

*Chapter 1*

## **BIOLOGY OF THAI HONEYBEES: NATURAL HISTORY AND THREATS**

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### **Abstract**

Honeybees play an important ecological role as pollinators of many plant species, and their products are the basis for a multi-million dollar commercial industry in the US and more than a thousand million Bath in Thailand. This chapter provides a summary of the natural history of Thai honeybees. We focus on the role of Thai honeybees in pollination ecology, potential threats to honeybees, and commercial applications of products derived from honeybees. This chapter covers how honeybees reproduce, variation in caste development among Thai species, and how sex determines the division of labor in these populations. The discovery of the oldest bee species and the evolution of honeybees also will be discussed. In addition to behavior related to nesting and colony defense. We will also examine honeybee pheromones: how honeybees produce pheromones, how they detect pheromones and other odorants, and how they respond after exposure to pheromones. Finally, we will focus on the role of parasites (i.e., wax moth, mites), predators and pathogens on the ecology of Thai honeybees, and explore the consequences for honeybee commercial product and pollination.

There are seven sections in this chapter on Thai honeybees, 1) natural history, evolution and taxonomy; 2) castes, development, and age polyethism; 3) anatomy, including the structure of the pheromone glands and exocrine glands; 4) olfaction, odor production, odor perception and pheromones, 5) pollination, 6) beekeeping, and 7) honeybee pathogens, parasites and predators. Our goal is not to exhaustively discuss each topic, but provide relevant information about native species of Thai honeybees in regional context. Much more information is known about the European honeybee (*Apis mellifera*) than other species of *Apis*. In some of these sections, we will therefore be necessarily brief.

## 1. NATURAL HISTORY, EVOLUTION, & TAXONOMY

### Life history of Thai honeybees

In recent years, interest in tropical bees has increased. This is appropriate because honeybees like originated in Tropical Africa and spread from South Africa to Northern Europe and East into India and China (Otis, 1990). The first bees appear in the fossil record in deposits dating about 40 million years ago in the Eocene. The oldest bee fossil is preserved in a piece of amber found from a mine in northern Burma. It is believed to date back as far as 100 million years to the time when bees and wasps split into two different lineages. The fossilized insect appears to share features both common to the bee and wasp, but is more similar to bees than wasps (Danforth et al., 2006). The earliest known honeybee fossil (genus *Apis*) was found in Europe dating back 35 million years. About 30 million years ago, honeybees appear morphologically very similar to modern honeybees (Koning, 1994). The genus *Apis* is evidently tropical in origin. It is native to Asia, Africa and Europe including such continental islands as Japan, Taiwan and the Philippines (Seeley, 1985). Honeybees did not appear in the Americas, Australia or New Zealand until European settlers introduced them in the 17th century (Zander and Weiss, 1964). Thailand is a tropical country that has a wide variety of flowering plants and animals. Perhaps due to high temperature, the native tropical bees generally build their nest as a single air open nest and rely upon aggressive behavior to defend these exposed nests (Collins and Kubasek, 1982).

Honeybees of the genus *Apis* are the most studied because of their fascinating and complex lifestyle, communication systems (Nieh, 1998; Nieh and Roubik, 1995), role as keystone pollinators of native plants, pollination of agricultural crops, and the valuable hive products that they produce, such as honey, royal jelly, bee wax, bee pollen, propolis and even bee venom. Honeybees belong to the order Hymenoptera, superorder Apocrita, infraorder Acuelata, superfamily Apoidea, family Apidae, subfamily Apinae, tribe Apini. There are more than 11 extant species of *Apis* worldwide (Michener, 2000). Four species are native to Thailand: *Apis andreniformis*, *A. cerana*, *A. dorsata* and *A. florea* (Oldroyd and Wongsiri, 2006). *A. mellifera* was introduced for beekeeping. Honeybees of Thailand are classified as follows:

Kingdom Animalia  
 Phylum Arthropoda  
 Class Insecta  
 Order Hymenoptera

## Family Apidae

Genus *Apis*Species *A. andreniformis**A. cerana**A. dorsata**A. florea**A. mellifera* (Ruttner, 1988)

Honeybees are hymenopterans, a group that generally feed on pollen and nectar and constitute about 20,000 species throughout the world, known taxonomically as the superfamily Apoidea (Michener, 2000). Although the question of how many honeybee species exist is still debated among taxonomists, at least four species are commonly recognized: the dwarf or midget bee (*A. florea*), the giant or rock bee (*A. dorsata*), the Asian bee (*A. cerana*), and the common European honeybee (*A. mellifera*). The existence of another giant bee (*A. laboriosa*), was recently confirmed in Nepal, but little is known about its biology (Seeley, 1985; Otis, 1990). *Apis* species are classified into two groups, based upon nesting. The first group builds single comb, open-air nests: *A. andreniformis*, *A. florea*, *A. dorsata*, *A. breviligula*, *A. binghami* and *A. laboriosa*. These bees are restricted to the Asian tropics and subtropics. The second group consists of species that nest inside cavities where they build multiple combs: *A. cerana*, *A. koschevnikovi*, *A. nigrocincta*, *A. nuluensis*, and *A. mellifera* (Hepburn and Radloff, 2011; Michener, 2000).

**Honeybees that build single-comb, open-air nests**

The architectural design of the comb of all honeybee species is essentially similar. It consists of adjoining hexagonal cells made of wax secreted by the workers' wax glands. The bees use these cells to rear their brood and to store their food. The general utilization of comb space is also similar among the species. Honey is stored in the upper part of the comb. Beneath the honey storage area, there are commonly rows of pollen-storage cells, worker-brood cells, and drone-brood cells, respectively. The larger queen cells are normally built at the lower edge of the comb (Seeley, 1985; Wongsiri et al., 1991; Otis, 1990).

**The black dwarf honeybee, *Apis andreniformis* Smith, 1858**

*Apis andreniformis* was reported in Thailand by Wongsiri et al. in 1990 from the coastal flats and near the foothill areas (1-100 meter above sea level) of Chantaburi province, Thailand to high mountainous and forest areas (approximately 1600 m altitude) in the northern parts of Thailand (Wongsiri et al., 1996a). This species was rediscovered in South China in the same habitat as a. It was the fifth honeybee species to be described of the eleven known *Apis* species, and its biology and geographic distribution remain relatively poorly understood (Maa, 1953; Turgari, 1971; Wongsiri et al., 1996a; Wu and Kuang, 1987). Only recently, this species has been diagnostically separated from the closely related *A. florea* because there are sites where both species live conspecifically. Both species are distributed throughout tropical and subtropical Asia, including Southeast China, India, Burma, Laos, Vietnam, Malaysia, Indonesia (Java and Borneo), and the Philippines (Palawan) (Akrotanakul, 1976; Maa, 1953; Otis, 1990; Turgari, 1971; Wongsiri et al., 1996; Wu and Kuang, 1987). In body size, *A. andreniformis* is smaller than *A. florea*.

Morphologically, *A. andreniformis* has stripes of black hairs on the metathoracic tibia and on the dorsolateral (back and side) surface of the metathoracic basitarsus (Wongsiri et al., 1996a). Additionally, the pigmentation of *A. andreniformis* is blackish, while that of *A. florea* is yellowish. Other distinguishing characteristics include a difference in cubital indexes, which is the ratio of two of the wing vein segments of honeybees. The pattern of the veins of the fore wings is specific for each breed of bees. The cubital index is consistent for a given race of bee. It can be used to distinguish between similar populations of honeybees and to determine degrees of hybridization: *A. andreniformis* has an index of 6.37, and that of *A. florea* is 2.86. The proboscis of *A. andreniformis* has a length of 2.80 mm, while that of *A. florea* is 3.27 mm. This physical difference may contribute to differences in the types of flowers visited by different species. Finally, there are differences in the barbs of the stinger, and in the basitarsus of the drones (Rinderer et al., 1995; Wongsiri et al., 1996a). *Apis andreniformis* nests in quiet forests, generally in darker areas where there is 25 to 30% of ambient sunlight. The hive is built in the branches of trees or shrubs usually 1-15 m above ground, although the average height is 2.5 m. The nest typically ranges from 70-90 mm in size (Wongsiri et al., 1996a).



Figure 1. The single open nesting of *Apis andreniformis*.

Almost nothing is known about the recruitment communication behavior of *A. andreniformis*. However, all studied *Apis* species can waggle dance to communicate food location (Nieh et al., 2003), and thus it is highly likely that *A. andreniformis* also uses this behavior (Dyer and Seeley, 1991; Dyer, 2000; 2002). Given, its similarity with *A. florea*, and the fact that it also builds single-comb, open nests it is reasonable to expect that *A. andreniformis* also waggle dances on exposed comb. Drone “dances” have been observed on the open comb surface of *A. andreniformis* nests (Wongsiri et al., 1996a), although the

significance of this behavior is unclear. Further studies of this species' communication abilities are needed.

### **The red dwarf honeybee, *Apis florea* Fabricius, 1787**

As its name implies, the dwarf honeybee is small in body size. This species does well in very hot, arid climates. A nest of *A. florea* consists of a single comb, typically built in small trees. *Apis florea* nests in the open, but nests are camouflaged. Most nests are hung from slender branches of trees or shrubs covered with relatively dense foliage, from 0.3 to 8 m above the ground (Hepburn, and Radloff, 2011; Wongsiri et al., 1996a). In Oman, where *A. florea* nests are frequently found in caves, such combs lack the crest that is the honey storage area and that surrounds the branch on which the comb is suspended (Akratanakul, 1976). *Apis florea* is generally distributed throughout Thailand and it is an economically important species as well as important to crop and wild plant pollination. The single comb nest contains cells of four sizes. The large storage cells for the honey 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 (2.7-3.1 mm) are located below the honey storage cells. The considerably larger drone cells (4.2-4.8 mm) are mostly found in the lower part of the comb. The pear shape queen cells, which are the largest of the cells, are located near the bottom. These can be observed when a colony loses its queen, and are emergency queen-cells (Ruttner, 1988).



Figure 2. The single open nesting of *Apis florea* showing ant barriers made of resin (propolis) at the edges of a branch.

This species applies a sticky resin (propolis) like substance to branches to support its comb and prevent ants and other insects from invading the nest (Akratanakul, 1976; Wongsiri et al., 1996a). The communication dance by scouts, announcing the discovery of a food source, also takes place on the platform of honey storage area and is a classic “figure eight” waggle dance. However, unlike the cavity-nest species, *A. florea* foragers dance upon the relatively flat upper comb area above the branch supporting the nest. Because they dance upon this horizontal surface, foragers orient towards celestial cues rather than to gravity or towards landmarks if the celestial cues are unavailable (Dyer, 2002). As a result, these foragers dance with the waggle phase pointing directly at the indicated resource, with visual cues (celestial or landmark) used to correctly orient the waggle phase (Lindauer, 1956; 1961).

### **The giant honeybee, *Apis dorsata* Fabricius, 1793**

This species has the largest individual body size of all honeybees (Michener, 2000). Interestingly, queen, workers, and drones of this species are all produced in cells similar in size and shape (Richards, 1953). The average cell diameter ranges between 5.42-6.35 mm (Dietz, 1992; Graham, 1992). The nest is made as a single comb about 1-2 meters long, approximately 0.5 m high on thick branches (20-40 cm diameter to support comb weight) on the upper parts of large trees that are 30-60 meters high. Other preferred nesting sites include overhanging rocks, cliffs, or cavities of large buildings (Lindauer, 1961). In general, *A. dorsata* may occur singly or several nests are formed aggregately, usually 20-50 nests in a single tree in the forests of Thailand.

Some populations of this species are very aggressive (Wongsiri et al., 1990; 1996b). About three-quarters of the worker population of a colony of giant honeybees is engaged in colony defense, forming a protective curtain that is three to four bees thick, similar to *A. florea*. While birds are common predators of *A. dorsata*, they are evidently well protected against ant invasion. Thus, the sticky bands of propolis around the nests of the dwarf honeybee are not found surrounding the nests of *A. dorsata*, nor are the nests hidden by dense foliage (Akaratanakul, 1976).

In Thailand, *A. dorsata* has relatively high levels of genetic diversity. There is a lack of genetic population differentiation between *A. dorsata* originating from geographically different regions when using microsatellite polymorphisms (Insuan et al., 2007). However, there are significant genetic differences between bees from the north-to-central region (north, northeast, and central regions), peninsular Thailand, and Samui Island (Insuan et al., 2007).

The range of the giant honeybee is similar to that of the dwarf honeybee. It occurs from Pakistan (and, perhaps, parts of southern Afghanistan) in the west, through the Indian subcontinent and Sri Lanka to Indonesia and parts of the Philippines in the east. Its north-south distribution spans southern China to Indonesia; it is not found in New Guinea or Australia. In Thailand this species is particularly found in forested areas with a large variety of wild plant species. The organization of the comb is similar to that in the other honeybee species: honey storage at the top, followed by pollen storage, worker brood and drone brood (Hepburn, and Radloff, 2011; Otis, 1990; Wongsiri et al., 1996b).

At the lower part of the nest is the colony’s active area, known as the mouth, where workers take off and land and where communication dances by scouts announcing the discovery of food sources take place (Akaratanakul, 1976). This dance takes place on the



vertical surface of the comb, and during its progress, the bees must have a clear view of the sky to observe the exact location of the sun. Unlike other species of honeybees, *A. dorsata* has the unusual habit of continuing foraging after sunset on bright moonlit nights (Wongsiri et al., 1991), a nocturnal ability that is not reported in other honeybee species (Suwannapong and Wongsiri, 1999, Suwannapong et al., 2010a). *Apis dorsata* foragers can fly at night in part because of large concentrations of visual pigment in the reticular cells of their ommatidia (Suwannapong and Wongsiri, 1999).



Figure 3. The aggregated single open nesting of *Apis dorsata*.

## Honeybees that Build Multi-Comb Nests in Enclosed Cavities

### The Asiatic hive honeybee, *Apis cerana* Fabricius, 1793

The Asiatic hive honeybee, *A. cerana* is widespread in temperate and tropical Asia (Smith et al., 2000). The range for this species is greater than that of *A. florea* and *A. dorsata*. It is found throughout Thailand. There are two subspecies of *A. cerana* in Thailand: *A. cerana cerana* and *A. cerana indica*. *Apis cerana* populations have high genetic diversity in the mainland (north, central, northeast and peninsular Thailand), but limited diversity in the Samui population, implying that genetic drift or founder effects may have occurred in this population (Hepburn et al., 2001; Hepburn, and Radloff, 2011; Otis, 1990; Wongsiri et al., 1996). There are five conspecific populations of *A. cerana* in Thailand. These are assigned to

four different genetic groups: north and central region, peninsular Thailand, Samui island and northeast (Sittipraneed et al., 2001).

*Apis cerana* provides honey, beeswax, and the invaluable service of crop pollination. They tend to swarm, abscond and migrate quite frequently (Akranakul, 1976; Maa, 1953; Morse and Moch, 1971; Otis, 1990; Richards, 2001; Smith et al., 2000; Wongsiri et al., 1996; Wu and Kuang, 1987). Among the native bees of Asia, *A. cerana* will likely become an increasingly important beekeeping resource. Its nest structure is similar to that of *A. mellifera* because it builds multiple combs inside a nest cavity (Figure 4). It also performs waggle dances inside the nest, usually in the dark, like *A. mellifera* (Lindauer 1956). Because *A. cerana* has not been domesticated to the same extent as *A. mellifera*, it poses some problems for apiculture such as high swarming and absconding rates, a different set of parasites, and more limited honey storage capabilities. However, they are strongly resistant to *Varroa jacobsoni* and predatory wasps (Kerr et al., 1974, Ruttner, 1988; Wongsiri et al., 1996a).



Figure 4. The cavity hive of *Apis cerana* in a clay jar.

In the wild, these bees construct their nests in dark enclosures such as caves, rock cavities and hollow tree trunks. The normal nesting site is usually close to the ground, not more than 4-5 meters high. The bees' habit of nesting in the dark enables one to keep them in specially constructed vessels. For thousands of years *A. cerana* has been kept in various kinds of hives (clay pots, logs, boxes, wall openings, etc). Despite the relatively recent introduction of Langstroth-frame hives, colonies of *A. cerana* kept in traditional hives are still common in the villages of most Asian countries (Akranakul, 1976; Hepburn, and Radloff, 2011). As a result, feral nests of *A. cerana* are less hunted by man than nests of dwarf and giant



honeybees (Akkratanakul, 1976; Maa, 1953; Otis, 1990; Tirgari, 1971; Wongsiri et al., 1996b; Wu and Kuang, 1987). The several combs in an *A. cerana* colony are built parallel to each other, and have a uniform distance known as the "bee space" between them. The body size of *A. cerana* workers is relatively small and there are two sizes of brood comb cells: smaller for worker and larger for drone brood. Queen cells are built on the lower edge of the comb. As in the other *Apis* species, honey is stored in the upper part of the combs, but also found in the outer combs, adjacent to the hive walls (Akkratanakul, 1976; Maa, 1953; Otis, 1990; Wongsiri et al., 1996b).



Figure 5. The cavity hive of *Apis cerana* in the hole of a coconut tree trunk.

### **The European honeybee, *Apis mellifera* Linnaeus, 1758**

*Apis mellifera* was brought to Thailand for beekeeping about 60 years ago (Suppasat et al., 2007). Three common and five rare composite haplotypes exist among colonies in North, Central, Northeast and South Thailand. This species builds multiple-comb nests in dark cavities (like *A. cerana*), has an intermediate individual body size, and shares a similar social organization and division of labor with other honeybee species (Akkratanakul, 1976; Maa, 1953; Otis, 1990; Tirgari, 1971). Beekeeping with *A. mellifera* in Thailand is quite successful. This species is used for honey production and is an integral part of Thai agriculture. It is used for pollination of longan, litchi, durian, rambutan and other crops. Although this species is one of the most studied, efforts over the past few decades to introduce *A. mellifera* into Asia have encountered a number of problems, such as the inter-species transmission of bee pests and diseases (Crane, 1990; Seeley, 1985). In addition, this species needs much more sugar

feeding during dearth periods and is highly susceptible cold temperatures (Partap and Verma, 1994; 1998).

This species builds their multiple combs nest in the cavity of the hole that provides protection of the colony against predators, and from climatic changing including rain (Schmidt and Hurley, 1995). However, this species displays the ancestral characteristics of open nesting behavior since they can also nest in the open (Butler, 1975; Butler et al., 1970). This open nesting behavior could indicate a swarming nest that honeybees choose for a temporary place before determining the final cavity nest site (Raffiudin, 2002; Raffiudin and Crozier, 2007).

With respect to communication, *A. mellifera* is the best studied of all species of honeybees. All species of honeybees share the ability to “dance,” performing repetitive cycling behaviors on the surface of the comb that indicate the presence of a resource outside the nest and, in the case of the waggle dance, the resource location (von Frisch 1967). Resources communicated include pollen, nectar, water, resin (propolis), and nest sites (Dyer 2002). Near the nest (the exact cutoff distance varies with the species and the bee but is generally less than 50 m for *A. mellifera*), bees perform a round dance which consists of the dancer moving in a circle and then periodically making a sharp turn to double back. Far away from the nest, bees perform a repeating waggle dance that consists of a figure-eight pattern in which bees waggle during the center portion. Generally, this occurs for resources greater than 100 m away from the nest (in *A. mellifera*, von Frisch 1967). The waggle portion communicates the distance to the resource and its direction.

There are differences in the coding of distance in different species of honeybees (Lindauer, 1956; Punchihewa et al., 1985) that are thought to be tuned to the foraging range of the species. Thus, species with different foraging ranges should have a different distance codings. Dyer and Seeley (1991) found no significant differences between the distance coding “dialects” of the different Asian species of honeybees, *A. florea*, *A. cerana*, and *A. dorsata* although, based upon reading dances for natural food sources, there were significant differences in the flight range. The much larger-bodied species, *A. dorsata*, had approximately twice the foraging range (95% of dances were for distances estimated to be less than 3.8 km) of *A. florea* (1.3 km for 95% point) or *A. cerana* (0.9 km for 95% point). The authors point out that observations over a variety of seasons is required for a more robust test of the dance dialect tuning hypothesis (Beekman et al., 2008; Dyer and Seeley, 1991; Lindauer, 1956; Otis, 1991; Punchihewa et al., 1985; Raffiudin and Crozier, 2007; Wongsiri et al., 1996b).

In general, much remains to be learned about the communication of Asian honeybees. For example, drone dances are reported in the dwarf honeybees (*A. andreniformis* and *A. florea*). Drones of *A. andreniformis* are reported to engage in runs on the surface of the colony’s protective curtain, running in circular loops (similar to the round dance) with their wings somewhat spread out. A dancing drone can attract another drone to follow its dance and result in both drone followers and the drone flying off together. Drones of *A. florea* are not reported to dance (Rinderer et al., 1995; Wongsiri et al., 1996a). Unfortunately, there have been no subsequent studies of this interesting phenomenon.



Figure 6. The multiple comb nest of *Apis mellifera*.

## 2. HONEYBEE DEVELOPMENT, CASTES, AND AGE POLYTHEISM

### Honeybee caste and development

There are three main forms or “castes” of honeybee in every honeybee colony: a single queen, a few hundred drones and several thousand workers (all female). The queen is a fertile, functional female that can produce males and females, the worker is an unfertilized female capable of only producing males (due to the haplodiploid sex determination system found in honeybees) and the drone is male (Tribe and Fletcher, 1977; Winston, 1979). Honeybees undergo complete metamorphosis, and all of the honeybee castes (worker, queen, and drone) pass through the same four stages during their development: egg, larva, pupa and adult. All three castes spend three days for their egg stages (Winston, 1979; 1987; 1992). The larval stages last for different amounts of time, depending caste, genetics, and the environment. The mean duration of the uncapped larval period is about 4.5 days for queen, 5.5 days for workers and 6.5 days for drones. The total developmental times average 16, 21 and 24 days for queen, workers and drones, respectively (Tribe and Fletcher, 1977; Winston, 1979; 1987; 1992).

**The Queen:** There is generally one queen in honeybee colony. The queen honeybee is effectively the “mother” of the colony. Her main role is to lay eggs. Through the production of queen pheromone, she influences the physiology and behaviour of the workers. Under normal circumstances, the queen is the only egg-laying bee in the colony. If there are enough cells available she will lay up to 2,500 a day (Winston, 1992). A queen starts out as a simple

fertilized egg that can be laid in a queen cell (for most species, a larger cell specially built for queen-rearing) or laid in a worker cell that is subsequently transformed (by the workers) into a queen cell. To create a queen cell, workers extend one or more existing worker cells in the honeycomb to form “queen cups”. These will usually be towards the bottom or edge of the main brood area of the comb. The queen lays an egg in these queen cups and once hatched (3-3½ days after being laid) the new larva is fed a rich diet of “royal jelly” by the “nurse” bees. Immediately after being laid, there is no difference between an egg that will become a queen or a worker. Whether or not an egg becomes a queen is largely due to worker actions. If the colony is ready to swarm, detects that it has a failing queen, or lost its queen, “house” worker bees will alter how they feed one or more larvae that hatch out. This rich food allows the larvae to grow larger and more quickly than a normal worker (Allen, 1955; Winston, 1979; 1992).

The new queen (known as the “virgin queen”) emerges 16 days after the egg was laid. This compares to 21 days for a worker bee or 24 by the drone. If a queen is lost for whatever reason (e.g. is killed accidentally by predators), the colony will resort to making emergency queen cells (Seeley, 1985; Winston, 1987; 1992). Providing there are eggs or very young larvae (up to 3 days old) present, the colony will use these young eggs to raise several new queens. They will build out existing cells and start feeding what would have been ordinary worker larvae with royal jelly. The rich diet fed to the developing queen larva alters the anatomy of the larva (from that of the worker). The abdomen becomes notably longer than that of her sister workers. The larger abdomen accommodates the enlarged reproductive organs, namely the ovaries and spermatheca (Anderson, 1963; Mackensen, 1943; Winston, 1992). When the virgin queen is ready to emerge, one of four fates await her; 1) She will be attacked and possibly killed by another queen that emerged ahead of her, 2) She will seek out and find other emerging queens and kill them (sometimes by stinging through the cell before the victim has emerged), 3) She will be ushered out of the colony by workers to fly off with a number of other bees from the colony (a swarm), or 4) She will be accepted by the colony, the workers of which will immediately start to care for her (Anderson, 1963; Mackensen, 1943; Winston, 1992).

The virgin queen will typically stay in the colony for a few days in order to feed and gain strength and allow her reproductive organs to mature a little further (Mackensen, 1943; Winston, 1992; Woyke, 1963; 1969, 1973). Following this initial “rest period,” the virgin queen emerges from the colony and makes a several reconnaissance flights. She may do this to ensure she knows where the hive is and also to find where drones congregate (Anderson, 1963; Mackensen, 1943; Winston, 1987; Woyke, 1969). Due to her large size, the queen is particularly vulnerable to being captured by predator like birds, and therefore avoids long flights (Anderson, 1963; Mackensen, 1943; Winston, 1992; Woyke, 1969, 1973).

When ready, usually any time up to around 21 days from emerging from her cell, the queen will make a “mating flight”. She will head straight for the drone congregation site usually in the vicinity of the queen’s colony and up to 30 metres from the ground. The queen flies up to the drones and mates repeatedly with several of them. Once her spermatheca is full, she heads back to the colony. She relies on the attendant workers for her every need. They feed her, groom her, and remove her wastes (Mackensen, 1943; Winston, 1987; 1992; Woyke, 1963; 1969). The queen will spend the remainder of her life laying eggs. The number she lays will largely depend on the activities of the colony. Workers control how many cells remain vacant for egg laying (increasing and decreasing the amount of stores of pollen and



honey according to seasonal variations) and will control her egg production by varying the amount of food she is fed (Anderson, 1963; Brouwers, 1982; 1983; Mackensen, 1943; Winston, 1992; Woyke, 1973).

The queen produces a pheromone called queen mandibular gland pheromone. As the workers clean her, they distribute queen substance around the colony. This serves to maintain the colony in a “normal” state. However, as the queen gets older, typically coinciding with a steady reduction in the number of eggs laid, she produces less queen substance (Winston, 1992; Woyke, 1969; 1973). The colony senses this and begins preparation for her ultimate removal. This occurs by a process of supercedure where a new queen is raised and the old one is killed off either by the workers or the new queen. Supercedure is a natural process that will face nearly every queen. Supercedure may occur within the colony or once a colony has swarmed. A prime swarm (the first to leave a colony) will have the incumbent queen. Once the swarm establishes its new home, a new queen is raised and the old queen is killed. Queens occasionally lay fertilized eggs in worker cells, which develop into males called diploid drones (Mackenson, 1943). Male diploid drones are quickly eaten by workers (Woyke, 1963; 1969, 1973).

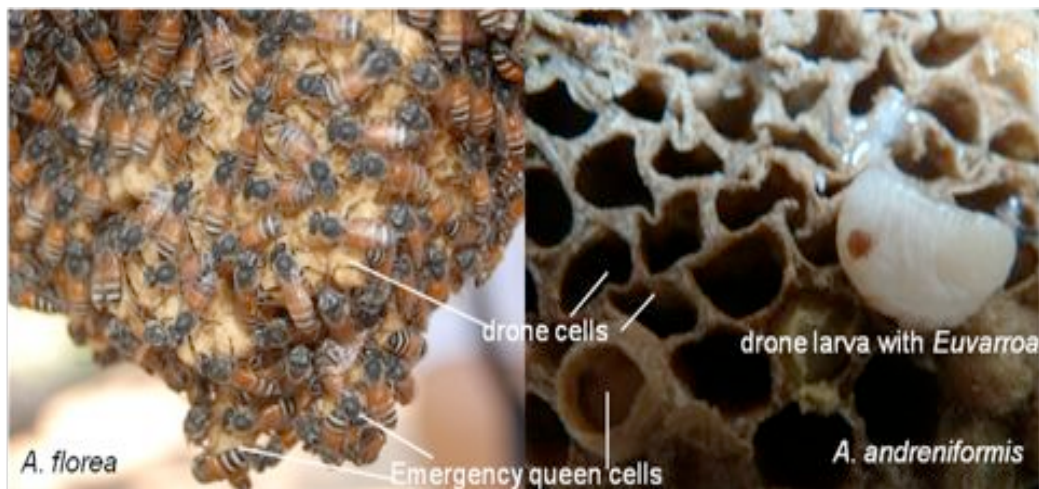


Figure 7. The emergency queen cell of *A. andreniformis* and *A. florea* surrounded by drone cells.

**Workers:** The worker bees, as the name implies, do almost all colony tasks. Workers are all sterile females. They carry out almost all the duties that go into building and maintaining a colony such as brood rearing, comb building, house cleansing, foraging, and colony defence (Winstons, 1992; Wongsiri et al., 1996a; 1996b). Workers generally have a smaller body size than either drones or the queen. There are about 8,000-25,000 workers in *A. andreniformis* and *A. florea* colonies, 40,000-50,000 workers in *A. mellifera* colony, 20,000-40,000 workers for *A. cerana* and, 50,000-80,000 for *A. dorsata* (Otis, 1990; Winston, 1992; Wongsiri et al., 1991, 1996). Worker bees, like the queen, possess a sting that is a modified ovipositor or egg

laying tube. Despite the presence of reproductive organs, the workers are infertile and lack the reproductive capacity of the queen.

The worker bee starts out as a normal egg laid by the queen. The larva is fed “brood food” which is largely a combination of nectar, pollen and enzymes from the saliva of the “nurse” bees (Anderson, 1963; Mackenson, 1943; Winston, 1992). The pollen provides the necessary protein required for rapid growth. Sugar is the energy source. A worker larva is fed approximately half the quantity of brood food fed to a queen larva. However, there is little difference in the ingredients of the food that is fed to each. Instead, the difference is related to the relative proportions of those ingredients. Research suggests that the queen larva is fed a significantly higher proportion of sugar in its food (royal jelly) and that this extra energy boost given to a larva within its first 3 days promotes an increase in certain growth hormones that cause what would otherwise become a worker to become a queen. Recently, Kamakura (2011) made a major breakthrough and discovered a 57 kDa protein, royalactin, that induces honey bee larvae to become queens. Workers may lay eggs, under certain conditions, which develop into drones since workers never mate and they have no sperm to fertilize their eggs (Anderson, 1963; Mackenson, 1943). However, in a normal queenright colony, worker policing occurs and workers consume eggs produced by other workers (Ratnieks, 1993). In *A. cerana*, unlike *A. mellifera*, there can be a relatively large number of laying workers in a queenright colony (Partarp and Verma, 1998).

**Drones:** Drone bees are males. They do not work in the nest, and die after workers stop feeding them and then drive them from the nest. They seemingly have little or no purpose within the colony. However, drones perform one vital role. Their primary purpose is to mate with virgin queens, contributing their genes to different colonies. The body sizes of drones are larger than workers, but normally smaller than the queen. They are characterised by their large compound eyes, which appear to come together at the top, and rounded stocky bodies. They arise from unfertilised eggs laid by a queen or worker. Drones are “haploid”, they only possess one half of the pairs of genes found in the “diploid” workers and queen (Anderson, 1963; Mackenson, 1943; Winston, 1992).

Usually, drone eggs are laid only by the queen as unfertilized eggs. The queen holds the eggs and sperm separately and has the choice as to whether or not an egg is fertilized as it is laid. When the queen lay an egg, she determines whether that egg is being laid in a worker or in a drone cell, likely by inspection of the cell within her forelegs prior to egg laying or by the angle of her abdomen during oviposition (Koeniger, 1979, 1983). The queen will lay drone eggs in drone cells that are larger in diameter in order to accommodate the larger body size of the drone. The queen can detect the difference in cell type and lays worker or drone eggs accordingly. If the egg is going into the drone cell, the queen does not release any sperm: the unfertilized egg is haploid (Winston, 1992). The drone larva is progressively fed by workers until it spins a cocoon within the cell and undergoes metamorphosis, turning into a pupa. The drone emerges as a young adult on day 24. The young drone, being unable to gather food for itself, is fed by the workers (Koeniger, 1969, 1970; Winston, 1992).

After a few days, the drone will begin to carry out reconnaissance flights. Their ability to navigate is probably the best of all three castes because they typically have around 75-80% more facets in their compound eyes than the workers or queen (Gary, 1963; Koeniger, 1969, 1970; Ruttner, 1966; Winston, 1992). This should give them better vision. Around 2 weeks after the drone emerges from the cell, it will be mature enough to mate. Drones typically

come together in areas known as “drone congregation areas” or “mating yards”. These areas are often several metres from the ground (up to 30 m) and will typically be in the vicinity of the apiary or cluster of natural colonies. However, drones can fly several miles to find other established congregation areas (Gary, 1963; Koeniger, 1969, 1970; Ruttner, 1966; Winston, 1992).

How drones find these congregation areas, particularly from kilometers away, remains a major mystery. They may be able to sense the odours of other drones over some distance using their enlarged and highly sensitive antennae. However, this may not account for their long-distance orientation to drone congregation sites. It is possible that all drones possess similar innate preferences for congregation sites that lead to choose a few locations. Once they are close enough to a favored site, the odor of other drones may then enhance their orientation. However, this explanation is speculative and requires experimental verification.

A congregation area may have several hundred to several thousand drones (Gary, 1963; Koeniger, 1969, 1970; Ruttner, 1966). A virgin queen will have already found such a congregation area from early reconnaissance flights. On her mating flight the queen will head straight for the congregation area (Gary, 1963; Koeniger, 1969, 1970). The drones will sense her arrival (their notably large antennae have 10 times more sensory receptors than those of workers or queens) and will immediately pursue her. Several drones will mate with the queen. Each drone mounts the queen in turn and inserts his endophallus into the queen. The drone’s sex organs inflate (almost explosively) within the queen, pumping sperm into her. This rapid inflation and expulsion of sperm causes him to spring backwards and fall away from the queen. The endophallus is gripped by the opening of the queen’s oviduct and results in the drone’s endophallus and part of the drone’s gut contents being ripped out of his abdomen. Each additional drone will remove from the queen the endophallus of his predecessor before undergoing the same fate. After mating, the drone will often fall to the ground where he will die (Gary, 1963; Koeniger, 1969, 1970; Ruttner, 1966; Winston, 1992).



Figure 8. The different developmental stages of *A. florea* workers.

### **Honeybee Development and Differentiation**

The first stage in the development of a worker bee occurs when the queen deposits a single fertilized egg at the bottom of a worker cell. The newly egg is shaped like an ellipsoid. It is elongated, rounded at the ends, and slightly convex in the center (Snodgrass and Ericson, 1992). After three days, the egg hatches into a first-instar larva and is then fed regularly by nurse bees. After successive stages of growth and molts, the larva completely covers the floor of its cell, and then changes position, stretching out along the depth of the cell. During its life, the larva goes through five larval instars (Benhof, 1925; Michener, 2000). When the larva is fully grown and no longer needs to be fed, house bees cap its cell with a thin layer of wax. At this stage, the larvae are, called "sealed brood." The sealed larva spins a cocoon around itself and begins to pupate. It sheds its last larval integument and differentiates into a pupa. The pupa has all the adult bee's distinct body parts, but they all adhere tightly to the body, and some appendages are not yet fully expanded. Before emerging, the pupa becomes gradually darker in color. Finally, transformed into an adult, it slowly chews its way out of the cell. In *A. mellifera*, the complete metamorphosis from newly laid egg to emerging adult worker requires 21 days: three as an egg, six as a larva, and 12 as a pupa (Snodgrass and Ericson, 1992; Tribe and Fletcher, 1977; Winston, 1979).

For a drone, life begins when the queen deposits an unfertilized egg in drone brood cell at the bottom of the comb. Like the eggs of worker brood, drone-brood eggs require three days to hatch. When the larvae are full grown, the nurse bees cease feeding them, their cells are capped, they spin their cocoons, and pupation takes place. It requires 24 days for a drone to develop from newly laid egg to emerging adult. Emerging drones are fed on honey and royal jelly until they are about a week old. Their flight activity begins when they are from 6 to 8 days old, but they are sexually mature only after 12-14 days.



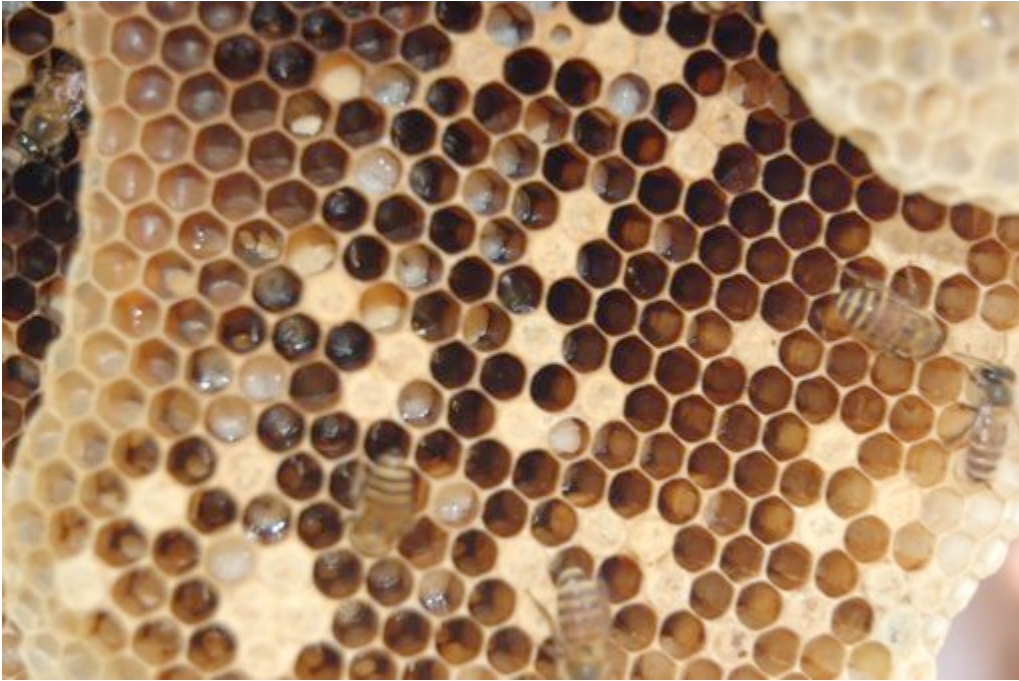


Figure 9. The different developmental stages of *Apis cerana*.

Whereas worker and drone brood are reared in hexagonal cells, queen development takes place in cells shaped somewhat like peanuts (Michener, 2000). There are three types of queen cells: swarm cells, supercedure cells, and emergency cells. Swarm queen cells are built along the lower edge of the comb, often in large numbers: as many as 20 cells of various ages may be seen in a colony. Supercedure queen cells, fewer in number, are generally about the same age and are perpendicular to the comb surface (Michener, 2000; Winston, 1987; 1992). They are usually formed from old, darker wax than swarm queen cells that, built at times of high food availability, consist of whiter, newly secreted wax. A distinctive feature of emergency queen cells is that they are expanded from ordinary worker cells already containing young larvae, and protrude directly from worker-brood cells on the surface of the comb. The development period of a queen is significantly faster than workers and drones: 16 days from egg to adult. The queen larva is well provided by nurse bees with royal jelly for her entire development. The food is deposited very frequently in the cell, and the queen larva simply lies on a bed of its food. The remains of uneaten royal jelly is often seen in the cell after the young queen emerges. Although larvae destined to become queens and workers are genetically similar (both are hatched from fertilized eggs), qualitative and quantitative differences in the diet they receive, particularly in the early stages of their larval lives, give rise to major differences in anatomical and physiological development (Michener, 2000; Winston, 1987; 1992).

### **Division of Labour**

A colony of honeybees usually consists of a queen, several thousand workers, and (during the breeding season, a few hundred drones). Among the members of the colony, there

is division of labor and specialization in the performance of biological functions (Winston, 1987). Workers can flexibly shift among different tasks, depending upon colony need (Ferguson and Winston, 1988; Smith et al., 2008). The tasks performed are primarily age related (Lindauer, 1961; Wang and Moeller, 1970). There is also a strong genetic component to division of labor with workers from different strains, races within the colony showing differences in task ontogeny (Winston and Katz, 1982). Both genetics and environment are important. Workers can perform a subset of multiple tasks at all ages (Lindauer, 1952; Winston, 1992).



Figure 10. Brood rearing by nurse bees in *Apis cerana* comb.

In general, young workers work inside the nest and older workers work outside or at the nest entrance (foraging or guarding, Winston and Ferguson, 1985). The youngest bees perform house cleaning and capping. Brood and queen rearing occupy slightly older workers, nurse bees. Comb building and food processing are handled by middle-aged workers (who serve as a general reservoir of labor that be channeled into performing different tasks inside the nest, as needed). Finally, nest temperature regulation and ventilation, defense, and foraging occupy the oldest bees (Winston, 1992). The caste structure in honeybees is closely linked with the development of brood food glands (hypopharyngeal glands), mandibular glands, and wax glands (King, 1933; Simpson, 1960, 1966; Simpson et al., 1968; Wang and Moeller, 1969).



Figure 11. *Apis florea* forager.

### **Colony cycle: migration, swarming, and absconding**

The annual colony cycle of honeybees in Thailand includes migration, swarming, and absconding. However, for beekeepers, the imported European honeybee, *A. mellifera* has been a popular option because this species has a prolific queen and both swarms and absconds less than native *Apis* species (Partarp and Verma, 1998). Migration from one habitat to another is normal part of seasonal cycle for *A. andreniformis*, *A. dorsata* and *A. florea*, (Wongsiri et al., 1996b). Migration may increase colony fitness by allowing the colony to move to an area with more food, enhanced outbreeding, and reduce parasite loads (Oldroyd et al., 1996). The migration period for *A. dorsata* in Thailand peaks in September to October (Wongsiri et al., 1996b). Absconding to escape predators (such as human honey hunters) can also occur.

Swarming is a natural mode of colony reproduction. If a colony is relatively safe from damage or destruction by its natural enemies, has an ample supply of forage, and queen and the workers have been performing their duties in an optimum manner, it will eventually outgrow its hive space. When this occurs, the colony is ready to reproduce itself by swarming. In temperate regions, natural food is most available to colonies in spring and summer, when warm ambient temperatures permit flights and active foraging (Partap and Verma, 1998; Wongsiri et al., 1996b). The colony is busily engaged in brood rearing during this period, until hive overcrowding and congestion signal the colony to swarm. During the cold autumn and winter months, however, colonies raise only a small amount of brood and depend on their stored food. Such a clearly defined annual cycle does not exist to the same extent in tropical



regions, where colonies of indigenous *A. cerana* and introduced temperate races of *A. mellifera* rear brood whenever their food supply is plentiful (Partap and Verma, 1998).

Overcrowding of the hive can thus occur at almost any time, and swarming under tropical conditions occurs whenever the forage is seasonally plentiful. Under normal conditions, a temperate-zone colony of *A. mellifera* will swarm at least once per year. However, tropical species such as *A. cerana* may send out several successive swarms. *Apis cerana* is prone to swarm excessively during times of food abundance. Such swarming appears to depend primarily upon environmental conditions rather than swarm genotype (Punchichewa et al., 1998).



Figure 12. The old open single comb nest of *Apis dorsata* after absconding has occurred.

In preparation for swarming, a colony builds queen cells, normally just about the time the virgin queens begin to emerge and rears young queens. At the same time, the old queen receives less food and loses weight, which facilitates her departure flight. Before the new queens emerge, the colony's worker population leaves the parent hive in search of a new home site. At the next point, the bees will 'decide' who is to leave and who is to stay (Tew, 2006; Passino and Seeley, 2008). The swarming behavior may partially be the result of the evolution of absconding behavior or migratory behavior. Just as in absconding, the old queen goes with the swarm rather than the colony waiting around for the new queen to emerge and then leave with the new queen. It is commonly said that honeybees have a tropical ancestry where migratory and absconding behavior are much more prevalent. Whatever reason, the behaviors of swarming, supercedure, and absconding have obvious characteristics in common (Tew, 2006).



### Foraging behavior

Honeybees have sophisticated foraging coordination and communication (von Frisch, 1971; Suwannapong, 2000). This activity is only performed by workers, known as foragers or foraging bees. Some foragers specialize on pollen foraging and some on nectar foraging. Between these extremes, there are a large number of generalists who collect both (Fewell and Page, 1993). The range for the onset of foraging ranges from 18.3 days (Sakagami, 1953) to 37.9 days of age (Winston and Ferguson, 1985). This food consists of carbohydrates and proteins (nectar and pollen, Seeley, 1985). Under normal conditions, worker bees begin to forage when they are about 2 to 3 weeks old. Foraging is the last chore in the life of a worker. Part of the colony's stored honey is consumed by foraging bees who need fuel and therefore consume a certain amount of honey to ensure that she will have a sufficient energy supply for her round-trip journey (Akranakul, 1976; Seeley, 1985). To obtain a full load of nectar and pollen (or both) in a single trip, she may have to visit several hundred flowers (Akranakul, 1976). The amount of energy she expends, related to the amount of food she collects, is determined largely by such factors as the amount of nectar obtained per flower, floral density per unit area, the distance from the hive, and weather conditions (Akranakul, 1976; Partap, 1992; Partap and Partap, 1997).

There are differences among flowering plant species with respect to nectar and pollen production. Not all plant species possess nectaries (glands secreting nectar) or have nectar that bees can reach with their proboscis (tongue) (Partap, 1992). Nectaries can be located in various areas of the flower and some species have extrafloral nectaries that may be visited by bees. In addition, some bees may perform nectar robbing, making a small hole at the base of a flower in order to obtain the nectar. In this case, the bee does not perform any pollination service for the "robbed" plant. A forager may prefer the nectar of one flower species. It is to her advantage to visit flowers producing greater quantities of nectar with a higher sugar concentration. The sugar concentration in the nectar of a given plant species may vary depending upon its location, time of day, and genotype. If nectar with a high sugar concentration is available, a forager of *A. mellifera* can carry as much as 70- 80 mg of nectar per load (Akranakul, 1976; Partap, 1992; Partap and Partap, 1997).

Workers of all honeybee species carry nectar internally. Part of their alimentary canal is modified to form a "honey sac" or "honey stomach". After returning to the hive, the forager regurgitates the nectar to one or more house bees, which then dehydrate the nectar and convert it into honey. They use the enzyme invertase, which splits sucrose in the nectar into fructose and glucose, the sugars predominant in honey. To dehydrate the nectar, house bees regurgitate a part of the nectar and hold the droplet in their mouthparts (Partap, 1992; Partap and Partap, 1997).

The entire body of a worker bee, particularly her thorax, is covered with fine, branched hairs, on which pollen grains are caught. She sometimes uses her mandibles to chew off the anthers, or deliberately rolls over the anthers to acquire the pollen. The tibiae of the bee's metathoracic legs are equipped with rows of short setae, which she uses to scrape the pollen from her body and to form it into pellets, sometimes regurgitating a slight quantity of nectar to provide moisture and adhesiveness. The pellets, attached to "pollen baskets" on the bee's rear tibiae, are carried back to the hive, where the load is deposited in a pollen-storage cell. Whereas cells containing ripe honey are capped, pollen-storage cells are not. The bees tightly pack pollen to about two thirds of the capacity of the cell and coat the top surface of the

pollen in each cell with honey (Partap, 1992). This helps protect the pollen against spoiling (Partap, 1992; Partap and Partap, 1997; Verma and Partap, 1998).

In addition to collecting nectar and pollen, foragers can collect plant gum (propolis) and water (Fanesi et al., 2009; Marcucci, 1995; Bankova et al., 1983, 2000). Propolis, which is a resinous hive product exuded by certain plants, often to protect wounds on their surface and against the bees' enemies, is rich in tannin, flavonoids, aromatic acids, esters, aldehydes, ketones, fatty acids, terpenes, steroids, amino acids, polysaccharides, hydrocarbons, alcohols, hydroxybenzene, and several other trace compounds. Propolis exhibits antibiotic activity (Fanesi et al., 2009; Marcucci, 1995; Bankova et al., 1983, 2000). It is also an adhesive material, which the bees use in comb construction, to coat the interior of the hive, and to seal cracks. To collect propolis, a bee uses her mandibles to bite the substance from the plant surface and carries it back on her rear legs (much like pollen). In the hive, workers use their mandibles to remove the resin from the forager (Cheng and Wong, 1996).

The honeybee colony needs water for two purposes only: to cool the hive and to dilute the honey fed to the larvae (von Frosch, 1967; Seeley, 1996). Like nectar, water is collected by the forager through her proboscis and is carried back to the hive in her honey stomach. Water is regurgitated to the house bees on arrival. During the heat of the day, some foragers may switch from nectar to water collection, or they may prefer to collect nectar with a low sugar concentration and higher water. Interestingly, this process is mediated by the willingness of foragers to accept nectar from the forager. If there is need for more water, workers that unload nectar (food unloading bees) will preferentially unload bees with water or more dilute nectar (von Frosch, 1967). Bees bringing back sweeter nectar will wait for increasingly long periods and will therefore reduce their rate of recruitment for the sweeter nectar source (Seeley, 1996).

It has been also reported that *A. mellifera* foragers use 2-heptanone to mark previously visited flowers, thereby signaling nectar depletion to other bees (Engels et al., 1997; Giurfa, 1991). However, the four native Thai species do not appear to use aversive pheromone marking during foraging (Suwannapong, 2000; Suwannapong et al, 2010c). For example, they may revisit the same flower briefly after the first visit and continue to forage on the same flower simultaneously with several bees of their own species or other species. Suwannapong (2000) observed *A. florea*, two to three bees of *A. cerana*, one to two bees of *A. dorsata* and one to two bees of *A. andreniformis* visiting the same flower (Suwannapong, 2000). It is also possible that honeybees, like bumblebees can learn to associate floral depletion or floral reward using olfactory cues, cuticular hydrocarbon "footprints" deposited while walking on the food source (Leadbeater and Chittka, 2007). However, this remains to be investigated.

The mandibular gland of *A. mellifera*, the source of this putative food-marking pheromone is primarily 2-heptanone. However, the primary component of mandibular gland secretions in Thai honeybees is (Z)-11-eicosanol. In general, the ten most abundant components in the mandibular glands of all these species are 80% similar (Suwannapong, 2000).



Figure 13. *Apis florea* foragers are foraging on palm flowers.

**Table 1. Comparison of the main composition of mandibular gland pheromone of Thai honeybees (Suwannapong, 2000).**

Pheromone	A. an	A. c	A. d	A. f	A. m
(Z)-11 eicosanol	+	+	+	+	+
1-butanol-3methyl-acetate	+	+	+	+	-
Dibutyle phthalate	+	+	+	+	+
Nonadecane	+	+	+	+	+
2-hexyl 1-decanol	+	+	+	+	+
Heneicosanol	+	+	+	+	+
Eicosane	+	+	+	+	+
1-octanol	+		+		
2-propyl 1-heptanol	+		+	+	+
2-butyl-1-octanol	+	+	+	+	+
Heneicosane		+			
Heptadecane				+	
Limonene	+	+	+	+	+
2-heptanone	Undetectable	undetectable	undetectable	undetectable	+

\*A. an, *A. andreniformis*; A.c , *A. cerana*; A.d, *A. dorsata*; A.f, *A. florea* and A.m, *A. mellifera*

### Thermoregulation

Honeybees are poikilothermic and have partial control over their internal body temperature. Unlike warm-blooded animals, honeybees can maintain lower body temperatures, but they can also elevate their body temperature through basking or muscular contractions (Woods et al., 2005). A populous honeybee colony can regulate the interior temperature of the hive, particularly within the area surrounding the developing brood (Nakamura and Seeley, 2006). In normal colonies, the brood-nest temperature is maintained at a remarkably constant 30-36 °C (Underwood, 1991). Thermoregulation by honeybee colony has a high energetic cost (Kronenberg and Heller, 1982; Heinrich, 1979; Heinrich and Esch, 1994). To generate more body heat, the worker bees will consume more food, especially honey: more heat is released because of the increased rate of food metabolism (Jones and Oldroyd, 2007).

By fanning their wings, evaporating the water regurgitated on worker mouthparts, and dispersing drops of water in empty cells, a honeybee colony can reduce its temperature. When water is available, a colony of *A. mellifera* can withstand external heat of up to 70° C. When the external temperature is low, bees reduce heat losses by clustering together, and the lower the temperature, the more compact the cluster (Jones and Oldroyd, 2007).



Figure 14. Thermoregulation in an *Apis florea* colony. Although the fanning wings are not visible in this still photograph, the way that the wings are spread out is characteristic of fanning.

The survival ability of honeybee colonies during severe winter months depends on whether the colony has enough workers adequately provisioned with food. Insulating the hive

wall and decreasing the volume of the hive can also improve the effectiveness of the colony's thermal regulation (Kronenberg and Heller, 1982; Heinrich, 1979; Heinrich and Esch, 1994). In the temperate regions, colonies of *A. mellifera* survive by forming clusters around the brood nest, the bees on the surface of the cluster and those within it exchange positions over time (Jones and Oldroyd, 2007). In this manner, *A. mellifera* colonies can survive temperatures as low as -40°C. The regulation of brood-nest temperature is not confined to the European races of *A. mellifera*. Tropical honeybee species and races can also regulate their brood-nest temperature to a certain extent, but they are able to survive only mildly cold temperatures, generally not below 0°C (Kronenberg and Heller, 1982; Heinrich, 1979; Heinrich and Esch, 1994).

### 3. HONEYBEE ANATOMY

Honeybees have many characteristics common to all insects. Insects have a hard outer covering called an exoskeleton that is made of a material called chitin that helps to protect the internal organs of the insect and prevent desiccation (Gary, 1992; Snodgrass, 1925). In order to grow, the insect must shed its exoskeleton. Its body can be divided into three sections: head, thorax, and abdomen. The head contains mouthparts, two compound eyes with three ocelli, and two antennae. The thorax contains the appendages for locomotion, the three pairs of legs and two pairs of wings. The abdomen contains the organs for digestion, reproduction, and defense (Gary, 1992; Snodgrass, 1925).

#### **The head of honeybees**

The head of the honeybee is triangular when viewed from the front. The two antennae arise close together near the upper center of the head. There are two compound eyes and three simple eyes located on the top of the head. The honeybee uses its proboscis, or long tongue, to feed on liquids and its mandibles to manipulate pollen and work wax in comb building (Gary, 1992; Snodgrass, 1925).



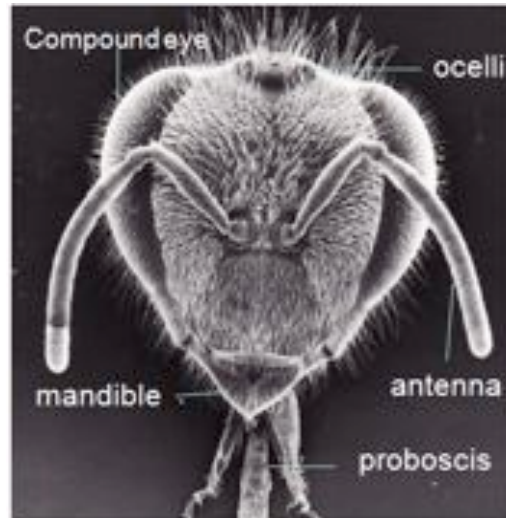


Figure 15. Scanning electron micrograph of the head of *Apis florea* worker.

### The antenna

Honeybee antennae are sensitive to temperature, humidity, air pressure, odor, gustatory stimuli, near-field sound vibrations, substrate vibrations, and tactile contact. Odor perception is particularly important. Each antenna contains a few thousand of antennal sensilla distributed over the third segment of the antenna, known as a flagellum (Figs 15 and 16). The insect antenna functions primarily as an odor receptor and secondarily as a taste receptor. The antennal form and arrangement of sensilla appear to be well adapted to the pheromone perception needs of a particular species (Gupta, 1992; Payne et al., 1970).

Each antenna consists of a single long joint connected to a prominent knob, which is inserted into an antennal socket. The honeybee can turn the antenna in 360° direction at the base (Agren, 1977; Snodgrass, 1925; Chapman, 1982; 1988). Each antenna has a segmented scape, a pivoted pedicel and a flagellum, which is composed of 11 segments in females, queens and workers, and 12 segments in drones. Sensory organs or sensilla on the antennae of honeybee can be divided into seven different types. They are sensilla basiconica, sensilla campaniforme, sensilla placodea, sensilla trichodea type A, B, C, and D (Snodgrass, 1925). Sensilla placodea measure air pressure and have olfactory functions. They are located on the last eight segments of the antennae (Guirfa, 1991; Gupta, 1992; Naik et al., 1995). The sensilla placodea which such consist of oval cuticular plates with numerous pores and each innervated by 15 to 30 olfactory receptor neurons containing large numbers of branched dendrites (Esslen and Kaissling, 1976).

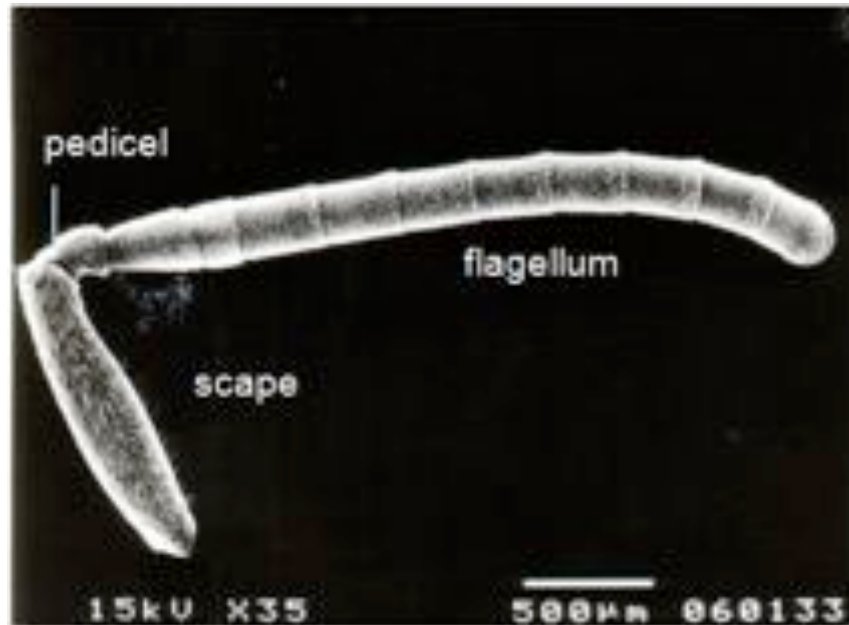


Figure 16. Scanning electron micrograph of the antenna of *Apis dorsata* consists of scape, pedicel and the flagellum.

Honeybees from different castes have different roles in their colony and exhibit different external and internal morphology (Snodgrass and Erickson, 1992; von Frisch, 1971). This is especially true for the olfactory sensilla. Honeybee queens primarily use their antennal sensilla to detect or perceive odor within the colony. Workers use olfactory sensillae to detect colony odors such as brood pheromone and queen pheromone in addition odors obtained outside the nest such as floral odors, which they can learn to associate with rewarding resources (von Frisch, 1971). Drones use these sensilla to detect queen pheromone. Antennal sensilla also play an important role in detecting various types of odors in *A. dorsata* foragers. Their foraging occurs during daytime and continues after sunset, a nocturnal feature that is not reported in other honeybee species (Suwannapong and Wongsiri, 1999; Suwannapong et al., 2010c).

Antennal sensilla each have multiple microscopic pores that are 10 to 50 nm in diameter (Steinbrecht, 1996; 1997; 1998) and allow the passage of volatile molecules (Slifer et al., 1959). The number of pores per sensillum (3-18,000 pores) and the diameter of pores (between 10-100 nm) vary depending on type of sensilla and insect species (Kaissling, 1971; 1972; 1974). Associated with each cuticular pore is a pore cavity or kettle from which extend four to eight filaments or tubules into the lumen of the sensillum and terminate at the receptor membrane (Slifer et al., 1959; Schneider, 1962; Schneider and Steinbrecht, 1968; Kaissling, 1971). Trichogen cells form the pore tubules. They may be single walled with whole tubules, or double walled with spoke canals. Their surface can be pitted or grooved longitudinally.

Most of the multiporous sensilla (MP) can easily be identified as multiporous pitted (MPP) or multiporous grooved (MPG). The MPP cuticles have many round holes or slits at the surface (Steinbrecht, 1997). The lumen is filled with sensillum lymph secreted by the trichogen (Tr) and tormogen (To) support cells. The thecogen support cell acts as a glial cell

for the olfactory neuron (Ernst, 1969; Ernst, and Boeckh, 1983; Farbman, 1992). This fluid contains a high concentration of odorant-binding proteins (OBPs), bathing the dendrites of the olfactory neurons. One to four olfactory neurons project dendrites into a single sensillum (Farbman, 1992). There are four types of olfactory sensillae: sensilla trichodea, sensilla basiconica, sensilla placodea, and sensilla coeloconica (Schneider and Steinbrecht, 1968). Each olfactory sensillum is connected to a single or multiple bipolar receptor neurons (ORNs) and auxiliary cells, trechogen (ensheaths the neuron and the peg), trichogen (synthesizes the peg) and tormogen cells (secrete the surrounding socket). Each sensilla cell envelopes the ORNs in each sensillum (Shanbhag et al., 1999, 2000; Steinbrecht, 1997).

### **The compound eye**

The honeybee eye is made up a large number of ommatidia or facets. Each ommatidium is composed a crystal line lens (the front surface of which makes up a single facet) that usually including light focusing elements (lens and a transparent crystalline cones). Within each ommatidium, light is focused onto eight light sensing cells (retinal cells) arranged in a radial pattern like sections of an orange (Giurfa, 1991; Giurfa et al., 1995). The pigment cells ensure that only light entering the ommatidium roughly parallel to its long axis reaches the visual cells and triggers nerve impulses. Thus, each ommatidium contributes information about only one small area in the field of view, just like a single pixel in a CCD of a digital camera. Each facet in a compound eye corresponds to one ommatidium and takes in one small part of the insect's vision. The brain then takes the image from each tiny lens and creates one large mosaic-like picture. Workers of *A. mellifera* have about 4,000-6,000 ommatidia but drones have from 7,000-8,600, presumably because drones need better visual ability during mating (Giurfa, 1991; Giurfa et al., 1995; Giurfa et al., 1996a; Giurfa et al., 1996b; Suwannapong and Wongsiri, 1999). There are approximately 6300 ommatidia in *A. dorsata* and *A. florea*, respectively. As in most insects, bee eyes are not designed to see high-resolution images like human eyes, but rather they see a mosaic lower resolution image. However, their compound eyes are better at motion detection than most camera lens eyes. Honeybees can adjust their light sensitivity and in dim light can adapt their eyes by concentrating the visual pigments of their ommatidia into the lower ends of the pigment cells. This shift enables light entering a single ommatidium at an angle to pass into and stimulate adjacent ommatidia. When multiple ommatidia respond to a single area in the visual field, the image becomes coarser and has reduced resolution (Giurfa, 1991; Giurfa et al., 1995; Giurfa et al., 1996a; Giurfa et al., 1996b; Suwannapong and Wongsiri, 1999).



Figure 17. Scanning electron micrograph of the compound eyes of *Apis dorsata* worker (left) and their facets (right)

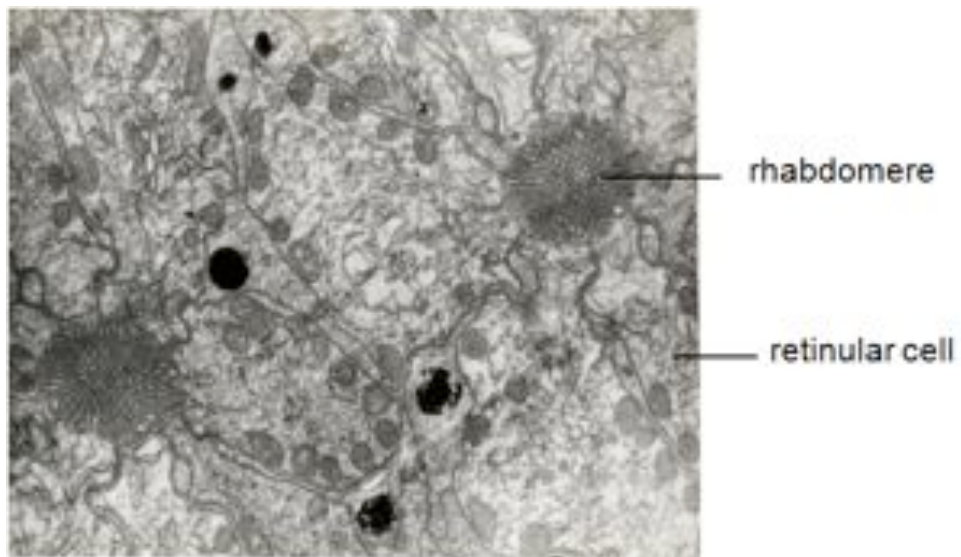


Figure 18. Transmission electron micrograph of the eight retinular cells surrounding the rhabdomere of *Apis florea* workers.



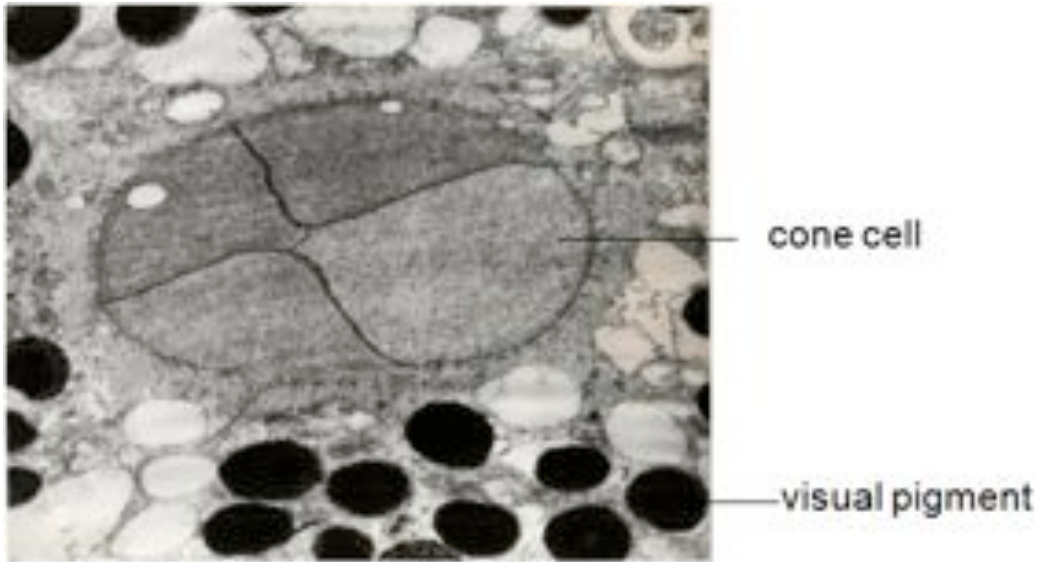


Figure 19. Transmission electron micrograph of the crystalline lens of *Apis dorsata* workers.



Figure 20. Scanning electron micrograph of the three ocelli of *Apis dorsata* workers.

Honeybees have trichromatic color vision. Each ommatidium consists of four cells that respond best to yellow-green light (544 nm), two that respond maximally to blue light (436 nm) and two that respond best to ultraviolet light (344 nm). This system enables the honeybee to distinguish colors, and this has been amply demonstrated in behavioral discrimination experiments (Chapman, 1988; Giurfa, 1991; Giurfa et al., 1996a, 1997). Although honeybees perceive a fairly broad color range, they strongly differentiate six major categories of color:

yellow, blue-green, blue, violet, ultraviolet, and also a color known as “bee purple”, a mixture of yellow and ultraviolet (Chapman, 1988; Giurfa, 1991; Giurfa et al., 1996a). Bees see red poorly. Differentiation is not equally good at all wavelengths and is best in the blue-green, violet, and bee purple colors. In addition, honeybees can discriminate various shapes and patterns, inability useful in recognizing different flowers and in local landmark orientation (Giurfa et al., 1995, 1996b). Honeybees can easily differentiate between solid and broken patterns, but show a preference for broken figures (Giurfa et al., 1995). Honeybees also have three smaller eyes in addition to their two compound eyes. These simple eyes that are called ocelli (singular: ocellus) and are located near the top of a bee’s head. The ocelli only provide information about light intensity. They cannot resolve images (Chapman, 1988; Giurfa, 1991; Giurfa et al., 1996a, 1996b).

**Table 2. Number of ommatidia in honeybee eyes.**

Honeybee species	Number of facet	Size of facet
<i>Apis florea</i>	7,933 7,760 queen	Un publish data (Suwannapong)
<i>A. cerana</i>	8,160 7,036 queen	Un publish data (Suwannapong)
<i>A. dorsata</i>	6,200 (worker) 5,700 (queen)	Rasmidatta et al., 1999 Suwannapong, 1999
<i>A. mellifera</i>	4,500	Gould and Gould, 1988
<i>A. andreniformis</i>	7,780	Un publish data (Suwannapong)



Figure 21. Scanning electron micrograph of the mandibles of *Apis dorsata* workers.

### **The internal organ of the head**

The main internal organs in the head are the brain, subesophageal ganglion, and the esophagus (food canal). The brain has a large area for receiving inputs from the two compound eyes, called optic lobes. The next largest input is from the antennal lobes. One important region in the middle of the brain is called the “mushroom body” because its cross section resembles a mushroom. This area is important for learning and memory formation (both consolidation memory and long term memory) (Snodgrass and Erickson, 1992).

Endocrine organs are also attached to the nerve cord, very close to the esophagus. One is called the corpora allata (in Latin it means the body beside the food canal). The corpora allata is the only source of a key important hormone, juvenile hormone, which is involved in the queen-worker differentiation and worker division of labor. The second key endocrine organ is called the corpora cardiaca (the body near the heart). This neurohemal organ stores and releases another hormone (PTTH, prothoracicotropic hormone). PTTH can stimulate the production of ecdysone in the prothoracic glands, located in the thorax (Snodgrass, 1956; Snodgrass and Erickson, 1992).

Lastly, there are several exocrine glands inside the head: mandibular, hypopharyngeal and salivary glands. Mandibular glands are a simple sac-like structure attached to each of the mandibles. In the queen, this is the source of the powerful queen pheromone (Boch and Shearer, 1971; Plettner et al., 1993; 2000). In young workers, the gland produces a lipid-rich white substance that is mixed with the secretion of hypopharyngeal glands to make royal jelly or worker jelly and fed to the queen or other workers. In old *A. mellifera* workers, (foragers) the gland also produces 2-heptanone, a component of the alarm pheromone (Boch and Shearer, 1971; Plettner et al., 1993; Snodgrass, 1956; Snodgrass and Erickson, 1992).



Figure 22. Scanning electron micrograph of the hypopharyngeal gland of an *Apis andreniformis* worker.

Other species of honeybees produce different compounds in their mandibular glands (see above). The hypopharyngeal glands produce protein-rich secretions in nurse bees, but produce invertase (an enzyme that breaks down sucrose into fructose and glucose) in foragers. The glands consist of a central duct with many of small grape-like spheres (acini, singular: acinus). The secretion flows to the mouth through the long duct. The glands are large (hypertrophied) in nurse bees (Deseyn, and Billen, 2005; Kubo et al., 1996; Ohashi et al., 2000; Suwannapong et al., 2010a). There is also a pair of salivary glands inside the head. The glands produce saliva, which is mixed with wax scales to change the physical properties of wax, making it easier to work (Snodgrass, 1956; Snodgrass and Erickson, 1992).

### Hypopharyngeal glands

Hypopharyngeal glands (HPGs) of honeybees are age-dependent structures that change with the size of acini and are correlated with social behavior (Feng et al., 2008; Kubo et al., 1996; Ohashi et al., 1999; Ohashi et al., 2000; Suwannapong et al., 2010a). Gland protein concentration increases progressively in nurse bees, and this has been correlated with the appearance of enriched protein granules in the cytoplasm. The glands are composed of several secretory units, each opened into a secretory duct that passed through the mouthparts. In pupae, the secretory cells are irregular in shape with low concentrations of proteins and carbohydrates, while the glands of nurse bees and foragers are fully developed with numerous secretory vesicles (Suwannapong et al., 2007). It has been also reported that the hypopharyngeal glands of *A. cerana* and *A. mellifera* workers had significantly larger than those of *A. florea* and *A. andreniformis* either guards or foragers, but without substantial



differences between these two species after counting for caste (Suwannapong et al., 2007; Suwannapong et al., 2010a).

The structure of honeybee HPGs depends on the development and age of individuals, which corresponds with age-specific tasks and is known as age polyethism (Deseyn and Billen, 2005; Robinson, 1987; 1994). Studies have shown that the histochemical structure of carbohydrate and protein in *A. cerana* and *A. mellifera* was correlated with honeybee age-specific tasks of the colony. Young worker nurse bees care for and feed their brood with royal jelly that is synthesized and secreted from the hypopharyngeal glands (Deseyn and Billen, 2005; Feng et al., 2008; Kubo et al., 1996; Ohashi et al., 1999). These hypopharyngeal glands were strongly positive to PAS and Ninhydrin Schiff's reagent reactions in this study (Suwannapong et al., 2007; Suwannapong et al., 2010). However older workers, when they became guards, had less positive staining to PAS as compared to nurse bees. This may be related to the development of the hypopharyngeal glands, which are fully developed when young workers take care of the brood by synthesizing and secreting royal jelly. Older workers no longer feed the brood, and thus gland atrophy is expected (Suwannapong et al., 2010a).

The development of HPGs in dwarf honeybee workers, *A. andreniformis* and *A. florea* primarily depends on age. These glands begin to differentiate at pupal stage and are largely undeveloped at emergence (Suwannapong et al., 2007). When workers become nurse bees, they perform brood rearing that is associated with HPGs development. The size of HPGs is correlated with glandular production and generally increases with age from 6 to 18 days in nurse bees (Deseyn and Billen, 2005; Hrassnigg and Crailsheim, 1998). HPGs synthesize and secrete proteinaceous substances and royal jelly that are fed to the queen and brood (Deseyn and Billen, 2005). The highest rate of protein synthesis occurs during nursing ages from 8 to 16 days (Ohashi et al., 2000; Knecht and Kaatz, 1990). In bees older than 18 days (guards and foragers), the HPGs decrease considerably in size and secrete enzymes such as  $\alpha$ -glucosidases, leucine arylamidase and invertase (Kubo et al., 1996; Ohashi et al., 1999; Feng et al., 2008). Forager gland size is reduced and correlated with gland activity (Deseyn and Billen, 2005; Furi et al., 1982; Ohashi et al., 2000).

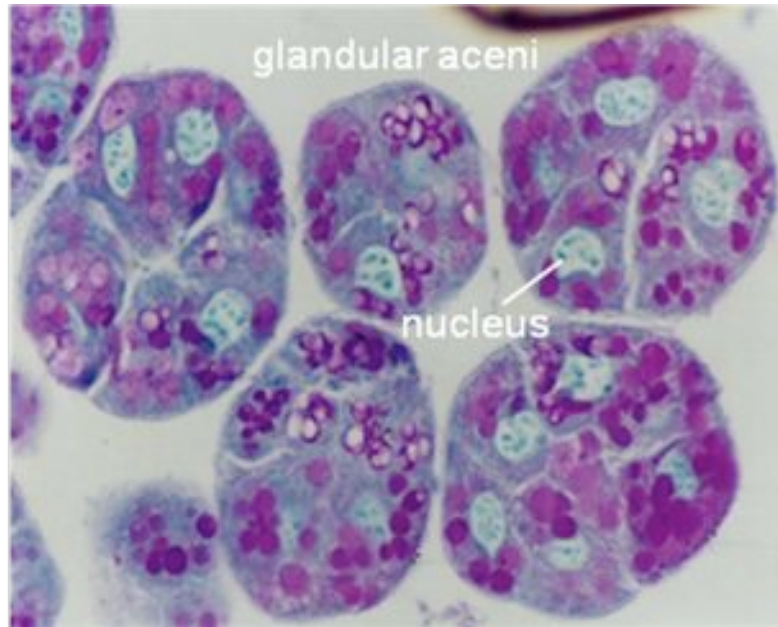


Figure 23. Light micrograph of the hypopharyngeal gland of an *Apis andreniformis* worker stained with PAS and counterstained with light green (200X).

Moreover, other studies have reported that the secretory units of the HPGs were filled with numerous vesicles that gave a strong positive staining with PAS and Ninhydrin Schiff's reagent (Suwannapong et al., 2010a). This indicates that the glands of nurses, guards, and foragers in four species of honeybees (i.e., *A. andreniformis*, *A. florea*, *A. cerana*, and *A. mellifera*), play an important role not only in secretion of carbohydrate rich substance but also in the secretion of enzymes for converting nectar to honey (Suwannapong et al., 2007; Suwannapong et al., 2010a; Simpson et al., 1968; Brouwers, 1982). However, there were also differences found in structure of the glands between the hive cavity nest honeybees, *A. cerana* and *A. mellifera*, and the single open nest honeybees, *A. andreniformis* and *A. florea*. The structure of the extracellular space between adjacent cells of *A. andreniformis* and *A. florea* was wider than that of *A. cerana* and *A. mellifera*. In addition, the secretory units of hypopharyngeal glands of *A. mellifera* from this study were different from results in the study of Deseyn and Billen (2005) who showed that the volume of acini decreased in foragers or displayed degenerative structure. This was not found by Suwannapong et al. (2010a). Hypopharyngeal glandular size is known to be positively correlated with gland activity and is influenced by larval feeding (Hrassnigg and Crailsheim, 1998; Ohashi et al., 2000; Crailsheim and Stolberg, 1989; Free, 1981; 1987). These glands gradually decrease in size when honeybees become guards, cease feeding, and begin defending the colony (Deseyn and Billen, 2005). However, the hypopharyngeal gland size of foragers was significantly larger than that of guards indicating that glandular development corresponds well with total protein synthesis in the hypopharyngeal glands at different adult life stages. Lastly, a number of reports indicate that the HPGs produce enzymes that are used to hydrolyze nectar into honey, including amylase,  $\alpha$ -glucosidases, glucosidase oxidase, galactosidase, esterase, leucine

arylamidase, and invertase (Hrassnigg and Crailsheim, 1998; Kubo et al., 1996; Li et al., 2008).

### The thorax

The thorax is the middle part of the bee. The thoracic contains the thoracic glands, which are derived from the cocoon-spinning gland of the larva and are well developed in workers, queens and drones (Snodgrass and Erickson, 1992; Snodgrass, 1956). Primarily, the thoracic is the anchor point for a bee's locomotory appendages for walking and flight. Three pairs of legs arise from the thorax: prothoracic (closest to the head), mesothoracic (center), and metathoracic (hind) legs. Honeybee metathoracic legs are modified to be pollen baskets. There are two also two pairs wings also located on the thorax of adult bees (Snodgrass, 1956).

**Legs:** The honeybee has three pairs of segmented legs. The legs of the bee are primarily used for walking. However, the legs have specialized areas such as the antennae cleaners on the prothoracic legs, and the pollen baskets on the metathoracic legs. These pollen baskets are also used to transfer propolis (Snodgrass and Erickson, 1992). Three pairs of legs divided into six segments by joints. The “foot” or tibia, of the insect has claws and a smooth pad to enable them to cling to surfaces. The mesothoracic leg has brushes for cleaning the thorax, and long spines at the end that bees use to loosen pollen from the pollen baskets and to clean the wings and the small breathing pores or spiracles (Snodgrass and Erickson, 1992; Snodgrass, 1956). In addition, the mesothoracic legs remove wax scales from the abdomen.

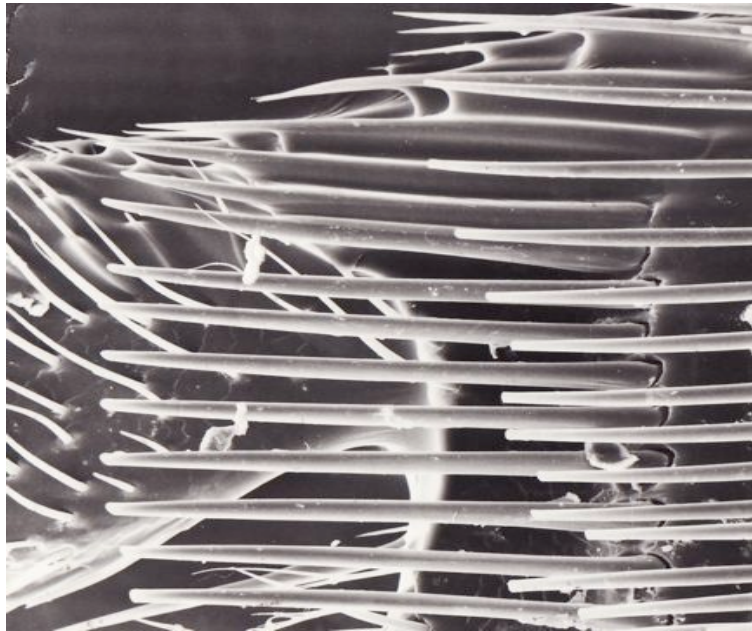


Figure 24. Scanning electron micrograph of the pollen basket of *Apis cerana*.

The metathoracic legs differ from the other legs in their larger size and broad flattened form. These legs differ in size in the queen, worker, and drone. Only the worker collects pollen. The legs have long curved hairs. The space enclosed by these hairs is called the pollen basket. This consists of a smooth, somewhat concave surface on the outer metathoracic leg that is

fringed with long, curved hairs to hold the pollen in place. This structure is known as a corbiculum and has 10 transverse rows of stiff hairs projecting backwards (Snodgrass and Erickson, 1992; Snodgrass, 1956). The deep notch between the upper and middle portion of the metathoracic leg transfers the pollen from brushes on the ventral section of each leg to the medial (inner) section of each leg. In this notch, there are short stiff spines called a rake. Bees use the rake by rubbing the leg on one side against the other leg.

The basitarsal hairs of *A. florea* are white while in *A. andreniformis* they are black. Drones of *A. florea* and *A. andreniformis* have a distinctive long inner lobe on the hind tibia (Maa, 1953; Wongsiri et al., 1996a; Wu and Kuang, 1987).

**Wings:** The honeybee has two sets of flat, thin, membranous wings, strengthened by veins (Crushman, 2010; Snodgrass and Erickson, 1992). The fore wings are much larger and stronger than the hind wings, and the two wings of each side work together in flight since small hooks called hamulae connect them. The bottom wing has hooks on the top edge. During flight, the front wings are drawn over the hind wings and held together by the hooks. Flight results from a propeller-like twist given to each wing during the upstroke and the down stroke. The wing vein measurement is very important for calculating the cubital index, the ratio of two wing vein segments. For instance, in Figure 25 points A, B & C should be judged to the centroid of the vein junctions concerned. The distance “AB” divides the distance “BC”. There is a tool for doing this known as the Herold Fan named after the individual who devised this method (Crushman, 2010).

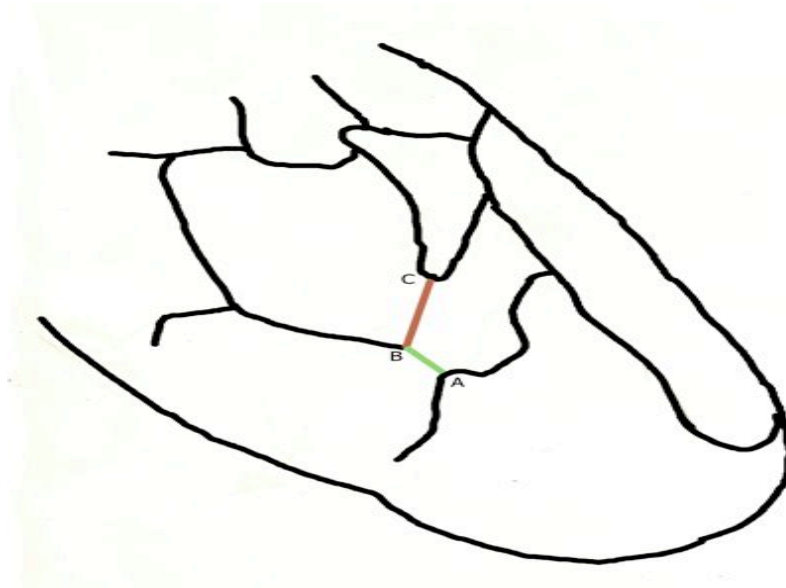


Figure 25. Wing venation of *Apis florea* worker.

The pattern of the veins of the fore wings and the cubital index is consistent for a given race of bee. The cubital index of *A. florea* is 2.78 (Wongsiri et al., 1990) and 2.86 (Rinderer et al., 1995). The wing length is  $6.26 \pm 0.10$  mm (Seeley, 1982). In *A. andreniformis*, the cubital index ranges from 6.28-6.37 (Rinderer et al., 1995; de Guzman et al., 1993). *Apis dorsata* workers have a rusty brown pubescence and dark tinge to the wings that are approximately

12.34 ± 0.34 mm in length (Seeley, 1982). The wing length of *A. cerana* is 7.54± 0.14 mm (Tingek et al., 1996) and the cubital index is 4.40 (Seeley, 1982). In *A. mellifera*, the forewing length is 7.64-9.70 mm and the cubital index is 2.30 (Seeley, 1982).

Honeybee species are clustered into three groups base on body and wing size. The largest size is *A. dorsata* group with a forewing length of 12-15 mm. Honeybees with a forewing length of 7-10 mm are a medium size of *A. mellifera* group while the smallest species, *A. florea* have fore wing length are from 5-7 mm (Maa, 1953; Ruttner, 1988).

### The abdomen

The honeybee abdomen is composed of nine segments and contains digestive organs, wax and some pheromone glands, reproductive organs, and the stinger which is a modified ovipositor that can both sting and deposit eggs in adult females. The body color is a distinctive characteristic among honeybee species and subspecies (Woyke, 1977). *Apis florea* has bright red-brown color on the first and the second abdominal segments, but *A. andreniformis* has a chestnut brown color, and other segments are yellow and banded in queen and workers, but are black in drones.



Figure 26. *Apis andreniformis*, *A. cerana*, *A. dorsata*, *A. florea*, and *A. mellifera* showing body color.

The gene responsible for body color expression of *A. florea* is designated Fl (Woyke, 1998). The pattern of yellow and black between queen and workers are different. The body color of *A. andreniformis* workers after the second segment is yellow although they are the darkest of all five honeybee species of Thailand. Queen and drones are black. In *A. dorsata* the workers



are yellow and black on abdomen segments (Sakagami et al., 1980): however, the queen and drones are brown color. The gene governing body color in this species is designated Do (Woyke, 1998). *Apis cerana* workers are yellow while queen and drone are brownish- black. The gene control color expression of this species is designated Ce (Woyke, 1998). The body color of *A. mellifera* is similar in workers, queen and drones, has yellow and black color abdomen, however the black area is different among subspecies which is control by Gene Y (Woyke, 1977; 1998).

**Stinger:** The stinger is located in a chamber at the end of the abdomen, from which only the sharp pointed shaft protrudes. The stinger is modified from an egg-laying organ, known as the ovipositor. However, the stinger can also be used to inject venom. Only females have a stinger/ovipositor. When the stinger is not in use, it is retracted within the sting chamber in the abdomen. The shaft of the sting is a hollow tube, like a hypodermic needle. The tip is barbed so that it sticks into the flesh or exoskeleton of the victim. The hollow needle has three sections. The top section is called the stylet and has ridges while the bottom has two pieces called lancets (Snodgrass and Erickson, 1992; Snodgrass, 1956).

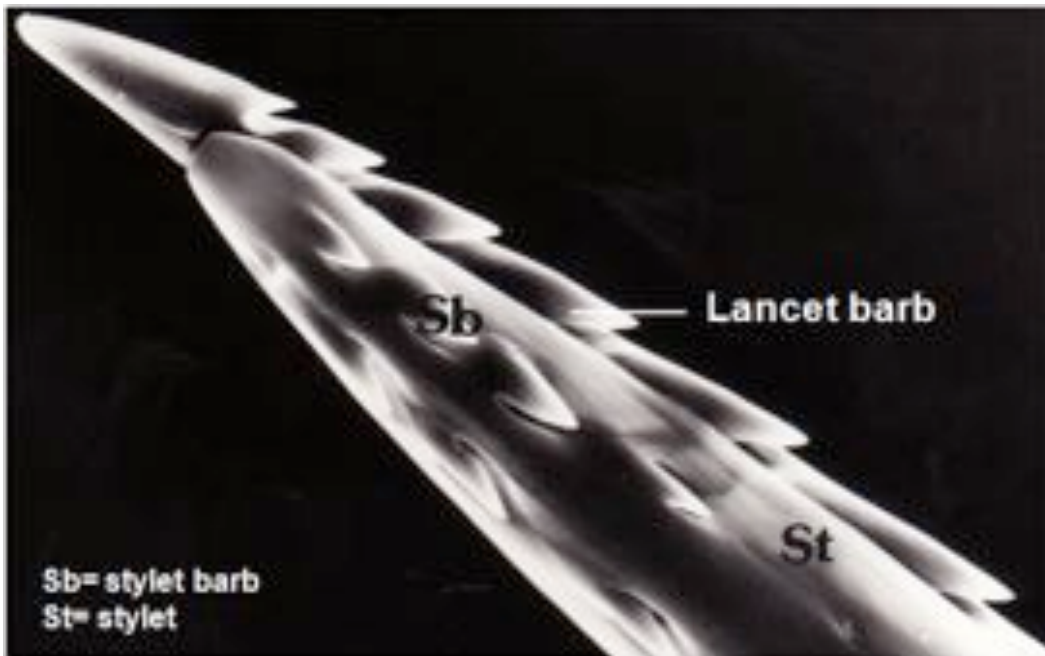


Figure 27. Scanning electron micrograph of the sting apparatus of *Apis cerana*.

When the stinger penetrates the skin, the two lancets move back and forth on the ridges of the stylet so that the whole apparatus is driven deeper into the skin. The enclosed tube of the two adjacent lancets creates the poison canal. In front of the shaft is the bulb. The ends of the lancets within the bulb are enlarged, and, as they move, they force the venom into the poison canal, like miniature plungers. The venom comes from two acid glands that secrete into the poison sac. During stinging, the contents of the alkaline gland are dumped directly into the poison canal where they mix with the acidic portion. When a honeybee stings another animal, the sting becomes embedded. In its struggle to free itself, a portion of the stinger is

left behind in the victim. This often damages the honeybee enough to kill her (Snodgrass and Erickson, 1992; Snodgrass, 1956).

Interestingly, although separated from the bee, the stinger continues to contract reflexively, continuously pumping venom into the wound for several seconds. The stinger of the queen is longer than that of the worker, and more solidly attached within the sting chamber. The lancets are fewer and the barbs are smaller than those found in a worker's stinger (and therefore less likely to remain inside the victim such as another queen), but the poison glands are well developed and the poison sac is very large (Snodgrass and Erickson, 1992). Queens typically only use their stinger upon other queens in the competition over who will take over the colony (see above section on castes and colony re-queening) (Snodgrass and Erickson, 1992; Snodgrass, 1956).

## 4. HONEYBEE PHEROMONES

### **Pheromone Glands and Pheromone Production**

Communication among insects is extremely important for their survival, especially for social insects that live in complex colonies. Honeybees are well known their chemical communication and use of olfactory cues (Free, 1987; Free et al., 1983; Leal 2010). For example, honeybees can navigate to food sources by detecting floral scents and by orienting towards food-marking pheromones or, possibly, cuticular hydrocarbons (cues) deposited as footprint (Balerrama et al., 1996). Honeybees smell or detect pheromone with their antennae using odorant-binding proteins in sensillum lymph. They produce volatile and non-volatile chemicals as signal molecules from their exocrine glands to communicate with others of the same species or with other species (Kaissling, 1987). These signaling chemicals are often called semiochemicals (Kaissling, 1972).

The secretory product of worker mandibular glands consists of 10- and 8-carbon acids that have an oily appearance. In young workers, this gland produces a lipid-rich white substance that is mixed with the secretion of the hypopharyngeal glands to make royal and worker jelly that is fed to the queen or workers. In old workers (foragers), the gland also produces 2-heptanone, a volatile substance that accumulates in the central reservoir, the amount of which progressively increases with increasing age (Engels et al., 1997). This compound can repel guard bees, and is a potential component of alarm pheromone. On guards, 2-heptanone has been reported to have either an attractive or a repulsive effect, according to the season. A foraging bee may mark a nectar-depleted flower with 2-heptanone (Balerrama et al., 1996; Boch and Shearer, 1962; 1971; Blum, 1969; 1982; 1992; Blum et al., 1978; Crewe and Hastings, 1976; Engels et al., 1997; Guirfa, 1991; Suwannapong, 2000). In foraging bees, 2-heptanone can have a temporary, repulsive effect on the visitation of flowers. Suggesting that, it is "forage marking" pheromone (Vallet et al., 1991). Mandibular glands of Thai honeybees mainly produce (z) -11-eicosanol instead of 2-heptanone (Suwannapong, 2000, Suwannapong et al., 2010a).

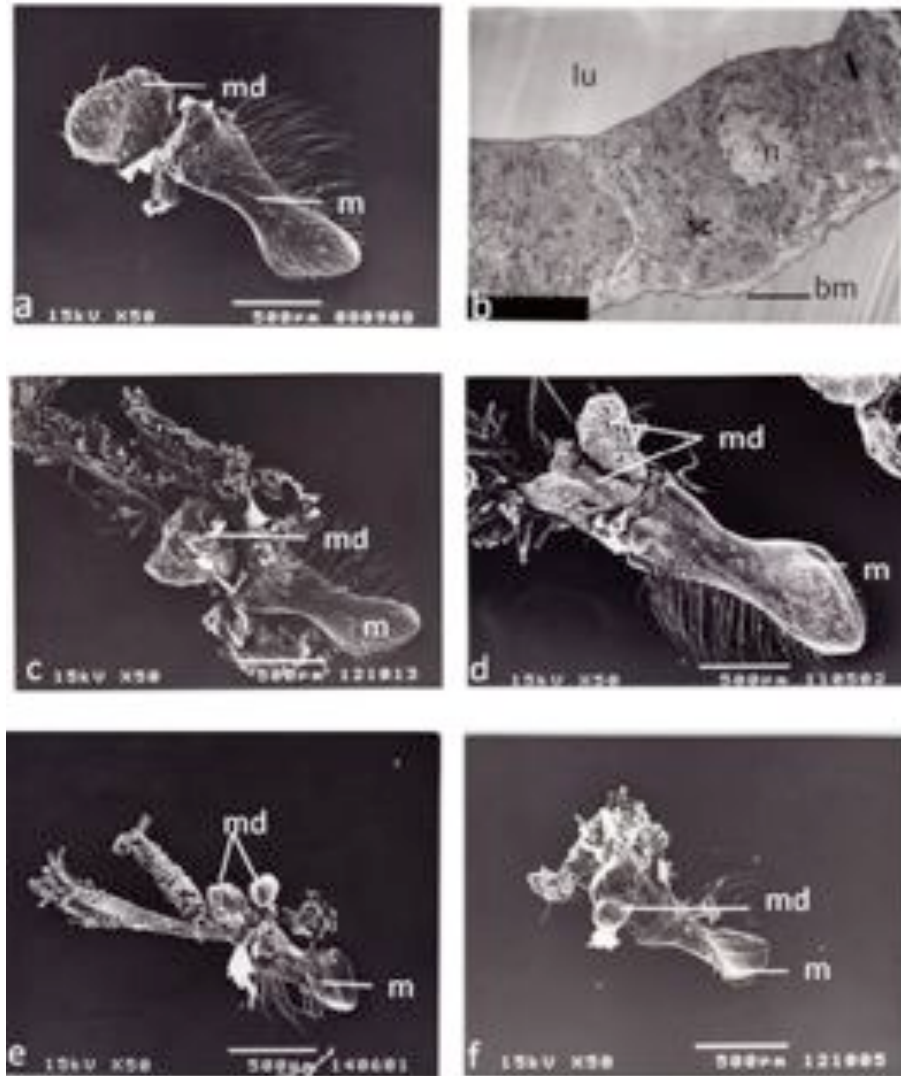


Figure 28. (a) Scanning electron micrographs of one mandibular gland of *Apis mellifera*, (b) the duct cells of that mandibular gland, (c) one mandibular gland of *A. cerana*, (d) a mandibular glands of *A. dorsata*, (e) mandibular glands of *A. andreniformis*, and (f) mandibular glands of *A. florea*; bm, basement membrane; lu, lumen; m, mandible; md, mandibular gland; n, nucleus; sc, secretory cell (Suwannapong and Benbow, 2011).

Maschwitz (1964) suggested that mandibular glands produce alerting pheromones (in Blum, 1969). Shearer and Boch (1965) identified 2-heptanone from mandibular gland secretions. When filter paper with 2-heptanone was placed at the hive entrance, guard bees were alerted and attacked the paper. This is consistent with suggestions by Boch and Shearer (1962) that 2-heptanone has two functions: as an alarm pheromone and as a foraging repellent pheromone. However, it is possible that cuticular hydrocarbons deposited by bees walking on a depleted food source are sufficient to indicate that food sources are depleted. It is possible that 2-heptanone is a repellent at high concentrations and an attractant at low concentrations (Boch and Shearer, 1971; Kerr et al., 1974; Vallet et al., 1991). The conflicting hypotheses of

2-heptanone's function on a depleted resource (repellent or attractant) require additional study.

In *A. florea* and *A. cerana*, the response of guards towards 2-heptanone was different from the response of foraging bees (Suwannapong et al., 2010a; Suwannapong et al., 2011). The response of antennal sensillum of *A. florea* to low concentrations of 0.1 % 2-heptanone was higher than the response to higher concentrations of 5% and 10% 2-heptanone (Suwannapong et al., 2011). In contrast, *A. cerana* foragers exhibited a stronger response to a 10% 2-heptanone compared to that of 0.1 and 1.0%. The membrane potential of foragers following exposure to 2-heptanone was higher than that of guards in both *A. florea* and *A. cerana* (Suwannapong et al., 2010c; Suwannapong et al., 2011). One of the components of sting pheromone, isoamyl acetate (IPA), releases strong alarm behavior in bees. Aggressive behavior can be observed when mandibular glands or crushed heads of worker bees are presented at the hive entrance (Shearer and Bosch, 1962; 1965). IPA is 20-70 times more effective as an alarm pheromone than 2-heptanone (Boch and Shearer, 1971; Boch et al., 1975).

All species of *Apis* have alarm pheromones and the compounds are generally similar among honeybee species with the exception of *A. laboriosa*, the giant Himalayan honeybee (Vander Meer et al., 1998; Harborne, 1993). Africanized bees secrete alarm pheromones with the same concentration of isopentyl acetate as other bee species, but with more 2-nonanol and decyl acetate. These differences may lead to a more aggressive response to alarm pheromone. Honeybee species that have open nests, such as *A. florea*, *A. andreniformis*, and *A. dorsata* tend to have alarm pheromones that persist longer, such as 2-decenyl acetate (Vander Meer et al., 1998). Workers of Thai *Apis* species also have these compounds, along with 9-HDA and ODA, which are normally not present in *A. mellifera* worker glands. Queens and workers of each different *Apis* species have different combinations of mandibular compounds (Plettner et al., 1982; 1996). The sting glands of *A. dorsata* and *A. florea* have an additional pheromone besides isopentyl acetate (IPA), 2-decen-1-yl-acetate (2-DA). Upon presenting this pure compound, these Thai species exhibited a prolonged alarm reaction as compared to the reaction for pure IPA. A mixture of IPA and 2DA had a similar effect on the behaviour and reaction time, as did sting extracts (Koeniger et al., 1979).

## 5. POLLINATION BY THAI HONEYBEES

Thailand is tropical country that has four main regions: (1) the northern mountainous region, (2) the north-east (including the semi-arid Korat plateau, the most desolate and least-visited part of the country), (3) central Thailand (including the fertile plains surrounding the Chao Phraya River which is the country's most populous region and its rice basket), and (4) the southern region which stretches for hundreds of miles along the Malay peninsula. Thailand has a tropical climate divided into three seasons: cool in November to February, hot in March to May, and rainy in June to October. The seasons are more extreme in the northern regions (Maksong, 2008).

In Thailand, more than a thousand plant species, including agricultural crops and native plants are pollinated by insects. Of these, several hundred plant species are visited by honeybees. For example, honeybee pollination increases the productivity of crops such as

corn, sunflower, lychee, mango, rambutan, longan, sesame, and durian. This section provides an overview of the plants upon which Thai honeybees forage, how to use pollen analysis to identify these plants, how to manage bee plants to the benefit of bees, and when different species flower (Maksong, 2008).

### **Honeybee Pollination in Thailand**

Honeybees play a major agricultural pollinators around the world (McGreger, 1976; Crane, 1991; Free 1993; Partap and Verma, 1994) play an important role in tropical ecosystems, such as in Thailand. All species of honeybees in Thailand tend to be good generalist pollinators for native plants. Honeybees typically do not nectar rob, their body size and proboscis lengths are suited for pollinating many types of crops and they can forage in a wide variety of weather conditions.

Thai honeybee species forage by visiting plants for nectar and pollen. As the bees forage for nectar, pollen sticks to the fuzzy hairs that cover their bodies. Some of this pollen rubs off on the next flower they visit, fertilizing the flower. Some plants will not produce fruit at all without the help of honeybees. This floral fidelity of bees is due to their preference for nectars having sugar contents and pollens with higher nutritive values. All species of honeybees in Thailand are very good pollinators for native plants due to their related morphological structure of the organs that fit and provide other functions important for pollination, such as a body covered with hairs that help carry nectar and pollen. Further the bees do not injure the plants, as the body size and proboscis length are very much suitable for many Thai crops (Pyramarn and Wongsiri, 1986).

The availability of natural insect pollinators in Thailand is decreasing rapidly as a result of increased and continued use of pesticides. There is timely need for better management of hive honeybees such as *A. cerana* and *A. mellifera* in rare pollinator areas to increase fruit production. Information on the role of honeybees in pollination leads to increase in the quality and yield of crops that has been reported worldwide (McGreger, 1976; Crane, 1991; Free 1993; Partap and Verma, 1994).

### **Categorization of Bee Flora**

Nectar content, odor, color, and shape of flowers affect honeybee foraging behavior, which is somewhat different among species. Honeybees forage on a variety of plant species to collect nectar and pollen (McGregor, 1976). However, not all plant species are available in one locality. A plant that produces nectar and pollen prolifically in one area may not yield the same amount of nectar and pollen in another area (Erdtman, 1966, 1969; Latif et al., 1960; Singh, 1981).

Bee flora or bee plants are the plants at which bees collect pollen and nectar. There are three types of bee flora: plants that only supply nectar, plants that only supply pollen, and plants that provide both (Allen et al., 1998; Baker, 1971; 1983; Bhattacharya, 2004; Crane et al., 1989; Partap, 1997). Some plants provide only resin, but these are less common. Floral nectar provides energy for flight activity, foraging activity and other activity in the colony. Honeybees also convert the nectar into honey and store in honey storage area of the comb. Pollen provides protein, lipids, minerals, and vitamins (Gary, 1975; 1992). Pollen from different plant species differs in nutritive value and attractiveness to honeybees (Baker, 1971; 1983; Erdtman, 1966, 1969; Shuel, 1992).



The five species of honeybees in Thailand differ somewhat morphology, differences that affect their foraging preferences. Nectar content, odor, color and shape of flowers affect to honeybee foraging behavior and they are different among honeybee species (Akkratanakul, 1976; Maksong, 2008; Pyramarn and Wongsiri, 1986). In Thailand, several plant species are most visited by these five species. These plants consist of agricultural and horticultural crops such as maize, rice, cucurbitaceous plants, bean, carrots, cabbage, litchi, mango, papaya and wild plants and forest trees such as rubber tree, Eucalyptus, teak, Burmese Ebony, and jambolan plum (Akkratanakul, 1976; Maksong, 2008; Pyramarn and Wongsiri, 1986). Studies of bee flora can be carried out by several techniques such as pollen load analysis, melissopalynology (identification bee flora from honey), identification from the midgut, and from observations of foraging activity on flowers of different local plants (Akkratanakul, 1976; Maksong, 2008; Pyramarn and Wongsiri, 1986).

The success of beekeeping essential depends on the abundance and management of bee flora in an area. However, a plant that produces nectar and pollen prolifically in one area may not yield the same amount of nectar and pollen in another area (Latif et al., 1958; Singh, 1981). The species of plants visited by Thai honeybees are shown in Table 3. There are more than 30 species of plants visited by *A. andreniformis* in Thailand such as *Anacardium occidentale* L., *Antigonon leptopus* Hook., *Balakara baccata* Roxb., *Brassica chinensis* Just var., *Castanopsis acuminatissima* Rehd., *Chrysal*, *Cocos nucifera* L., *Coriandrum sativum* L., *Conyza sumatrensis* Retz., *Cucurbita citrillus* L. *Cucumis sativus* Linn, *Cuphea hyssopifolia* H.B.K., *Dimocarpus longan* Lour., *Eugenia javanica* and *Mimosa pigra* (Maksong, 2008).

The plants visited by *A. florea* include more than 40 species such as *M. pigra*, *Callistemon viminalis*, *Vetchia merrillii* (Becc.) H.E. Mosre, *Cocos nucifera* L., *Melampodium divaricatum*, *Zea mays* L., *C. hyssopifolia* H.B.K., *D. longan* Lour., *Durio zibethinus* L., *E. javanica*, *Eupatorium odoratum* L., *Euphoria longana* Lamk., *Fragaria ananassa* Guedes, *Hopea odorata* Roxb (Maksong et al., 2011). *Apis dorsata* reportedly uses fewer food plants than *A. florea*. Only 38 species are reportedly used by *A. dorsata*: *Ageratum conyzoides* L., *Amomum xanthioides* Wall., *Anacardium occidentale* L., *Blumea balsamifera* L. DC., *Bidens biternata* Merr. and Sherff., *Celosia argentea*, *Cinnamomum kerrii* Kosten, *Citrus aurantifolia* Swing., *C. maxima* (J. Burman ) Merr., *Cocos nucifera* L. (Maksong 2008).

There are more than 68 plant species visited by *A. cerana*. These include *Aeschynomene americana* L., *Ageratum conyzoides* L., *Amomum xanthioides* Wall., *Anacardium occidentale* L., *Antigonon leptopus* Hook. *Balakara baccata* Roxb., *Bidens biternata* Merr. & Sher, *Brachiaria ruziziensis* Germain & Evrard, *Castanopsis acuminatissima* Rehd., *Cinnamomum kerrii* Kosten, *Coccinia grandis* CL.Voigt, *Cocos nucifera* L., *Coffea Arabica* L., *Conyza sumatrensis* Retz. The number of bee *florea* of the introduced honeybee species in Thailand are more than 54 species such as *Ageratum conyzoides* L. , *Durio zibethinus* L., *Euphoria longana* Lamk., *Fragaria ananassa* Guedes, *Leersia hexandra* Sw., *Macadamia integrifolia* maiden & Betche, *Mikania cordata* Roxb., *Mimosa pigra*, *Musa acuminata* Colla., *Nephelium lappaccum* L., *Ocimum basillicum* L., *Oryza sativa* L., *Oxalis acetosella* L., *Prunus mume* Sieb., *Psidium guajava* L., *Sesamum indicum* L., *Schoenoplectus juncooides* (Roxb.) Palla, *Raphanus sativus* L. (Maksong, 2008).

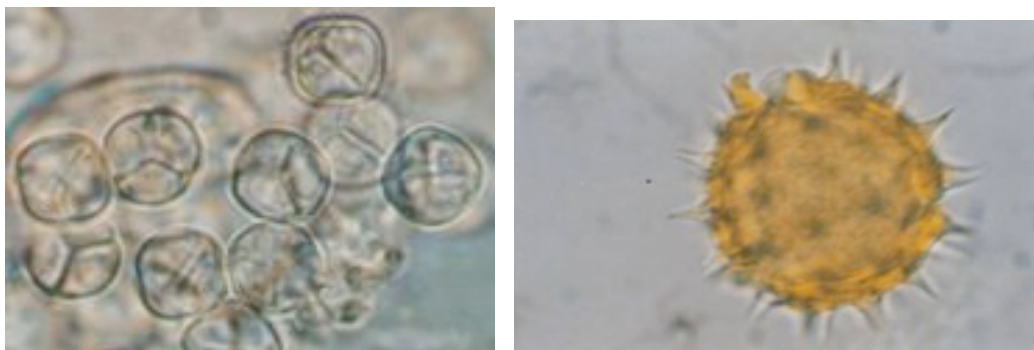


Figure 29. Pollen of bee flora, *Mimosa pudica* L. (left) and *Tagete erecta* L. (right) from the midgut of *Apis dorsata*.

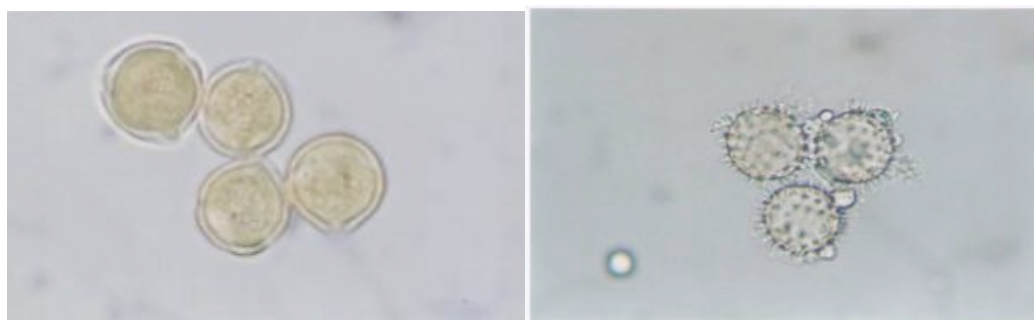


Figure 30. Pollen of bee flora, *Muntingia calabure* L. (left) and *Melampodium divaricatum* (right) from the midgut of *Apis andreniformis*.

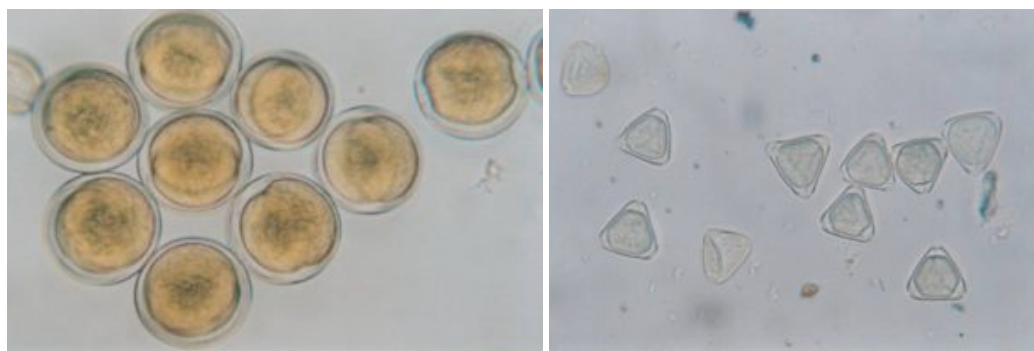


Figure 31. Pollen of bee flora, *Nymphaea nouchali* Borm. F (left) and *Callistemon viminalis* (right) from the mid gut of *Apis florea*.

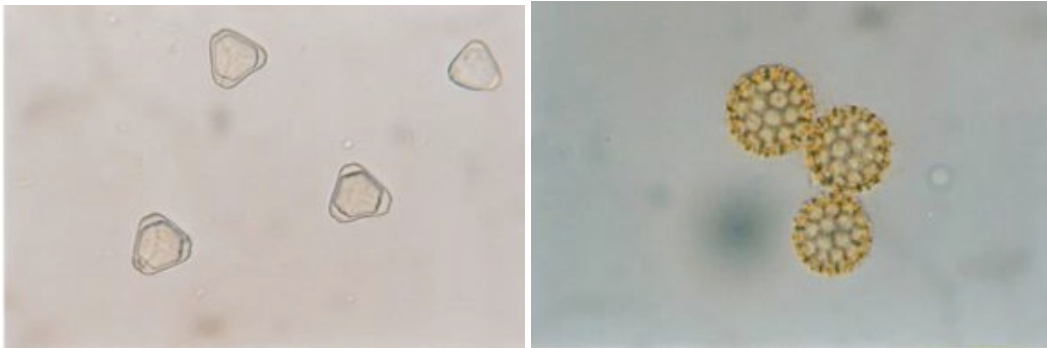


Figure 32. Pollen of bee flora, *Syzygium malaccense* L. (left) and *Gomphrena globosa* (right) from the mid gut of *Apis cerana*.

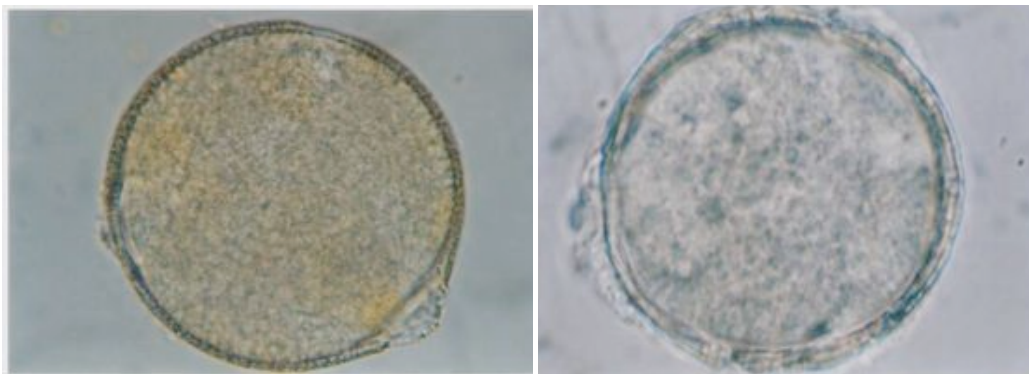


Figure 33. Pollen of bee flora, *Luffa cylindrical* Roem (left) and *Coccinia grandis* CL.Voigt (right) from the mid gut of *Apis cerana*.

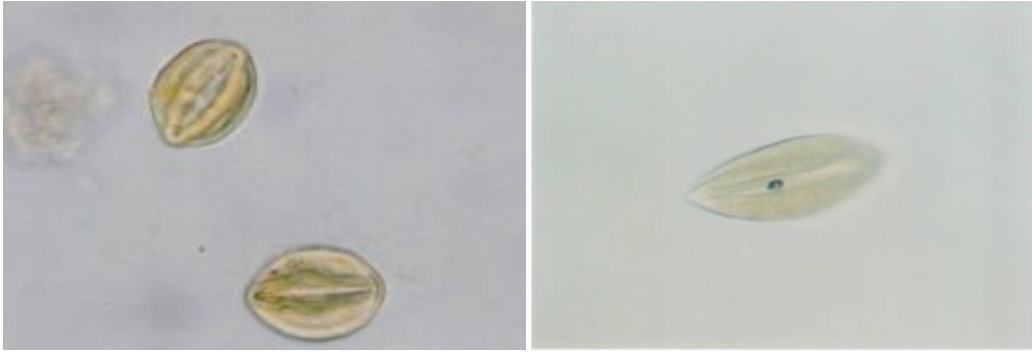


Figure 34. Pollen of bee flora, *Euphorbia millii* Desmoul. (left) and *Vetchia merrillii* (Becc.) H.E. Mosre (right) from the mid gut of *Apis mellifera*.

**Table 3. Bee flora of honeybees in Thailand study by pollen load and honey analysis.**

Plant species	Honey bee species			
	<i>A. cerana</i>	<i>A. dorsata</i>	<i>A. florea</i>	<i>A. mellifera</i>
<i>Aeschynomene Americana</i> L. <b>Common names:</b> American jointvetch, joint-vetch, shyleaf, deervetch	+	-	-	-
<i>Ageratum conyzoides</i> L. <b>Common names:</b> Catinga de Bode, Mexican ageratum, Erva de Sao Joao, Aru batu, Bandotan, Berokan, Rumput tahi ayam, Rompesaraguelo, Wedusan	+	+	-	+
<i>Amomum xanthioides</i> Wall. <b>Common names:</b> Bastard Cardamom, Tavoy Cardamom	+	+	-	-
<i>Anacardium occidentale</i> L. <b>Common names:</b> cashew	+	+	+	+
<i>Antigonon leptopus</i> Hook. <b>Common names:</b> Chain of Love; Coral Creeper; Coral Vine; Mexican Rose.	+	-	+	-
<i>Balakara baccata</i> Roxb.	+	-	+	-

<b>Common names:</b> Pho bai (Thai)				
<i>Blumea balsamifera</i> (L.) DC. <b>Common names:</b> Blumea camphor, Ngai Camphor, Sambong	+	+	-	-
<i>Bidens biternata</i> Merr. & Sherff. <b>Common names:</b> Spanish Needles, yellow flowered blackjack, black jack, five leaved blackjack, beggar ticks	+	+	+	+
<i>Brachiaria ruziziensis</i> Germain & Evrard <b>Common names:</b> Ruzi grass, Congo grass, Congo signal grass, prostrate signal grass	+	-	-	+
<i>Brassica chinensis</i> Justl var. <b>Common names:</b> Chinese cabbage, Bai-Cai, Sawi-Putih, Bok Choy, Pak-Choi	+	-	+	+
<i>Callistemon lanceolatus</i> DC. <b>Common names:</b> Bottle brush plant	-	-	+	-
<i>Castanopsis acuminatissima</i> Rehd. <b>Common names:</b> Castanopsis chestnut, Gon, Ko duel	+	-	+	+
<i>Ceiba pentandra</i> (L.) <b>Common names:</b> Kapok tree, silk cotton tree, ceiba de lana, bois coton, kapokier, pacaе, sumauma, kankantri.	-	-	-	+
<i>Celosia argentea</i> <b>Common names:</b> Will cockcomb, Cockcomb	+	+	-	-
<i>Chrysalidocarpus lutescens</i> H. Wendl <b>Common names:</b> Butterfly palm, Cane palm, Madagascar palm, Golden feather palm, yellow palm, Bamboo palm, Areca palm.	+	-	+	-
<i>Cinnamomum kerrii</i> Kosten <b>Common names:</b> -	+	+	-	-
<i>Citrus aurantifolia</i> Swing.		+	-	+



<b>Common names:</b> Common lime, Lime				
<i>Citrus maxima</i> (J. Burman ) Merr.	+	+	-	+
<b>Common names:</b> Pomelo				
<i>Citrus eticulate</i> Blanco	-	-	-	+
<b>Common names:</b> orange				
<i>Coccinia grandis</i> CL.Voigt	+	-	+	+
<b>Common names:</b> Ivy gourd				
<i>Cocos nucifera</i> L.	+	+	+	+
<b>Common names:</b> Coconut palm				
<i>Coffea Arabica</i> L.	+	-	-	+
<b>Common names:</b> Kofi, coffee, koffie, Brazilian coffee				
<i>Coriandrum sativum</i> L.	-	-	+	+
<b>Common names:</b> Chinese Parsley				
<i>Conyza sumatrensis</i> Retz.	+	-	+	+
<b>Common names:</b> fleabane, tall fleabane, broad-leaved fleabane, white horseweed, Sumatran fleabane, Guernsey fleabane				
<i>Cosmos sulphureus</i> Cav.	+	-	-	-
<b>Common names:</b> Yellow cosmos, Orange cosomos				
<i>Crataeva magna</i> Lour.	-	+	-	-
<b>Common names:-</b>				
<i>Croton oblongifolius</i> Roxb.	+	-	-	+
<b>Common names:</b> Oblong-leaved croton				
<i>Cucurbita citrillus</i> L.	+	+	+	-
<b>Common names:</b> Pumpkin				
<i>Cucumis sativus</i> Linn	-	-	+	-
<b>Common names:</b> Cucumber				
<i>Cuphea hyssopifolia</i> H.B.K.	-	-	+	-
<b>Common names:</b> Mexican Heather, False Heather, Hawaiian Heather				
<i>Dalbergia oliveri</i> Gamble ex Prain	-	+	-	-
<b>Common names:</b> Burma Rosewood, Tamalan (Thai), Mai				

Kham Phii )Lao				
<i>Datura metel</i> L. <b>Common names:</b> angel's trumpet, devil's trumpet, metel.	+	-	-	+
<i>Dillenia ovata</i> Wall. <b>Common names:</b> Ovate dillenia, Kadah Simpoh, Simpoh Beludu	-	-	-	+
<i>Dimocarpus longan</i> Lour. <b>Common names:</b> longan	+	+	+	+
<i>Diospyros glandulosa</i> Lacc. <b>Common names:</b> Kluai-ruesi (Thai)	-	+	-	+
<i>Diospyros areolata</i> King& Gamble <b>Common names:</b> phlap, kayu arang, maphlap(Thai)	-	+	-	-
<i>Duabanga grandiflora</i> Walp. <b>Common names:</b> lampatti, lamphupa (Thai)	-	+	-	-
<i>Duranta erecta</i> L. <b>Common names:</b> Golden Dewdrop, Pigeon Berry, Skyflower	-	-	-	-
<i>Durio zibethinus</i> L. <b>Common names:</b> Durian	+	+	+	+
<i>Elaeagnus latifolia</i> L. <b>Common names:</b> Oleaster, silverberry	+	-	-	+
<i>Erythrina suvumbrans</i> Merr. <b>Common names:</b> thong-lang (Thai), December-tree	-	+	-	-
<i>Eugenia javanica</i> <b>Common names:</b> wax apple, love apple, java apple, chomphu (Thai)	+	+	+	
<i>Eupatorium odoratum</i> L. <b>Common names:</b> Siam Weed, Christmas Bush, Common Floss Flower	+	+	+	+
<i>Euphorbia millii</i> Desmoul <b>Common names:</b> Crown-of-thorns, Christ Plant	+	-	-	-

<i>Euphoria longana</i> Lamk. <b>Common names:</b> longan, longyan	+	+	+	+
<i>Fragaria</i> × <i>ananassa</i> Guedes <b>Common names:</b> Strawberry	+	-	+	+
<i>Gmelina arborea</i> Roxb. <b>Common names:</b> Beechwood, Gmelina, Goomar teak	+	+	-	+
<i>Gomphrena globosa</i> L. <b>Common names:</b> Globe amaranth, Bachelor's bottons, Gomphrena	+	-	-	-
<i>Helianthus annuus</i> <b>Common names:</b> Sunflower	-	-	+	+
<i>Hopea odorata</i> Roxb. <b>Common names:</b> Takian, white thingan	-	+	-	-
<i>Jacaranda filicifolia</i> D.Don <b>Common names:</b> Blue Jacaranda	-	+	-	-
<i>Leersia hexandra</i> Sw. <b>Common names:</b> Swamp rice grass, swamp cut grass, Southern cutgrass	+	-	-	+
<i>Leucaena leucocephala</i> de Wit. <b>Common names:</b> White Leadtrees, Jumbay, White Popinac	+	-	+	+
<i>Litchi chinensis</i> Sonn <b>Common names:</b> lychee	+	-	+	+
<i>Luffa cylindrica</i> Roem <b>Common names:</b> sponge gourd, Smooth loofah, Loofah	+	-	-	-
<i>Lxora stricta</i> Roxb. <b>Common names:</b> Chinese ixora, Needle flower, Jungle flame	+	-	-	-
<i>Macadamia integrifolia</i> maiden & Betche <b>Common names:</b> macadamia nut, Australian brush nut, Bopple nut	+	-	-	+
<i>Mangifera indica</i> L. <b>Common names:</b> Mango, Mangot, Manga	+	-	+	-

<i>Melampodium divaricatum</i> <b>Common names:</b> butter daisy, melampodium	-	-	+	-
<i>Mikania cordata</i> Roxb. <b>Common names:</b> heartleaf hempvine	+	+	+	+
<i>Mimosa diplotricha</i> C. Wright. <b>Common names:</b> Giant sensitive plant, creeping, nila grass	+	+	+	-
<i>Mimosa pigra</i> <b>Common names:</b> Giant Sensitive Tree, bashful plant, catclaw mimosa	+	+	+	+
<i>Mimosa pudica</i> L. <b>Common names:</b> Sensitive plant, Sleeping grass, shame plant	+	+	+	+
<i>Muntingia calabura</i> L. <b>Common names:</b> strawberrytree, Jamaican cherry, takhop farang (Thai)	+	+	+	+
<i>Musa acuminata</i> Colla. <b>Common names:</b> Dwarf Cavendish Banana	+	+	-	+
<i>Musa sapientum</i> L. <b>Common names:</b> Banana	+	-	-	+
<i>Nephelium lappaccum</i> L. <b>Common names:</b> Rambutan	+	-	-	+
<i>Nymphaea nouchali</i> Borm. F <b>Common names:</b> Lotus	+	-	+	-
<i>Ocimum sanctum</i> L. <b>Common names:</b> Holy Basil	-	-	+	-
<i>Ocimum basilicum</i> L. <b>Common names:</b> Sweet Basil	+	-	-	+
<i>Oryza sativa</i> L. <b>Common names:</b> Rice	-	-	-	+
<i>Oxalis acetosella</i> L. <b>Common names:</b> Wood Sorrel, Shamrock	-	-	-	+
<i>Passiflora laurifolia</i> L.	+	+	-	-

<b>Common names:</b> passion fruit, bell apple, yellow granadilla				
<i>Pithecellobium dulce</i> (Roxb.) Benth. <b>Common names:</b> monkeypod	+	-	+	-
<i>Portulaca oleracea</i> L. <b>Common names:</b> Verdolaga, Pigweed, Little Hogweed	-	-	+	-
<i>Prunus cerasoides</i> D.Don <b>Common names:</b> sour cherry, wild Himalayan cherry	-	-	-	+
<i>Prunus mume</i> Sieb. <b>Common names:</b> Japanese Apricot, Black plum, Mume	-	-	+	+
<i>Psidium guajava</i> L. <b>Common names:</b> guava, goiaba, guayaba, djamboe	+	+	-	+
<i>Pyrostegia venusta</i> (Ker-Crawl.) <b>Common names:</b> flame flower, flame vine, orange creeper	-	-	-	+
<i>Raphanus sativus</i> L. <b>Common names:</b> cultivated radish, jointed charlock, wild radish	+	-	+	+
<i>Schoenoplectus juncooides</i> (Roxb.) Palla. <b>Common names:</b> Sedge, Rock bulrush	+	-	-	+
<i>Sesamum indicum</i> L. <b>Common names:</b> Sesame seed, benne seed, til	-	-	+	+
<i>Shorea siamensis</i> Miq. <b>Common names:</b> Dark Red Meranti, Red Lauan		+	-	+
<i>Solanum torvum</i> SW. <b>Common names:</b> Turkey berry	+	-	-	+
<i>Spilanthes paniculata</i> Wall. Ex DC. <b>Common names:</b> phak khraat	+	-	+	-



(Thai),yari sennichimodoki (Japanese)				
<i>Synedrella nodiflora</i> (L.) Gaerth. <b>Common names:</b> Synedrella, cerbatana	+	-	+	-
<i>Syzygium malaccense</i> L. <b>Common names:</b> Mountain Apple, Malaysian Apple, 'Ohi'a 'Ai, Rose Apple, Malay Apple, Pomerac, Otaheite-apple	-	+	-	-
<i>Tagete erecta</i> L. <b>Common names:</b> Marigold	+		-	-
<i>Tamarindus Indica</i> L. <b>Common names:</b> Tamarind	+	+	+	+
<i>Vetchia merrillii</i> (Becc.) H.E. Mosre <b>Common names:</b> Manila palm, Christmas palm, Merrill palm	+	-	+	-
<i>Wedelia trilobata</i> (L.) Hiteh. <b>Common names:</b> Climbing wedelia, Creeping daisy, Singapore daisy	+	+	+	-
<i>Wrightia religiosa</i> <b>Common names:</b> Water Jasmine, Wild Water Plum	-	-	-	-
<i>Wrightia arborea</i> (Dennst.) Mabb. <b>Common names:</b> Woolly Dyeing Rosebay	+	-	-	-
<i>Zea mays</i> L <b>Common names:</b> Corn	+	+	+	+
<i>Zizyphus mauritiana</i> Lamk. <b>Common names:</b> Indian jujube, common jujube	+	-	-	+

**Table 4. Nectar, pollen and Nectar and pollen source plants of Thai honeybees**

Number	Plant species	Nectar source	Pollen source
1	<i>Ageratum conyzoides</i> L.	+	+
2	<i>Amomum xanthioides</i> Wall.	+	+
3	<i>Balakara baccata</i> Roxb.	+	+

4	<i>Blumea balsamifera</i> (L.) DC.	+	+
5	<i>Bidens biternata</i> Merr. & Sherff.	+	+
6	<i>Brachiaria ruziziensis</i> Germain&Evrard	-	+
7	<i>Brassica chinensis</i> Jusl var.	+	+
8	<i>Castanopsis acuminatissima</i> Rehd.	-	+
9	<i>Ceiba pentandra</i> (L.)	+	+
10	<i>Cinnamomum kerrii</i> Kosten	+	+
11	<i>Citrus aurantifolia</i> Swing.	+	+
12	<i>Citrus maxima</i> (J. Burman ) Merr.	+	+
13	<i>Coccinia grandis</i> CL.Voigt	+	+
14	<i>Cocos nucifera</i> L.	+	+
15	<i>Coffea Arabica</i> L.	+	+
16	<i>Coriandrum sativum</i> L.	+	+
17	<i>Conyza sumatrensis</i> Retz.	+	+
18	<i>Crataeva magna</i> Lour.	+	+
19	<i>Croton oblongifolius</i> Roxb.	+	+
20	<i>Cuphea hyssopifolia</i> H.B.K.	+	-
21	<i>Dalbergia oliveri</i> Gamble ex Prain	+	+
22	<i>Datura metel</i> L.	+	+
23	<i>Dillenia ovata</i> Wall.	+	+
24	<i>Dimocarpus longan</i> Lour.	+	+
25	<i>Diospyros glandulosa</i> Lacc.	+	+
26	<i>Diospyros areolata</i> King & Gamble	+	+
27	<i>Duabanga grandiflora</i> Walp.	+	+
28	<i>Elaeagnus latifolia</i> L.	+	+
29	<i>Erythrina suvumbrans</i> Merr.	+	+
30	<i>Eucalyptus camaldulensis</i>	+	+
31	<i>Eugenia javanica</i>	+	+
32	<i>Eupatorium odoratum</i> L.	+	+
33	<i>Euphoria longana</i> Lamk.	+	+
34	<i>Fragaria ananassa</i> Guedes	+	+
35	<i>Gmelina arborea</i> Roxb.	+	+
36	<i>Hopea odorata</i> Roxb.	+	+
37	<i>Jacaranda filicifolia</i> D.Don	+	+
38	<i>Leersia hexandra</i> Sw.	-	+
39	<i>Leucaena leucocephalade</i> Wit.	-	+
40	<i>Litchi chinensis</i> Sonn	+	+
41	<i>Macadamia integrifolia</i> maiden & Betche	+	+
42	<i>Mangifera indica</i> L.	+	+
43	<i>Mikania cordata</i> Roxb.	+	+

44	<i>Mimosa diplotricha</i> C. Wright.	+	+
45	<i>M. pigra</i>	+	+
46	<i>M. pudica</i> L.	-	+
47	<i>Muntingia calabura</i> L.	+	+
48	<i>Musa acuminata</i> Colla.	+	+
49	<i>M. sapientum</i> L.	+	+
50	<i>Ocimum sanctum</i> L.	+	+
51	<i>Oryza sativa</i> L.	-	+
52	<i>Oxalis acetosella</i> L.	+	+
53	<i>Passiflora laurifolia</i> L.	+	+
54	<i>Prunus cerasoides</i> D.Don	+	+
55	<i>P. mume</i> Sieb.	+	+
56	<i>Psidium guajava</i> L.	+	+
57	<i>Raphanus sativus</i> L.	+	+
58	<i>Schoenoplectus juncooides</i> (Roxb.) Palla.	-	+
59	<i>Shorea siamensis</i> Miq.	+	-
60	<i>Solanum torvum</i> SW.	+	+
61	<i>Spilanthes paniculata</i> Wall. Ex DC.	+	+
62	<i>Synedrella nodiflora</i> (L.) Gaerth.	+	+
63	<i>Wedelia trilobata</i> (L.) Hitch.	-	+
64	<i>Wrightia arborea</i> (Dennst.) Mabb.	+	+
65	<i>Zea mays</i> L.	-	+
66	<i>Zizyphus mauritiana</i> Lamk.	+	-

## 6. BEEKEEPING IN THAILAND

### Introduction

Beekeeping is an important component of agriculture and rural development programs in many Asian countries (Ahmad, 1992; Partap, 1992; Partap and Verma, 1998). It is mainly conducted using the European honeybee, *A. mellifera* and the Asiatic honeybee *A. cerana*. The benefits provided by beekeeping include enhanced nutritional, economic and ecological security to rural communities of Asia (Partap and Verma, 1998). Thai people have learned how to hunt honey from wild bee colonies. However, their methods tend to destroy the colony. It is important that education is enhanced that can teach sustainable ways of harvesting honey. This training should teach beekeepers the following basic activities: checking general bee colony health, checking for the general presence of the queen, how to establish new colonies, control colony pests and diseases, moving bee hives to food sources, feeding colonies (if necessary), routine maintenance of bee hives, and how to harvest hive products.

## History

The first report of Western-style beekeeping in standard Langstroth hives was recorded in 1940 by Professor Supachai Wattana (Wongsiri et al. 2000). He imported foreign honeybees to study at Chulalongkorn University in Bangkok. In 1953, Professor Saman Worakitta (who at the time served as the Dean of Agriculture at Kasetsart University) introduced European honeybees from Australia and raised them on campus. However, the operation was not successful (Wongsiri et al. 2000). Later, Thailand established a cooperative agreement with Taiwan to exchange and share knowledge related to beekeeping. Taiwan sent bee experts to advise farmers in northern Thailand; however, beekeeping was still quite limited. Until about 1976-1979, private companies hired specialists from Taiwan for management and operation of beekeeping in Thailand (Wongsiri et al. 2000).

Since 1980, the Thai government has recognized the importance of bees to the national economy. And as a result the government has a policy to encourage and promote agricultural apiarists. The Ministry of Agriculture and Cooperatives is an agency responsible for promoting and educating farmers on beekeeping. New apiarists can obtain information about beekeeping from government resources, take training courses, and receive advice provided by organizations distributed throughout the country that include the following:

1. Department of Agriculture
2. Department of Agricultural Extension
3. Agricultural Extension and Development Center Chiang Mai (Beekeeping)
4. Agricultural Extension and Development Center Phitsanulok (Beekeeping)
5. Agricultural Extension and Development Center Khon Kaen (Beekeeping)
6. Agricultural Extension and Development Center Chanthaburi (Beekeeping)
7. Agricultural Extension and Development Center Chumphon (Beekeeping)

Many universities in Thailand also have researchers who study honeybees, educate, and advise beekeepers. Some research units have their own apiary. Others cooperate with beekeepers and local communities. To receive information about bee biology and beekeeping you can go to any of the universities shown below:

1. Bee Biology Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330.
2. Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand 10900.
3. Department of Agricultural Technology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand 10900.
4. Department of Biology, Faculty of Science, Burapha University, Chon Buri, Thailand 20131.

5. Department of Entomology, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand 40000.
6. Department of Entomology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand 50000.
7. Department of Agricultural Science, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand 65000.

There are also beekeeping associations with regional and local branches, which help beekeepers: the Beekeeper Association of Northeastern Thailand, the Beekeeper Association of Thailand (<http://thailandbee.net>), and the Thai Organic Beekeeper Association (<http://www.thaiorganicbee.net>). Some private bee farms, such as Supha Bee Farm in Chiang Mai (<http://www.suphabeefarm.com>) are good beekeeping resources.

### **Status of beekeeping in Thailand**

Beekeeping is chiefly conducted to produce honey, but some beekeepers focus on producing royal jelly, beeswax, bee colonies, or queens. Some beekeepers provide bees to pollinate crops. Each type of operation requires specific experience and management techniques, and thus beekeepers often specialize on one type of production. Only two-thirds of honeybee colonies in Thailand are used for crop pollination. Primarily, these colonies are used to pollinate longan, lychee, sesame, sunflower and bitter weed (Pyramarn and Wongsiri, 1986). The number of beekeepers rearing *A. mellifera* across the country according to information provided by the Agricultural Extension and Development Center is 887 as shown in Table 5.

The cavity nesting honeybee *A. cerana* and the introduced *A. mellifera* are the primarily species used for beekeeping. In Thailand, there are more than a thousand beekeepers keeping more than two hundred thousand colonies of *A. mellifera*. *Apis cerana* has been recognized as native to eastern Asia including Laos, Thailand, and Malaysia. Most races of *A. cerana* are slightly smaller than *A. mellifera* and have smaller colonies. Unlike *A. mellifera*, *A. cerana* provides fewer products for beekeepers. *Apis cerana* does not gather propolis; however, it is more resistant to mites than *A. mellifera*. In nature, *A. cerana* build their nest in logs or other cavities. Many local beekeepers keep this species in traditional beehives. They drill out the core of tree trunks and cover both ends with wooden lids. Three to five inches from the bottom of the hive, they make a hole for bee entrance. However, most honeybee farms use movable-frame Langstroth-style hives for *A. cerana*. Management of *A. cerana* is relatively simple. Beekeepers need to provide sufficient food and protect the colony from its enemies (ants, wasps) to minimize absconding. Major beekeeping areas for *A. cerana* are in Southern Thailand: Chumphon, Surat Thani, Nakhon Si Thammarat, Trang, Phattalung, Songkhla, Pattani, and Satun.

**Table 5. Number of beekeepers and honeybee colonies according to center**

Province	No. of beekeepers	Total Colonies	Average
<b>Agricultural Extension and Development Center Chiang Mai</b>			
Chiang Mai	127	31,170	245
Chiang Rai	62	21,270	343
Lamphun	75	18,870	252
Lampang	27	1,510	56
Phayoa	12	6,870	573
Phrae	106	33,450	316
Nan	54	7,000	130
<b>Total</b>	<b>463</b>	<b>120, 140</b>	<b>259</b>
<b>Agricultural Extension and Development Center Phitsanulok</b>			
Chai Nat	1	150	150
Kamphaeng Phet	1	20	20
Nakhon Sawan	1	150	150
Phetchabun	2	1,300	650
Sukhothai	1	100	100
Phichit	12	1,590	133
Phitsanulok	24	6,750	281
Uthai Thani	4	180	45
Uttaradit	55	12,051	219
<b>Total</b>	<b>101</b>	<b>22,291</b>	<b>221</b>
<b>Agricultural Extension and Development Center Khon Kaen</b>			
Khon Kaen	20	2,920	146
Udon Thani	32	5,586	175
Roi Et	4	1,470	368
Nong Khai	3	126	42
Loei	17	3,349	197
Chaiyaphum	6	580	97
Maha Sarakham	4	400	100
Sri Sa Ket	5	420	84
Amnat Charoen	3	510	170



Nong Bua Lam Phu	9	1,468	163
Nakhon Ratchasima	12	1,530	128
Buri Ram	4	160	40
Surin	1	850	850
Ubon Ratchathani	1	88	88
<b>Total</b>	<b>121</b>	<b>19,457</b>	<b>161</b>
<b>Agricultural Extension and Development Center Chanthaburi</b>			
Chanthaburi	18	1,480	82
Sa Kaeo	3	210	70
Saraburi	35	970	28
Lop Buri	27	12,540	464
Kanchanaburi	12	1,180	98
<b>Total</b>	<b>95</b>	<b>16,380</b>	<b>172</b>
<b>Agricultural Extension and Development Center Chumphon</b>			
Chumphon	24	1,970	82
Surat Thani	21	354	17
Nakhon Si Thammarat	18	323	18
Trang	3	57	19
Phattalung	24	72	3
Songkhla	12	720	60
Pattani	3	255	85
Satun	2	210	105
<b>Total</b>	<b>107</b>	<b>3,961</b>	<b>37</b>

### Conserving *Apis cerana* and others native species in Thailand

At present, conservation of native Thai honeybee species is of primary importance. In terms of practicality for beekeepers, *A. cerana* is the best choice when compared to *A. dorsata*, *A. florea* and *A. andreniformis* because swarming and absconding is less frequent in *A. cerana*. However, *A. cerana* populations are declining because of competition with *A. mellifera*. The conservation of *A. cerana* is being promoted by the Agricultural Extension and Development Center of Thailand, particularly in southern areas such as Chumphon and Samui Island. A key point is to convince Thai farmers to think about crop pollination in addition to honey production (which is traditional and for which *A. mellifera* is superior). *Apis cerana* may be better suited for pollinating certain kinds of crops. This is an area of current Thai research. In addition, Thai honeybee researchers and agriculturalists are educating people to preserve natural bee habitat and increase honeybee habitat by planting bee flora.



Figure 35. A beekeeper wears a tight suit with veil to protect her from being stung by bees.

### **Beehive**

A beehive is an enclosed structure in which beekeepers keep bees. Traditionally, beehives are often made from wood. A modern beehive is generally a box of wood with a bottom board, a brood box or brood chamber, a honey box (top box where most of the honey is stored), and frames (where bees build combs for egg laying and honey storage). In addition, a queen excluder made of slotted zinc, plastic, or wire keeps the queen in the brood chamber, an inner cover or crown board (which prevents the hive lid from sticking due to wax or propolis collected by bees), and the lid. However, in Thailand, beehives are generally built in one piece: the base, brood box, and honey box are united together. This box contains 8 to 9 removable frames. This hive is then set on a stand, which is usually 0.5-0.6 m in height to separate the hive from damp ground and to exclude ants (with oil or similar material applied to the legs). Hive stands can be made from wood or metal Wongsiri et al., 2000).



Figure 36. Beekeeper is removing *Apis cerana* brood frame from coconut trunk hole to keep in the box hive.

## 7. HONEYBEE PATHOGENS, PARASITES, AND PREDATORS

### Introduction

Four main factors contribute to honeybee decline in Thailand: honeybee pests and diseases, deforestation, pesticides, and human management and honey hunting practices. Over the past two decades, these four factors have affected *A. mellifera* and the native species, *A. dorsata*, *A. florea* and *A. andreniformis* (Wongsiri et al. 2000).

### Honeybee mites

Parasites such as bee mites have been spread, in part, by human management practices because beekeepers move hives for commerce and pollination (Anderson, 1999; Oldroyd and Wongsiri, 2006). Such parasites are now a global problem, causing significant reduction in bee populations (particularly *A. mellifera*) and problems for fruit and vegetable producers who rely on bee pollination. The outbreak of parasite and disease has the potential to destroy beekeeping in Thailand (Wongsiri et al., 2000; Oldroyd and Wongsiri, 2006). The commercial beekeepers are usually preferring to switch to *A. mellifera* colonies to explore different bee flora leading to an increased exchange of disease and parasite within bee species and colonies (Boecking et al., 2000). In Asian countries including Thailand, honeybees are kept almost exclusively for honey production, in fact, pollination of crop plants by honeybees is much more important than honey production. It is estimated that honeybees account for 80 per cent of all crop pollination (Robinson et al., 1989). Mites are the largest and most diverse

group of honeybee parasites. *Apis dorsata*, *A. cerana*, *A. florea*, *A. andreniformis*, and *A. mellifera* are parasitized by a wide variety of ectoparasitic mites, particularly *Acarapis woodi* (Acarine tracheal mites), *Varroa jacobsoni*, *V. destructor*, *Tropilaelaps clareae*, *T. koenigerum*, *T. mercedesae*, and *T. thaii*. Consequently, mites constitute a major threat to all beekeeping in Thailand (Wongsiri et al., 2000; Oldroyd and Wongsiri, 2006).

**Table 6. Mesostigmatic mites parasitizing bees, arranged according to host species (modified from Koeniger, 1996).**

Honeybee species	Mite species	Reference
<i>A. andreniformis</i>	<i>Euvarroa sinhai</i> <i>Euvarroa wongsirii</i>	Delfinado-Baker, Baker and Phoon, 1989 Lekprayoon and Tangkanasing, 1991
<i>A. florea</i>	<i>Euvarroa sinhai</i> <i>Tropilaelaps clareae</i>	- Delfinado-Baker, Baker and Phoon, 1989
<i>A. cerana</i>	<i>Tropilaelaps clareae</i> <i>Varroa jacobsoni</i> <i>Varroa underwoodi</i> <i>Acarapi woodi</i>	Delfinado-Baker, Baker and Phoon, 1989 - Delfinado-Baker, Baker and Phoon, 1989
<i>A. koschevnikovi</i>	<i>Varroa rindereri</i> <i>Varroa jacobsoni</i>	de Guzman and Delfinado-Baker, 1996 Delfinado-Baker, Baker and Phoon, 1989
<i>A. dorsata</i>	<i>Tropilaelaps clareae</i> <i>Tropilaelaps koenigerum</i>	Delfinado-Baker, Underwood and Baker, 1985
<i>A. laboriosa</i>	<i>Tropilaelaps clareae</i> <i>Tropilaelaps koenigerum</i>	- Delfinado-Baker, Baker and Phoon, 1989
<i>A. mellifera</i>	<i>Tropilaelaps clareae</i> <i>Varroa jacobsoni</i> <i>Euvarroa sinhai</i> <i>Acarapi woodi</i>	Delfinado-Baker, Baker and Phoon, 1989 Delfinado-Baker, Baker and Phoon, 1989 Koeniger, Koeniger, de Guzman and Lekprayoon, 1993

The relationship between different bee species and their acarine parasites is still being explored (Otis, 1991; Smith et al., 1991). Recently, several new mites have discovered parasitizing older and newly recognized honeybee species (see Table 7). *Apis andreniformis* and *A. florea* are attacked by *Euvarroa* spp. (Lekprayoon and Tangkanasing, 1991; 1993). *Apis cerana*, *A. koschevnikovi*, and *A. mellifera* are parasitized by different *Varroa* species (de Guzman and Delfinado-Baker, 1996). Finally, *A. dorsata* and *A. laboriosa* are parasitized by *Tropilaelaps* species (Delfinado-Baker et al., 1985; 1987). This pattern also seems to apply to *Varroa rindereri rindereri* (Anderson, 1999; Lekprayoon and Tangkanasing, 1991; 1993).

More and perhaps unexpected associations between honeybees and parasitic mites will likely be discovered. More species of *Varroa* and *Tropilaelaps* are likely to be found in Southeast Asia. In addition, significant differences have been found between *E. sinhai* from India and from Thailand (Morin and Otis, 1993). In Borneo, de Guzman and Delfinado-Baker (1996) reported finding multiple *Varroa* species similar to *V. underwoodi* on *A. nuluensis*.

### ***Tropilaelaps clareae***

This species of mite was originally discovered in the rat (Delfinado and Baker, 1961), but is also found on five species of honeybees (see below), is primarily found on *A. dorsata*, and occur on *A. mellifera* (Laigo and Morse, 1969). Currently, *T. clareae* is restricted to Asia and ranges from Iran to Papua New Guinea, including India, Pakistan, Philippines, Nepal, and Burma (Mattheson, 1993; 1996). *Tropilaelaps koenigerum* has been reported in Sri Lanka and Nepal (Delfinado-Baker, 1985), Borneo (Koeniger et al., 2002), and Thailand (Tangjingjai et al., 2003). *Tropilaelaps clareae* is now found in five honeybee species: *A. mellifera*, *A. dorsata*, *A. cerana*, *A. florea*, and *A. laboriosa* (Aggarwal, 1988). After the European honeybee (*A. mellifera*) was introduced to Asia and subsequently to Thailand, this species was parasitized by *T. clareae*, resulting in a significant annual decrease in commercial honey production (DeJong, 1990; 1997). Interestingly, *T. clareae* is more harmful to the exotic species, *A. mellifera* than to its native host, *A. dorsata* (Eickwort, 1988). Because of the importance of this parasite, we have included detailed information about its biology.

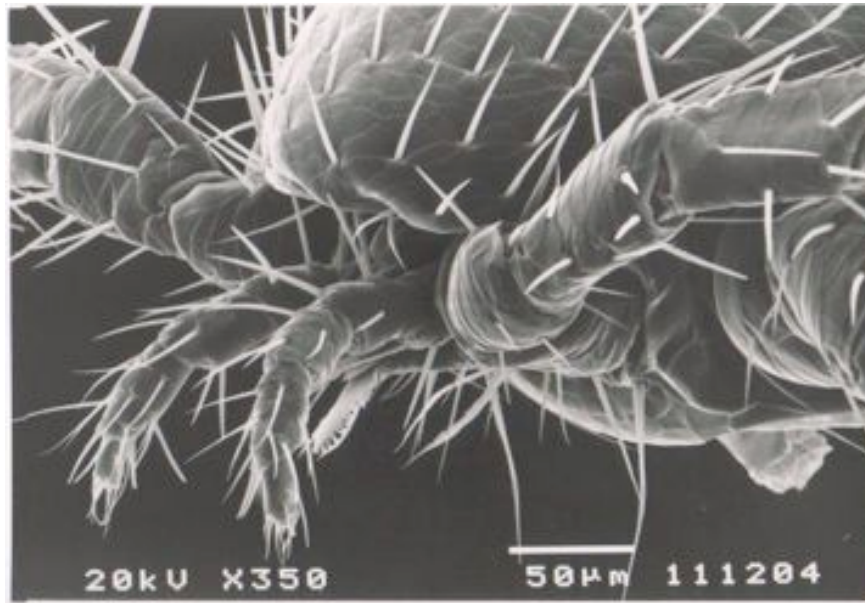


Figure 37. Scanning electron micrograph shows the mouth part of *Tropilaelaps clareae*.



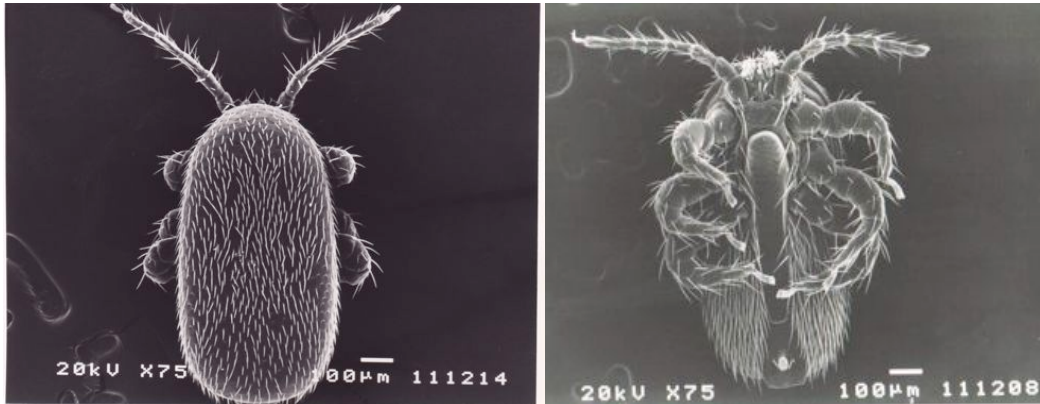


Figure 38. Scanning electron micrograph of the dorsal surface (left) and ventral surface (right) of *Tropilaelaps clareae*

#### Taxonomy of *Tropilaelaps clareae*

Kingdom Animalia  
 Phylum Arthropoda  
 Class Arachnida  
 Subclass Acari  
 Order Parasitiformes  
 Suborder Mesostigmata  
 Family Laelapidae  
 Genus *Tropilaelaps*  
 Species *T. clareae*  
 (Delfinado and Baker, 1962)

#### General biology and life cycle of *Tropilaelaps clareae*

Females are medium sized (< 1 mm), elongated, and light reddish brown. Males are similar but less sclerotized (Lekprayoon and Tangkanasing, 1991; 1993; Sammataro, 1997). They are visible to the naked eye. The foundress mite places three to four eggs on mature bee larvae shortly before they are capped and the progeny feed only on developing bee larvae. The mite requires about one week to develop, and the adults, including the foundress mite, emerge with the adult bee and search for new hosts. This short life cycle is one reason why *T. clareae* populations increase more rapidly than those of *Varroa* species. *Tropilaelaps clareae* out competes the latter when both infest the same colony of *A. mellifera* (Sihag, 1988). Nevertheless, populations of both mites can survive in the same apiary for 12 months, probably because their niches are not completely congruent (Rath et al., 1995). Like *Varroa*, female *T. clareae* are dispersed by bees, but can only survive in this dispersal phase for a short time. Gravid female mites die within two days unless they deposit their mature eggs (Woyke, 1987; 1994a, 1994b). Even adult *T. clareae* chelicerae (jaws) cannot pierce the integument of adult bees. The mouthparts are stubby, with an apically bidentate fixed upper digit, and a longer, unidentate and pointed moveable digit. This piercing-grasping structure is more suitable to piercing soft brood tissue. This implies that *Tropilaelaps* can feed only on



soft tissues, such as honeybee brood (Griffiths, 1988). However, the tearing-sawing jaws of *Varroa* can penetrate the harder adult *Apis* exoskeleton (Griffiths, 1988).

### **Pest status: Symptoms and distribution**

*Tropilaelaps clareae* causes severe damage to *A. mellifera*, *A. cerana*, *A. dorsata*, and *A. florea*. Honeybees infested by this parasite may become unable to fly. Heavily infested bees may crawl on the hive floor or form a parasitized cluster in the hive. Bee lifespans are shortened by heavy mite infestations, creating a condition called acarine disease or acariosis (Anderson and Morgan, 2007). This also leads to reduced brood production. However, this does not generally cause colony death, only reduced colony health, and productivity because bees in the warm Thai climate can produce brood throughout the year, unlike bees in colder climates (Anderson and Morgan, 2007).

### ***Varroa jacobsoni***

*Varroa jacobsoni* is another important and dangerous ectoparasite of Thai honeybees and can feed on the bodily fluids of larvae, pupae, and adult bees. This parasite has a strong economic impact and places stress on the Thailand's commercial and wild honeybee populations. The pathology it causes is commonly called varroasis (also seen as varroatosis or varrosis). The *varroa* mite was first discovered in Southeast Asia around 1904. Since then, they have spread worldwide and from their original host, *A. cerana* (Sasagawa et al., 1999) to European honeybees (*A. mellifera*). The name *Varroa destructor* has been proposed (D, Anderson, personal communication) and may be recognized if several species and strains are lumped under the name *V. jacobsoni* (Anderson, 1999; de Guzman et al., 1997; 1998; De Jong and Goncalves, 1999). *Varroa* became an economic concern in Japan and China in the 1950s and 1960s, in Europe in the late 1960s and 1970s, and in Israel and North America in the 1980s. Because it is a significant pest of honeybees, we provide more details about its biology below.

#### **Taxonomy of *Varroa* mite**

Kingdom Animalia  
 Phylum Arthropoda  
 Class Arachnida  
 Subclass Acari  
 Order Parasitiformes  
 Suborder Mesostigmata  
 Family Varroidae  
 Genus *Varroa*  
 Species *V. jacobsoni*  
 (Anderson and Trueman, 2000)

### **General biology of *Varroa***

Adult females are a reddish-brown color. Mites are dorsoventrally compressed, allowing them to fit beneath a bee's abdominal sclerites (Yoder et al., 1999). The average female mite is approximately 1.1 mm in length and 21.6 mm in width and approximately 0.14 mg in mass (Sammataro, 1997; Sammataro et al., 1994). Adult males are smaller and lighter in color than

adult females. The mite feeds on the honeybee haemolymph by making a soft hole through the honeybee's exoskeleton between the soft intersegmental tissues of the bee's exoskeleton. Mites have modified chelicerae that contain a moveable digit that is like a saw blade. It can pierce and tear open the host's integument (Delfinado and Baker, 1987).

*Varroa* mites reproduce on a 10-day cycle. Females ride on adult bees during dispersal (phoresy). These mites prefer young "house" bees to older workers, probably because of the lower titer of the Nasonov gland pheromone geraniol in older workers, which strongly repels the mite (Hoppe and Ritter, 1989). The female mite enters a honeybee brood cell, one to two days before capping and produces its first egg 60 hours after the cell is sealed (Ifantidis, 1983). These eggs then hatch to typically produce one haploid male and several females. Mites go through the following instars: pharate larvae, mobile protonymph, pharate deutonymph, mobile deutonymph, pharate adult, and adult (De Jong, 1997, Donze and Guerin, 1997). The young mites hatch at about the same time and leave the cell with the host. Young females mature in 6.5 -6.9 days (De Jong, 1997). When the young bee emerges from the cell after pupation, the *Varroa* mites also leave and spread to other bees and larvae. They can survive off of the host for 18 -70 hours, depending on the substrate (de Guzman et al., 1993). The mites preferentially infest drone larvae over workers because they contain higher quantities of fatty acid esters (Le Conte et al., 1989), more aliphatic alcohols and aldehydes, and a larger amount of hemolymph (Donze et al., 1998).

There are several signs of infestation. The pale or dark red-brown mites can be easily seen on white pupae. Infested drone or worker brood develops into disfigured, stunted adults with deformed legs and wings (De Jong, 1997; Gerson et al., 1988; Le Conte et al., 1989). Additionally, workers bees are seen discarding infested larvae. Because mite populations increase in proportion to the available bee larvae, *Varroa* can destroy bee colonies within months (Gerson et al., 1988). *Varroa* mites attack adults and brood, weakening and shortening the life span of infested bees. Losses due to these parasitic mites are sometimes attributed to standard winter mortality or queenlessness. Beekeepers in Thailand tend to treat *Varroa* using natural products such as extracts of the snake root plant. However, commercial products such as Coumaphos®, Bayer Bee Strips® or CheckMite® are also used (De Jong, 1997; Gerson et al., 1988; Le Conte et al., 1989).

### ***Acarapis woodi***

This mite lives inside a bee's tracheal tubes and was discovered and first named *Tarsonemus woodi* (Rennie, 1921, Rinderer et al., 1999). However, it was later renamed *Acarapis*, from *Acarus*, mite, and *Apis*, bee (Hirst, 1921). The disease was then called Isle of Wight disease. This mite is found worldwide and is a serious pest of *A. cerana* and *A. mellifera*. Beekeepers have reported heavy colony mortality due this mite in *A. cerana* colonies in southern Thailand and *A. mellifera* in northern Thailand. Beekeepers suspect that *A. cerana* colonies are more susceptible to this mite than *A. mellifera* colonies (Partap and Verma, 1998).

#### **Taxonomy of Tracheal mite**

Kingdom Animalia

Phylum Arthropoda

Class Arachnida

Subclass Acari

Order Trombidiformae

Family Tarsonemidae

Genus *Acarapis*Species *A. woodi*

(Anderson and Trueman, 2000)

### **General Biology and Life cycle**

The mite's entire life cycle is spent within the thoracic tracheal system of adult honeybees, except for brief migratory periods (Sammataro and Needham, 1996; Smith et al., 1991). Mites are occasionally found in air sacs in the thorax and abdomen. Female tracheal mite length ranges from 120-190  $\mu\text{m}$  and the width ranges from 75-84  $\mu\text{m}$  (Delfinado-Baker et al., 1989). Females each weigh 5.5-10.4 mg. Male range in length from 125-136  $\mu\text{m}$ , in width from 60-77  $\mu\text{m}$ , and weigh 2.61-10.4 mg. Males complete their development in 11-12 days. Females complete development in 14-15 days. Like other members of the prostigmatic Heterostigmata, *A. woodi* has a foreshortened life cycle. It has only three apparent stages: egg, larva, and adult. However, the mite has an apdous nymphal instar that remains inside the larval skin (Lindquist, 1986).

These mites feed on bee hemolymph, which they obtain by piercing the tracheae with their closed-ended, sharply pointed stylets, operated by internal chitinous levers (Hirschfelder and Sachs, 1952). Once a trachea is pierced, the mites' mouth, located just below the stylets, is pressed against the wound, and the mite sucks host hemolymph into its pharynx.

Mites are spread within the colony as a result of bee-to-bee contact. Mated female mites leave the breathing tubes where they develop and climb to the tip of a body hair. As bees come in contact with one another, the mites attach themselves to the hairs of a passing bee and enter the tracheae through the thoracic spiracles. Dispersing female mites are attracted to air expelled from the prothoracic (first thoracic) spiracle of young bees (Hirschfelder and Sachs, 1952), as well as to specific hydrocarbons from the cuticle of callow bees, less than four days old (Phelan et al., 1991). This mite is less attracted to older bees and prefers drones to workers (Royce and Rossignol, 1991). If the mite does not locate a new host within 24 hours, it will die. Once the female mite enters honeybee's spiracle, she lays 4-8 eggs within a couple days. The mite's small size is critical to its survival. The tiny mites can hide under the flat lobe that covers the bee's first thoracic spiracle. Mites begin to disperse when the host bee is more than 13 days old. Dispersal peaks when they are 15- 25 days old. Mites leave the tracheae after the death of the bee. Drifting bees between hives and swarms from infested colonies can spread the mite the apiary (Publication 1753, Extension Service of Mississippi State University, cooperating with U.S. Department of Agriculture).

### **Pest status: symptoms and distribution**

It is difficult to determine if tracheal mites are present in honeybee colonies: the parasites are very small, and they infest the host bees internally. Adult bees infested by *A. woodi* show no noticeable signs, but their lifespan is shortened. Infested bees are whitish in color, and have a shiny cuticle with a few long fine hairs on the body and legs (Fyg, 1964; Morse, 1978). The only reliable diagnostic method is the microscopic examination of dissected tracheae. If present, the mites are usually found within the trachea closest to the bees' thoracic spiracles. The infested tracheae display a color darker than normal. Infested queens can live

for many years (Fyg, 1964). Morse (1978) estimated that mite infestation reduces colony size by approximately five percent. Honey production and pollen collecting are correspondingly reduced. In addition, honeybees infested by this parasite may become unable to fly (Fyg, 1964; Morse, 1978).

### The parasitic mite, *Euvarroa*

*Euvarroa sinhai* is a parasite of *A. florea*, and ranges from Iran through India and Sri Lanka (Sammataro et al., 2000). The mite infests capped drone brood (Mossadegh and Komeili, 1986), but can be reared in the laboratory on *A. mellifera* worker brood (Mossadegh, 1990). Development requires less than one week, and each female produces four to five offspring. Mites disperse by riding on drones and workers. The female mite overwinters in the colony, probably feeding on the clustering bees. Colony infestation by *E. sinhai* is somehow hindered by the construction of queen cells (Aggarwal and Kapil, 1988), and its population growth is inhibited in the presence of *T. clareae* and *V. jacobsoni* (Sihag, 1988). Transfer experiments (Koeniger et al., 1993) confirmed that *E. sinhai* can survive and thus potentially cross-infest *A. mellifera* and *A. cerana*. The closely related *Euvarroa wongsirii* parasitizes drone brood of *A. andreniformis* in Thailand and Malaysia. Its biology appears similar to *E. sinhai* and it can live for at least 50 days on worker bees outside the nest (Morin and Otis, 1993).



Figure 39. *Euvarroa sinhai* feeding on drone larva of *Apis florea*.



Figure 40. *Euvarrao singhi* collected from *Apis florea*.



Figure 41. *Euvarrao singhi* collected from an *Apis florea* worker.

### Viral Diseases

Honeybees are subject to many viruses (Allen and Ball, 1996; Ball and Bailey, 1997), five of which are associated with *Varroa* and one with tracheal mites. Several viral diseases affect *A. cerana*. Thai honeybees suffer from the Kashmir bee virus (KBV) (Anderson, 1991; Bailey, 1962; Ball and Bailey, 1997). This disease is similar in size to several picornavirus-like agents. KBV may be activated in the presence of *Varroa*, multiplying to lethal levels. Another important virus is *Apis* iridescent virus (AIV). Recent reports state that AIV has been causing serious damage to commercial colonies of *A. cerana* in northern India and Pakistan, the virus being associated with "clustering disease" (Aemprapa and Wongsiri, 2000). The bees are unusually inactive and frequently form small, detached clusters of bees that do not fly. Many individual bees are observed crawling on the ground and are lost. At first, these symptoms were associated with the presence of the tracheal mite *Acarapis woodi* on some diseased bees, but it was later shown that AIV is the major causative agent (Anderson, 1991; Ball and Bailey, 1997).

**Table 7. Viral infections in Thai bees (modified from Allen and Ball, 1996; Anderson, 1991).**

Honeybee species	Viruses	Reference
<i>Apis andreniformis</i>	-	-
<i>A. cerana</i>	Thai sac brood virus Deformed wing virus <i>Apis</i> iridescent virus Kashmir bee virus	Abrol and Bath, 1990; Bailey et al., 1982; Oldroy and Wongsiri, 2007
<i>A. dorsata</i>	Thai sac brood virus	Abrol and Bath, 1990; Oldroy and Wongsiri, 2007; Vermat et al., 1990
<i>A. florea</i>	Black queen cell virus	Abrol and Bath, 1990; Oldroy and Wongsiri, 2007; Verma et al., 1990
<i>A. mellifera</i>	Acute bee paralysis virus Kashmir bee virus Sac-brood virus (SBV) Deformed wing virus	Sanpa and Chantawannakul, 2009

Thai Sac-brood virus (TSBV) is a spherical virus that infects the cytoplasm of fat cells of honeybee larvae (Aemprapa and Wongsiri, 2000; Ball and Bailey, 1997). It was found among colonies in mountainous areas of Northern Thailand, for example in *A. cerana indica* collected from Doi pui, Chiang Mai province (Aemprapa and Wongsiri, 2000). In 1990, there was an outbreak in the southern Thailand in Chumporn province (Jarungjit et al., 1990). The pupae turn into sac-like structures filled with lemon-colored liquid at the posterior end. Later, the larvae change their appearance from yellowish to brownish to black color. No discernible foul odor can be detected. Many Indian bee colonies were destroyed by TSBV in South India during early 1990's. TSBV was first reported in *A. cerana* colonies in Thailand and has since been found other Asian countries. Its natural range may cover the entire Asian continent. In Thailand, the disease is found in colonies experiencing stress: lack of food, excessive



humidity, low worker population, poor-laying queens, etc. TSBV is also called Chinese sacbrood virus (CSBV), which occurs in *A. cerana* colonies in China (Allen and Ball, 1996; Anderson, 1991; Yan et al., 2009). Molecular data from TSBV from India (Genbank Accession # EU156753) and CSBV from China (Genbank Accession# AF469603) suggest that these viruses are very closely related and can be regarded as different varieties of the same virus (Hepburn and Radloff, 2010).

### **Bacterial diseases**

There are two main bacterial diseases found in Asian honeybees: American Foul Brood disease (AFB) caused by *Paenibacillus larvae* (Nakamura, 1996; Oldroyd and Wongsiri, 2006) and European Foul Brood (EFB) caused by *Melissococcus pluton* (Bailey and Collins, 1982). Because EFB is a stress-related disorder, colonies that are heavily infested with *Varroa* are susceptible to EFB. Colonies of *A. cerana* are occasionally infested with bacterial diseases such as AFB and EFB (Bailey and Collins, 1982). Other microbial diseases have also been reported.

#### **American Foul Brood (AFB)**

American foul brood is caused by the spore forming gram-positive bacterium, *Paenibacillus larvae* (formerly classified as *Bacillus larvae*). It is the most widespread and destructive of the bee brood diseases (Genersch, 2005; Genersch et al., 2006). *Paenibacillus larvae* are rod-shaped bacteria, which are visible only under a compound microscope. Ingesting spores that are in their food infects larvae up to 3 days old, with larvae less than 24 hours most susceptible. Spores germinate in the gut of the larva and the vegetative form of the bacteria begins to grow, taking its nourishment from the larva. Spores will not germinate in larvae over 3 days old. Infected larvae normally die after their cell is sealed. The vegetative form of the bacterium will die but not before it produces many millions of spores. Each dead larva may contain as many as 100 million spores (Alippi et al., 1995). AFB is spread worldwide in *A. mellifera* and *A. cerana* (Hansen et al., 1999).

#### **European foulbrood (EFB)**

*Melissococcus plutonius* is a bacterium that infests the mid-gut of an infected bee larva. European foulbrood is less deadly to a colony than American foulbrood. *Melissococcus plutonius* does not form spores, though it can overwinter on comb. Symptoms include dead and dying larvae which can appear curled, brown or yellow, melted or deflated with tracheal tubes dried out and rubbery (Bailey and Ball, 1991; Shimanuki, 1990). European foulbrood is often considered a “stress” disease, a disease that is dangerous only if the colony is already under stress for other reasons. An otherwise healthy colony can usually survive European foulbrood. An outbreak of the disease may be controlled chemically with oxytetracycline hydrochloride, but honey from treated colonies could have chemical residues from the treatment (Waite et al., 2003). The ‘Shook Swarm’ technique involves replacing all brood frames with new frames in a single operation, thus removing all potentially diseased equipment and minimizing disease transfer. The advantage is that chemicals are not used. Prophylactic treatments are not recommended as they may lead to resistant bacteria (Waite et al., 2003).

## Fungal diseases

### *Nosema* disease (*Nosemosis*)

*Nosema* is a fungus in the class Microsporidia (Nosematidae), a large group of obligate intracellular parasites that are highly widespread in nature. They frequently infect insects, including honeybees. This parasite enters via the ventriculus of honeybees (Bailey, 1952a; 1952b; Chen et al., 2008, 2009; Fries et al., 1996). *Nosema* is also associated with black queen-cell virus. There are two species of *Nosema*: *Nosema apis* (Hassanein, 1952, 1953; Higes et al., 2007; Rinderer and Elliott, 1977) and *N. ceranae* (Fries et al., 1996). The differences between these two species can be detected in their SSUrRNA sequences. Moreover, their spores also differ. Spores of *N. ceranae* have shorter and fewer polar filament coils than those of *N. apis* (Fries, 1989a; 1989a).

*Nosema* spore infections are found only in adult bees. Infected workers, drones, and queen bees become weak and suffer early mortality (Fries et al., 1996; Webster, 1993; Webster et al., 2004). Queens suffer damage to reproductive organs and, consequently, the colony bee population can drop dramatically. Nurse bees infected with *Nosema* do not fully develop their hypopharyngeal glands, reducing the production of royal jelly and brood food. Infected foraging bees have reduced foraging activity (Anderson and Giacon, 1992; Clark, 1978, 1980; Goodman, 2007; Hassanein, 1951; Malone and Gatehouse, 1998; Malone et al., 1995; 2001). Many compounds have been tested against *Nosema*, but currently the only effective product is the antibiotic fumagillin (Moffet et al., 1969). This product inhibits the development of *N. apis* in honeybees (Katznelson and Jamieson, 1952; Liu, 1973).

Recently, *N. apis* has become more widely distributed by cross infection from *A. mellifera* to *A. cerana*. The closely related *N. ceranae* was first found in *A. cerana* Fabricius, 1793 by Fries et al. (1996). Recently, *N. ceranae* was found in managed *A. mellifera* Linnaeus, 1758 colonies (Huang et al., 2005; Higes et al., 2006; Huang et al., 2007; Klee et al., 2007). *Nosema ceranae* also infects three native species of Thai honeybees: *A. dorsata*, *A. cerana* and *A. florea* (Suwannapong et al., 2010b; Suwannapong et al., 2011).

*Nosema* invades and destroys cells in the bee gut, resulting in drooping wings, lack of hair, dysentery marked by brown fecal marks in the comb and early mortality. *Nosema* infections are acquired by the uptake of spores during feeding or grooming (Bailey, 1969; Ellis and Munn, 2005; Fries, 1983, Fries et al., 1992, 2006; Huang et al., 2007; Matheson, 1993). The parasite invades the posterior region of the ventriculus, giving rise to large numbers of spores within a short period of time. *Nosema* levels generally increase when bees are confined (Goodman, 2007; Higes et al., 2007). The spores are also transmitted among bees via the ingestion of contaminated comb material and water, and by trophallaxis via honey stores. Crushed infected bees may also play a role in disease transmission. The pathological consequences of *N. ceranae* in *A. mellifera* are not well known, however they proved highly pathogenic in infected *A. florea* (Suwannapong et al., 2010b; Suwannapong et al., 2011). Newly reported *N. ceranae* has recently jumped hosts to *A. mellifera* Linnaeus, 1758 (Higes et al., 2006). However, in *A. mellifera* colonies, *Nosema* is normally only a problem when the bees cannot leave the hive to eliminate waste (Higes et al., 2006).

### *Nosema* in Thailand

*Nosema ceranae* was first observed in Thailand by Suwannapong et al in 2007 in *A. cerana* and can now be found throughout the country infecting *A. florea*, *A. dorsata* and *A.*

*andreniformis* (Suwannapong et al., 2010b, 2011). *Nosema* spores can be found in pollen removed from pollen storage area in honeybee comb of *A. cerana*, *A. florea* and *A. dorsata*. The presence of *Nosema* spores in corbicular pollen may be due to self-contamination during the process of pollen collection. As the forager bee moistens the surface of its body with its protruding tongue and brushes the collected pollen, the spores inside its body become mixed with the pollen, via an unknown mechanism. Spores can either come directly from the intestines after regurgitation or be present in the saliva. *Nosema* spores also are found in the honey of four species, *A. cerana*, *A. florea*, *A. dorsata* and *A. mellifera*. Experimental cross infection of *A. cerana* and *A. florea* with *N. ceranae* isolated from both *A. cerana* and *A. florea* workers has been reported by Suwannapong et al. in 2010, where they reported that the parasite rapidly divided in the honeybee midgut.

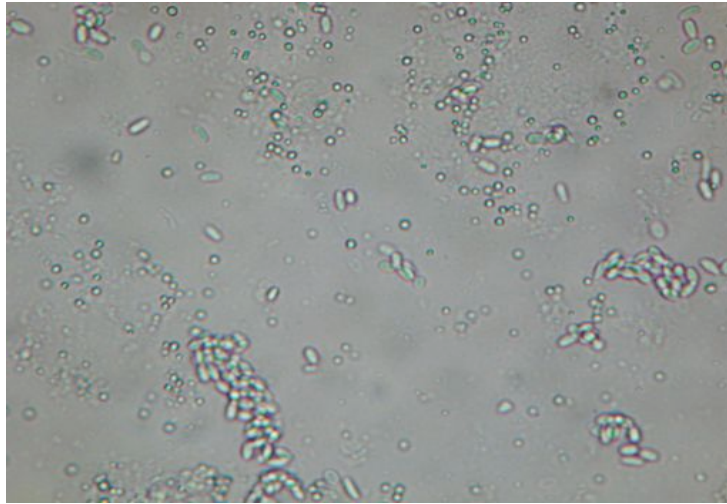


Figure 42. Light microscope image of *Nosema ceranae* spores.



Figure 43. Removing the midgut of *Nosema* infected *Apis florea* worker

### Control of *Nosema* disease

*Nosema* spores may be killed by heating hive equipments or tools to a temperature of at least 60°C for 15 minutes. Another treatment involves heating the equipment to 49°C for 24 hours. This is best conducted in a room where the temperature is uniform and thermostatically controlled (Morse and Shimanuki, 1990). Fumigation with acetic acid is effective, especially when the bees are transferred as early as possible in the season from contaminated equipment to fumigated equipment. An efficient method is to intersperse absorbent material with acetic acid between groups of hive bodies containing the combs (Bailey and Ball, 1991; Shimanuki et al., 1992). Fumigation with ethylene oxide (ETO) has also been demonstrated to kill spores on combs (100 mg ETO/l for 24 hours at 37.8 °C). However, there are a number of safety issues associated with the use of ETO (Shimanuki et al., 1992). Fumagillin has been found to be effective against *N. apis* (Katznelson and Jamieson, 1952). In addition, this chemical inhibits DNA replication of the microsporidian without affecting the DNA of the host cell (Hartwig and Przelecka, 1971; Liu, 1973). Fumagillin's activity remains high in honey kept at 4°C for several years and for at least 30 days at 30°C (Furgala and Sugden, 1985).

Several natural compounds have been examined for potential efficacy against *Nosema*: thymol, vetiver essential oil, lysozyme, and resveratrol. Lysozyme was not effective. However, resveratrol and thymol have high potential for the control of *Nosema* (Maistrello et al., 2008). Resveratrol (trans- 3,5,4'-trihydroxystilbene) is a phytoalexin produced by certain plants in response to infections caused by phytopathogens. It is known for its anti-cancer and anti-inflammatory effects (Fremont, 2000). Recent studies have shown that resveratrol can inhibit the development of the microsporidian *Encephalitozoon cunicoli* in vitro experiments (Leiro et al., 2004).

Thymol (3-hydroxy-p-cymene) is a constituent of the essential oil found in thyme and other plant species. It has been shown to suppress *N. vespula* disease in *Helicoverpa armigera* caterpillars. Some evidence suggests that thymol may suppress *Nosema* disease in honeybee colonies (Rice, 2001; Yucel and Dogaroglu, 2005). It also inhibits the growth of pathogenic

bacteria and fungi such as *Salmonella typhimurium*, *Staphylococcus aureus* (Juven et al., 1994), *Aspergillus flavus* (Mahmoud, 1999), and *Cryptococcus neoformans* (Viollon and Chaumont, 1994). In apiculture, it is known to suppress the parasitic mite *Varroa destructor* (Chiesa, 1991). Recent research has shown that thymol fed orally to adult bees is not toxic (Ebert et al., 2007).

Other treatments also show promise. Protofil is a natural product obtained from plants through hydro-alcoholic extraction. Protofil prevents the development cycle of *N. apis*, inhibits intestinal pathogens, and stimulates the digest enzymes. In general, it impairs the development of bee colonies (Chioveanu et al., 2004). The addition of acetic acid into winter food may have positive effects in preventing different diseases. An experiment in Norway found that acetic acid in food reduced the occurrence of chalk brood, but these results should be replicated (Pederson, 1976). Laboratory experiments in Belgium suggested that acidified food decreases the development of *N. apis* in the midgut, but field studies performed in France demonstrated no impact of acidified food on *Nosema* development (Chioveanu et al., 2004). The chemical composition of food may have an impact on of *N. apis* the spore germination. Changing the chemical environment (i.e. lowering the pH) may reduce spore germination (Crane, 1975).

### **Wax moths**

Two varieties of wax moth infest honeybee colonies: the lesser wax moth, *Aphomia sociella* which has a length of 10-13 mm, a wingspan of 11-14 mm and is silvery-grey to buff in color with a yellow head. When seen, it flies, runs very quickly or holds onto the comb vibrating its wings. Each female can lay 250-300 eggs hatching into larvae that are similar in appearance to greater wax moth larvae but not as large being up to 20 mm in length. Though larvae consume honey, pollen and wax they are not found in comb occupied by bees and do not damage hive components. Lesser wax moth larvae are unable to compete with greater wax moth larvae because the latter will eat them (Gambino, 1995). In the live bee situation the best preventative against wax moths is strong healthy colonies. If not controlled wax moth infestations can rapidly multiply, (which is mottled grey in color and 0.5-1.9 cm long) is found worldwide. In southern India, it causes severe damage in the plains and lower altitudes but it is rare at high altitudes. The greater wax moth is a natural scavenger of honeybee comb and their contents. Beeswax combs are vulnerable to wax moth damage anytime they are unprotected by bees: whether in a weak and declining colony or in shed storage. It is one of the most important enemies of the bee colony, causing serious damage particularly to weak colonies where the number of bees is not sufficient enough to cover all the combs. They will not attack the bees directly, but feed on the wax used by the bees to build their honeycomb. Their full development requires access to used brood comb or brood cell cleanings for protein (Ali et al., 2009; Gambino, 1995).

A strong hive generally needs no treatment to control wax moths. The bees themselves will kill and clean out the moth larvae and webs. Damaged comb may be scraped out and replaced by the bees. The moths prefer to mate and lay their eggs at night. In Thailand, wax moths occur throughout the year and are found in all species of honeybees. In nature, wax moths are valuable members of the ecosystem because they clean abandoned cavities of old comb (potentially contaminated with disease) and render it clean for the next occupying swarm (Ali et al., 2009; Gambino, 1995).

**Life cycle of wax moths**

Wax moth development goes through three consecutive stages: egg, larva and pupa. This sequence is only interrupted if the temperature is too low or when there is no food. Therefore, the cycle can last between 6 weeks and 6 months depending on temperature and food. According to the literature, over-wintering can take place as egg, larva or pupa. The females start laying eggs between days 4 and 10 after emergence (Shimanuki, 1981). At dusk, the females attempt to enter the beehive to lay their eggs. Normally, females lay their eggs into crevasses and gaps. If the colony is strong enough to repel the wax moth, the moths lay their eggs outside in cracks in the wood. This puts them out of reach of the bees and prevents their destruction. After hatching, the young larva immediately searches for a comb to feed and to build the silk-lined feeding tunnels. Speed of growth is directly dependent on temperature and food supply. Under ideal conditions, larval weight can double daily during the first 10 days. Newly hatched larvae are white, but successive instars are medium to dark gray on the top with creamy white undersides. The larval head capsule is brown. Heat, which is created by this rapid growth, can increase the temperature in the spun silk nests far above the ambient environmental temperature (Ali et al., 2009; Gambino, 1995; Shimanuki, 1981).

The larvae feed on wax, impurities in wax, the cocoons of bee larvae, and remnant pollen. Larvae that have been reared exclusively on pure wax (foundation and fresh comb), do not complete their development. Dark, old combs that contain many old bee cocoons provide the most food to wax moth larvae. The larvae grow rapidly and will migrate toward the edges of the frames or corners of the supers to spin a cocoon and pupate. At the end of the larval stage, the larva spins a very strong silk cocoon on a firm support. Frequently the larva spins its cocoon in a hollow it had bored into the wood (Ali et al., 2009; Gambino, 1995; Shimanuki, 1981).





Figure 44. Larvae of wax moth in an *Apis florea* nest.

Damage occurs as the larvae burrow into the comb feeding on the wax, larval skins, pollen and honey. As the larvae chew through the comb they spin a silk lined tunnel through the cell walls and over the face of the comb. These silk threads can tether emerging bees by their abdomens to their cells and they die of starvation because they are unable to escape from their cell (Ali et al., 2009; Shimanuki, 1981). This phenomenon is termed galleriasis. In severe infestations, the wax comb, wooden frames, and sides of the hive bodies can be heavily damaged. After hatching, the larvae, if not removed, by house bees can destroy a hive that is in weak. Adult wax moths cause no direct damage because their mouthparts are atrophied. They do not feed as adults. Only larvae feed and destroy combs. However, adult wax moths and larvae can transfer disease pathogens. In colonies infested with foulbrood, the feces of wax moths contain large amounts of *Paenibacillus larvae* spores (Ali et al., 2009)



Figure 45. Larvae and pupae of wax moths collected from an *Apis andreniformis* nest.

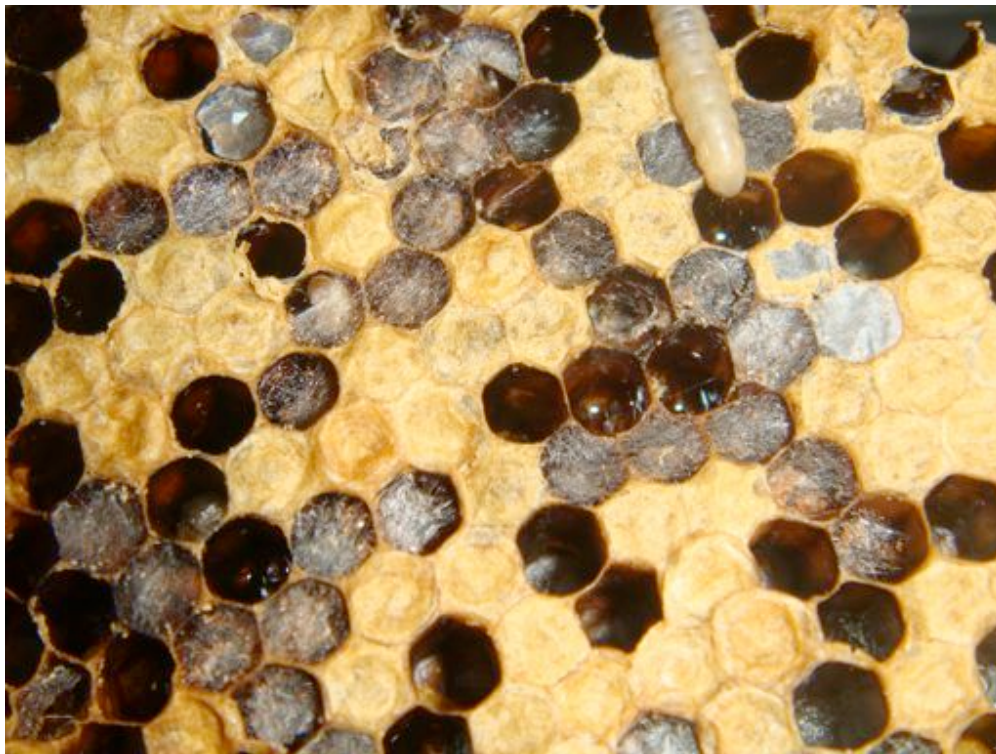


Figure 46. *Apis cerana* brood comb infested by wax moths.

The most effective method for preventing wax moth damage is to maintain strong colonies. The bees will remove the moth larvae and repair damage as it occurs. Stored equipment can be protected against wax moths by fumigating it with para-dichlorobenzene crystals or by stacking honey supers in a criss-cross fashion in open sheds. The penetrating air and daylight discourage colonization by moths. Some beekeepers store supers in enclosed barns with a lighted bug-zapper running constantly to kill emerging adult moths. This practice can eventually eradicate moths from the room. Control of wax moths by other means includes the freezing of the comb for at least 24 hours (Ali et al., 2009; Gambino, 1995; Shimanuki, 1981).

### **Wasps, Hornets and Ants**

Ants are the most common predators of honeybees in Thailand. They will collect honey, brood, and adults (dead or alive). Black ants (*Camponotus compressus*) and red ants (*Dorylus labiams*) are dangerous enemies of bees (Akranakul, 1976). They attack weak colonies and carry away the honey, pollen and the brood. Strong colonies are able to withstand the ants. In weak colonies, ant attack will result kill the colony. Beekeepers sometimes kill ant nests in the proximity of the apiaries. A common practice is to rub the hive stand posts with engine oil or any lubricants. A more reliable method of defense is to place the hive stand posts in plastic pots or cans filled with water or oil. Liquids require replenishment regularly and removal of all vegetation which can be the foundations of bridges that can be crossed by ants (Akranakul, 1976; Hirai et al., 1981).

Wasps and hornets are also enemies of bees. The yellow-banded hornet, *Vespa cincta* F., is a large wasp with a broad transverse band on its abdomen. They attack a weak colony en masse, using their strong mandibles to bruise the guardian bees at the hive entrance. Hornets drop the dead and dying bees to the ground. The colony under attack will eventually lose its defenders. Hornets will then invade the hive and carry away honey and brood to store in their nest (Akranakul, 1976; Hirai et al., 1981).

The Asian giant hornet, *Vespa mandarinia* is a relentless hunter that preys on Thai honeybees. They often attack honeybee nests with the goal of obtaining the honeybee larvae. A few scouts will cautiously approach the nest, giving off pheromones that lead other hornets to the hive's location. The hornets can devastate a colony of honeybees. A single hornet can kill as many as 40 honeybees per minute. It takes only a few of these hornets a few hours to kill thousands of bees. *Apis mellifera* has relatively small stings that do little damage to hornets, since they are five times the size of honeybees and twenty times their weight. The honeybees make futile solo attacks without mounting a collective defense, and are easily killed individually by the hornets. Once a hive is emptied of all defending bees, the hornets feed on the honey and carry the larvae back to feed to their own larvae (Hirai et al., 1981).

Adult hornets cannot digest solid protein, so the hornets do not eat their prey, but chew them into a paste and feed them to their larvae (Hunt et al., 1982). The larvae produce a clear liquid, *Vespa* amino acid mixture, which the adults consume. Larvae of social Vespidae produce these secretions. The exact amino acid composition varies considerably among species (Hunt et al., 1982). In many parts of Asia, including Thailand hornets, hornets are reported to be serious pests of honeybees. Making efforts to catch hornets that come near hive entrances can prevent serious destruction. However, the best way of dealing with the problem

is reduce hornet nest habitat by removing nearby trees until the hornet population is much reduced (Akarakul, 1976). This method, however, is not practical for forest beekeepers.



Figure 47. The Asian giant hornet, *Vespa mandarinia* and *Apis florea* nest.

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