a mixture compounds.

The secretions of the mandibular glands of queens, workers and drones of *A. mellifera ligustica* contain various biologically active substances, especially the queen bee gland secretions (queen substances) which act as both a sexual pheromone for drones and as an inhibitory pheromone on the ovaries of workers, as well as affecting social activities such as retinue behaviour of workers. The major component and the most important of the queen mandibular gland secretion is 9-oxo-trans-2-enoic acid (9ODA) (Butler, Anderson and Holzer, 1964; Engels et al., 1997; Free, 1987; Free, Ferguson and Simpson, 1988; Plettner et al., 1993). The secretory product of the worker's mandibular glands has an oily appearance. The major component is 2-heptanone, a volatile substance which accumulates in the central reserviour, the amount of which progressively increases with increasing age (Engels et al., 1997). This compound showed effect of repelling guard bees and foragers. It is probable that a foraging bee marks a visited flower with 2-heptanone thus signaling other bees as to nectar depleted flowers (Balerrama et al., 1996; Boch and Shearer, 1971; Blum et al., 1978; Crewe and Hasting, 1976; Engels et al., 1997; Guirfa, 1991).

Moreover, it was found that the mandibular gland pheromone from workers of *A. mellifera* consists of two majors components: 10-hydroxy-(E)-2-decenoic acid (10HDA) and 10-hydroxydecenoic acid (10HDAA) (Free and Ferguson, 1988; Hepburn, Jone and Kirby, 1994; Lensky et al., 1985; Plettner et al., 1993; Vallet et al., 1991). However, Rottener and his coworkers discovered that *A. mellifera capensis* workers were capable of producing 9-keto-(E)-2-decenoic acid (ODA). In 1996, Hepburn and Radloff reported that the mandibular gland secretions of *A. mellifera capensis* were composed of methyl-4- hydroxy benzoate, 4-hydroxy benzoic acid, decenoic acid, (E)-9- ketodecen-2-enoic acid, 2-4-hydroxy-3-methoxy

ethanol, tetradecenoic acid, 10-hydroxydecenoic acid and hexadecenoic acid (Hepburn and Radloff, 1996). Matsuyama and his coworker reported that the mandibular gland secretions of *A. cerana japonica* was composed of R-(-)-3-hydroxy octanoic acid (Matsuyama, Suzuki and Sasagawa, 2000).



Formula; $C_7H_{14}O$ and M.W.=114

Fig.2.4 Chemical structure, formula and molecular weight of 2-heptanone.

Previous investigations have shown that the mandibular gland of honeybee workers of *A. mellifera* both Africanized and European honeybees, produce 2-heptanone and this increases with age (Sakamoto et al., 1990). Foragers with pollen showed a higher 2-heptanone concentration than foragers without pollen and guard bees (Sakamoto *et al.*, 1990). Some researchers found that high concentrations of 2-heptanone in the worker mandibular glands of *A. mellifera* were positively correlated with amplified of aggressive behaviour at the hive entrance (Balerrama et al., 1996; Boch and Shearer, 1971; Crewe, 1970; Engels et al., 1997; Free et al., 1974; Free, Ferguson and Simpson, 1988; Hepburn, Jone and Kirby, 1994; Kerr et al., 1974).

Additionally, it was reported that 2-heptanone, a forage marking-pheromone secreted from worker mandibular gland varies in bee workers of different age. Africanized and European honeybee colonies were studied. Younger bees produced no 2-heptanone or produced it at almost levels undetectable, by using GC-analysis. Moreover, 2-heptanone production increased with age. The amount did not different significantly between Africanized honeybee and European honeybee colonies (Sakamoto et al., 1990). The level of 2-heptanone progressively increased with age, peaking in foragers (Lensky et al., 1985)

Maschwitz (1964) suggested that mandibular glands produce alerting pheromones, although a less effective one than the sting pheromones. Shearer and Boch (1965) identified 2-heptanone from mandibular gland secretions and when filter papers carrying 2-heptanone were put at the hive entrance, the guard bees were alerted and attacked them. This is consistent with suggestions by Boch and Shearer (1965) that 2-heptanone has two pheromonal functions; alarm, with lower efficacy than that of the sting apparatus and releasing sting behaviuor as efficiently as isopentyl acetate; the second function includes some repellent properties affecting foraging bees. Although 2-heptanone is generally considered as an alarm pheromone (but the report from Vallet et al. In 1991 did not support this), it also showed the effects of repellence toward guard and foraging bees. It was also found that compound elicited both repellent and attractant responses in bees: 2-heptanone may be repellent at high concentrations and is probably always deposited when a bee visits flowers, but is an attractant at low concentrations (Boch and Shearer, 1971; Kerr et al., 1974; Shearer and Boch, 1965; Vallet et al.,1991).

Therefore, in 1966, Butler suggested that the mandibular gland also presences some repellent properties, affecting foraging bees which are due to presence of 2-heptanone in the glands. Indeed, 2-heptanone was applied to alfalfa flowers, a short term repellence to foraging bees was observed (Butler, Anderson and Holzer, 1964; Reith et al., 1986). In contrast, Free and Simpson (1968) found that 2-heptanone was as effective as isopentyl acetate in eliciting attacks on cotton balls. As the mandibular gland used for grasping an intruder it seems likely that the main function of 2-heptanone is to label the intruder to be attacked. However, in 1970 Boch and Sherer found that isopentyl acetate was 20-69 times more potent than 2-heptanone in alerting bees, so such a function for 2-heptanone seems doubtful (Boch and Shearer, 1971; Free, 1984).

Guirfa and his co-workers were the first to analyze the problem of foraging oreintation by mean of pheromones. They demonstrated that individual honeybee, *A. mellifera ligustica* foragers mark with a repellent scent recently visited and nectar depleted flowers to save time and energy while foraging. Moreover, they also

showed that honeybee foragers can recognize and respond to the repellent scent mark left by other individuals (Giurfa, 1991).

Therefore, it was suggested that the mandibular gland of nurse bees of *A. mellifera* worker produced 10-hydroxy-(E)-2-decenoic acid, this compound is the main component of the brood food fed to larvae. In addition, simple fatty acid (e.g. hexanoic, octanoic acid) are produced in this secretion, and it is suggested that these compound may contribute to the antibiotic activity of royal jelly (Boch et al., 1975), which was previously identified with 10-hydroxy (E)- 2-decenoic acid (Blum *et al.*, 1959). When workers become guard bees or begin foraging, they produce a very odourous compound, 2-heptanone in their mandibular glands. The glandular content of this compound, which can reach 40 µg/bee, is dependent on the Physiological rather than the chronological age of the bee ((Boch and Shearer, 1962). The repellent effect of 2-heptanone for bee workers by applying this compound to the hands before opening a hive has also been reported. At a concentration of 0.5-2.0%, bees are effectively repelled and they do not exhibit aggressive behaviour. Curiously, 2-heptanone stimulates food hoarding behaviour in bees (Rinderer, 1982) in much the same way as do volatile a compound in used comb (Free and Willium, 1982; Rinderer, 1982), the real significance of this activity for 2-heptanone is not known.

Furthermore, this compound is reported to inhibit workers near the queen is a swarm from secreting a pheromone that normally guides workers to the swarm (Morse, 1972). Since 2-heptanone is produced in the mandibular glands of workers, it may identify robbers (Simpson, 1966) and attract workers to the intruder. Similarly, a foreign queen could be bitten and marked with 2-heptanone and thus labeled for attack by other workers (Boch and Shearer, 1971; Kerr et al., 1974; Shearer and Boch, 1965).

In 1991 Giurfa and Nunez reported that flower-marking by the honeybees (*A. mellifera ligustica*) while they were foraging on a artificial patch of flower yielding a continuous and equal flow of sucrose solution. Honeybees marked with scent and rejected all recently visited and nectar depleted flowers (Giurfa, 1991).

2.5 ELECTRON MICROSCOPY

A great contribution to biology has been the electron microscope in fine structural studies of cell organelles. One of the important generalizations to emerge from the explosive advances in knowledge of cell fine structure concerns the ubiquity of complex membrane system. The various organelles such as mitochondria, plastics and Golgi apparatus emerge as sharply defined objects with intricate internal structures. A number of previously unknown organelles have also been found (lysozomes). This detail is seen because of the thousand fold increase in resolving power of electron microscope. There are two major types of electron microscopes, TEM (Transmission Electron Microscope), which has resolution about 0.2-0.4 nm and SEM (Scanning Electron Microscope), which has resolution about 2-4 nm (Annpreece, 1972).

2.5.1 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a wildly useful accepted technique for obtaining accurate and undisturbed image of fine structure in biological specimens. This technique was developed by Knoll in 1935. This technique uses solid pieces of tissues rather than sections and allows the perception of three dimension views of the sureface of the cells or tissues. An electron beam then scans the specimen and electron produced from the sureface are used to reconstruct a fine three dimensional representation of the sureface. The specimen before examined by SEM need careful preparations, cutting in piece approximately 2 mm2, fixing by glutaraldehyde and post-fixed by osmium tetroxide, dehydration by grade series of ethanol (50-100%), critical-point drying (CPD), mounting on stubs, coating with gold in an ion sputtering (Annpreece, 1972). This research was used this technique for studying the surface of the mandibular glands of the honeybee foragers. Moreover, the micro-scale in SEM was used for measuring the sizes of mandibular glands (Annpreece, 1972).

2.5.2 Transmission Electron Microscopy (TEM)

This technique is used for studying the cellular morphology to the macromolecular level from ultra-thin tissue sections in the range of 10 to 90 nm thick Their preparation involves careful fixation and embedding of the tissues in plastics. Specimens are most generally fixed in osmic acid or double fixed in glutaraldehyde-osmic acid, dehydrated with acetone or alcohol series, embedded in liquid plastic, polymerized to the solid state, and cut on a special ultramicrotome (Annpreece, 1972).

2.6 GAS CHROMATOGRAPHY (GC) AND GAS CHROMATOGRAPHY MASS SPECTROSCOPE (GC-MASS)

These two techniques have been used for studying qualitative and quantitative of mandibular gland pheromones of *Apis mellifera* by Lensky et al, 1991 and others, but never study in honeybees in Thailand before.

The power of GC arises from its capacity to determine qualitatively many individual component presents in a mixture in one, single analytical procedure. Its versatility cames from its capacity to handle a very wide variety of samples that may be gaseous, liquid or solid in nature. In addition, the sample can range in complexity from a single substance to a multi-component mixture containing widely differing chemical species (Scott, P.W.R., 1994). The separated components of a sample mixture are characterized by individual retention time and retention (Braun, 1987; Scott, 1994; Skoog and West, 1980).

If gas chromatography, which can be recorded at different carrier gas flow rates, are to be compared, it is advantageous to use retention volumes rather than retention times, because retention volumes are independent of flow rate. Retention times are inversely proportional to flow rate. A separation process that achieved by the distribution at substance between two phase, a stationary phase and a mobile phase. Those solutes distributed preferentially in the mobile phase will move more rapidly through the system than those distributed preferentially in stationary phase. Thus, the solutes will elute in order of their increasingly distribution coefficients with respect to the stationary phase (Braun, 1987; Scott, 1994; Skoog and West, 1980).