

CHAPTER II

LITERATURE REVIEW

2.1 The Ants: As Predators

Ants are the major group of the eusocial insect hymenoptera. Their natural distribution includes all continents and major islands. Ants are classified in a single family, the Formicidae, within the order Hymenoptera, and are social insects which have been evolving successfully since the Cretaceous. The known living ants involve 16 subfamilies, 296 genera and 15,000 species (Bolton, 1995), around 12,000 of which are described (Wilson, 2006). At present, 9 subfamilies of ants are recorded in Southeast Asia and all of those are found to be native to Thailand with the estimated number of 800-1,000 species (Jaitrong and Nabhitabhata, 2005). Ants can be found in any type of habitats from the Arctic Circle to the Equator (Brian, 1978), although they are absent from Iceland, Greenland and Antarctica (Hölldobler and Wilson, 1990) and some smaller islands lack native ants (Wilson and Taylor, 1967). The number of species declines with increasing latitudes, altitudes, and aridity (Kusnezov, 1957; Fowler and Claver, 1991; Farji-Brener and Ruggiero, 1994; Samson *et al.*, 1997). Despite the fact that tropical areas and continental forests are amongst the poorest known, these areas have the greatest recorded species diversity (Hölldobler and Wilson, 1990).

The Asian Weaver Ants, *Oecophylla smaragdina*

Taxonomic Classification (Hölldobler and Wilson, 1990)

The weaver ant tested in our experiments is classified into:

Class: Insecta

Order: Hymenoptera

Superfamily: Vespoidea

Family: Formicidae

Subfamily: Formicinae

Tribe: Oecophyllini

Genus : *Oecophylla*

Species: *Oecophylla smaragdina*



Figure 2.1 A nest of weaver ant, *O. smaragdina*. Photo by O. Duangphakdee.

The weaver ants are classified in the Subfamily Formicinae, genus *Oecophylla*. There are two species: *O. longinoda* Latreille, distributed in tropical and subtropical Africa, and *O. smaragdina* Fabricius which is widespread from southern Asia to northern Australia, including many tropical western Pacific islands (Wheeler, 1922; Cole and Jones, 1948; Lokkers, 1986, Bolton, 1995). Weaver ants are arboreal and play an important role in rainforest ecosystems as a keystone predator of invertebrates and small vertebrates. They establish mostly on tree canopies and sometime dominate across territorial habitat. They can control the entire tree surface from the ground up and prey on virtually all animals found within their territory (Hölldobler and Wilson, 1994). To build their nests (fig 2.1), worker ants form chains along the edge and pull the edges together by shortening the chain by one ant at a time. They hold the leaves in place and glue them by the silk produced by their larvae. When the leaves die eventually they move and build the new nest. This trait of nesting behavior allows them to escape the necessity to locate a nest cavity and enables *O. smaragdina* to nest wherever leaves are found and conditions are suitable within their large and extended territory. Moreover, they can also grow or move if the leaves die or avoiding the unappreciated conditions. The single queen is located in one nest and her eggs are distributed to the other nests which dispersed over several nests which

could expand to several trees. They also co-exist with the scale insects (Hemiptera: Coccoidea) which produce the honeydew, source of carbohydrates, for them. One colony can contain 500,000 or more workers, and control a territory of a dozen nests or more large trees (Hölldobler and Wilson, 1994). This species have high variation in the color of worker body. In Southeast Asia, their workers have red-brown color, vary from light to dark, while they have intense green color abdomen in Australia. Therefore, they have been named as “green tree ants” in Australia. Generally, they eat small animals but they also can capture and kill much larger animals compared to the size of themselves. Although they do not have sting, they have powerful jaws and can inflict a painful bite in combination with formic acid to human body. Further, the effective recruitment system of *O. smaragdina* contributes significantly in the final success to kill a larger prey. Weaver ants have been cited as a major ant pest of the honeybees (Seeley, 1985, Wongsiri *et al.*, 1996, Morse and Flottum, 1997, Oldroyd and Wongsiri, 2006).

The Red Wood Ants, *Formica polyctena*

Taxonomic classification (Hölldobler and Wilson, 1990)

The red wood ant is classified into:

Class: Insecta

Order: Hymenoptera

Superfamily: Vespoidea

Family: Formicidae

Subfamily: Formicinae

Tribe: Formicini

Genus : *Formica*

Species: *Formica polyctena*

The red wood ants, *F. polyctena*, are a common species widely distributed in temperate regions of the northern hemisphere. Colonies build part of their nest underground, the other part are large mounds. As their name implies, they live in the wooded area, normally found in coniferous plantations, and build their nests by twigs, leaves or fir needles (fig 2.2). They build the nest above the ground in coniferous forests, and contrast below the ground in deciduous forests (M.G. Nielsen, personal

communication cited in Kristiansen and Amelung, 2001). For defense they secrete formic acid and can squirt the acid from its acidopore several feet if alarmed. There are omnivorous, feeding on invertebrates and honeydew. The colony consists of thousands of individuals. Ant galleries and chambers are dug down to a depth of >50 cm. They have a great influence on the cold-temperate forest ecosystem. In ant colonies, mature workers mainly feed on carbohydrate food, and retrieval of animal prey is usually mainly related to the protein demands of queens (who need protein for egg production) and developing larvae. Wood ants hunt in trees as well as on the forest floor (Skinner and Whittaker, 1981). Thus, they probably encounter regularly colonies of honey bees, *A. mellifera*. They have been cited as predators of European honey bees, *A. mellifera mellifera* (Szczuka and Godzińska, 2004).



Figure 2.2 A nest of red wood ant, *F. polycтена*. Photo by O. Duangphakdee.

2.2 The Bees: As Preys

Social bees are an “amazing” example of adaptation. Within the honey bee colony an extraordinary elaboration of caste system is displayed which includes the reproductive castes, queen and drone, and the labor caste (workers). Within the labor caste, honey bee workers have a complex division of labor. They switch their main activity at least four times. After hatching the worker bee starts with cell cleaning, passing through brood care and food storage in mid-life and ending with foraging.

When bees need a new home, in case of over population or disturbance by their enemies, worker bees scout for new nest sites and later they choose the best position through sort of plebiscite (Lindauer, 1961; Seeley, 1985).

Nests of social bee contain often a large amount of brood and high quantities of stored food (pollen and honey). Therefore, their nests are a very attractive target for all kind of predators (Seeley, 1985).

The honey bees and stingless bees are comprised in the superfamily Apoidea. According to Michener (2000), they have been reported to fall closest to the sphecoid wasps. They are categorized into the family Apidae, subfamily Apinae which belong to a family of bees living in social communities, tribe Apini, genus *Apis* for honeybees and tribe Meliponini for stingless bees. Within the tribe Meliponini, we chose the genus *Trigona* in this research as they are found in Southeast Asia (Ruttner, 1988).

Tribe Meliponini

Taxonomic classification (Michener, 2000)

Stingless bees were classified into the same family as honeybees but under the tribe Meliponinae. We collected samples from the nest of six bee species in the genus *Trigona*.

Class: Insecta

Order: Hymenoptera

Superfamily: Apoidea

Family: Apidae

Subfamily: Apinae

Tribe: Meliponini

Genus: *Trigona*

Species: *Trigona apicalis*

Trigona collina

Trigona terminata

Trigona melanocephala

Trigona laeviceps

Trigona minor

Like the honeybees, stingless bees (tribe Meliponini) are highly eusocial insects living in the permanent colonies. They have morphologically as well as behaviorally very different female castes, queen and worker. They are found in

tropical and southern subtropical areas throughout the world (Michener, 2000). The colony comprises of many individuals ranging from few hundreds to several thousands (Sakagami, 1982). The tribe Meliponini is a very diverse taxon and comprises several hundred species (Michener, 2000). The classification of stingless bees has undergone principal changes, starting with Michener (1944) and Schwarz (1948) who recognized only two main genera, *Melipona* and *Trigona*. Later Michener (2000) presented 23 genera because some subgenera have been elevated to the genus level according to Lepeletier (1835, 1841 cited in Michener, 2000). Here I will follow Michener (1974) and present the genera recognized by him:

(1) *Melipona*, consisting of a number of tropical American species varying from smaller to larger than *A. mellifera*.

(2) *Meliponula*, consisting of a single African species having the appearance of a small *Melipona*.

(3) *Trigona*, a large pantropical genus of long wing bees, ranging from almost as *A. mellifera* size down to the shortest of all bees, 2 mm in length.

(4) *Dactylurina*, another group which have slender bodied African species.

(5) *Lestrimelitta*, a genus of African and tropical American robber bees. This group lives dependently upon the products of nests of *Trigona*, *Melipona* or even *Apis*.

Sakagami and collaborators (1985) found 41 species of the genus *Trigona* in the Indo-Pacific area, out of which 22 species were found in Thailand. Later, many more species have been discovered and recently 32 species of the genus *Trigona* have been recorded in Thailand (Schwarz, 1939; Sakagami *et al.*, 1985 and Michener and Boongird, 2004; Klakasikorn *et al.*, 2005).

The majority of Meliponini nest in cavities of approximate size, into which they enter through a small aperture (Roubik, 2006). In the nest brood cells and food containers (honey and pollen are stored in separate pots) are built (Roubik, 2006). Regularly, nest cavities are found in hollow tree trunks or branches. Some species, for example, *Melipona nigra* and *Trigona fulviventris*, nest on the foot of a tree in hollow roots. Other species nest in the soil taking advantage of the abandoned nests of ants, termites, or subterranean rodents (Michener, 1974). There are various species, principally *Trigona*, that nest in man-made cavities, in towns and cities. Nest

structures of stingless bees are made out of a mixture of wax with large amounts of resin from plants and in several species also mud (Michener, 1974). Some *Trigona* species, like *T. laeviceps*, also collects oil or grease from the machinery whereas one Australian species, *Trigona hockiningsi*, collect the fresh colour paint to build the nest (Michener, 1974). The entrance into the cavity of the nest is often elaborated in various ways. The entrance tube often extends into the open air from the nest entrance. It may be made of bristle cerumen, soft or even sticky. Since the entrance tubes are vary depending on the species, therefore, the information is often used in the bionomical and ecological studies (Sakagami *et al.*, 1985).

Tribe Apini

Taxonomic classification (Michener, 2000)

The social bees belong to the family Apidae, one of the most diverse families of bees. Both honeybees and stingless bees are members of the subfamily Apinae. The honeybee species which we tested in this research is classified into:

Class: Insecta

Order: Hymenoptera

Superfamily Apoidea

Family: Apidae

Subfamily: Apinae

Tribe: Apini

Genus: *Apis*

Species: *Apis florea*

Apis andreniformis

Apis mellifera

The true honeybees consist of the single genus *Apis*. The *Apis* consists of small (7 mm long) to large (19 mm long). Tribe Apini comprises with nine species, dwarf honey bee (*A. florea* Fabricius 1787), small dwarf honey bee (*A. andreniformis* Smith 1858), common giant honey bee (*A. dorsata* Fabricius 1793), giant mountain honey bee, (*A. laboriosa* Smith 1871), eastern hive bee (*A. cerana* Fabricius 1793), red honey bee, (*A. koschevnikovi* Enderlein 1906), mountain honey bee (*A. nuluensis* Tingek, Koeniger, and Koeniger 1996), Sulawesi honey bee, (*A. nigrocincta* Smith 1861) and Western honey bee (*A. mellifera* Linnaeus 1758) (Oldroyd and Wongsiri, 2006). They are difference in size of individual and nest architecture. Consequently, phylogenetic analysis indicated three groups which are as follows (Michener, 2000):

(Oldroyd and Wongsiri, 2006). Accordingly, this dwarf honeybee species has shared several common characteristics with its sister taxa, *A. florea*. The most significant morphological characteristic used for identification of these species are black stripes on the legs, specifically on the tibia and on the dorsolateral (back and side) surface of the basitarsus. The hind tibia and dorsolateral surface of the hind basitarsus have black hair while of which *A. florea* are white color. Additionally, the pigmentation of *A. andreniformis* is mostly blackish, while that of *A. florea* is yellowish (Wongsiri *et al.*, 1996).



Figure 2.3 The *A. florea* nest. Photo by S. Wongvilas.

The *A. andreniformis* distribution overlaps with *A. florea*. However, they found only *A. andreniformis* in seven Thai provinces, Malaysia, Indonesia (Java and Borneo) and the Philippines Palawan where *A. florea* was not reported (Wongsiri *et al.*, 2000). The specimens of *A. florea* had been reported in Java, but these maybe the result of human-assisted introduction (Wongsiri *et al.*, 1996). Not only morphological characteristics have been separated, they also exhibit vastly different behavior, for example the defensive behavior, *A. florea* reacts to the smoke by clumping together and form protective curtain but *A. andreniformis* is more defensive than *A. florea*. They immediately attack the intruders by guard bees. The protective curtain is loosen and flying up from the comb. The drone flight time, is also different, *A. andreniformis*

fly just after the sun passes its zenith and *A. florea* flying later in the afternoon (Rinderer *et al.*, 1993). There are a small differences in nest site selection, *A. andreniformis* is mostly found near undisturbed, mixed deciduous to evergreen forests. Their nesting habitat is usually a dark and shady place while *A. florea* nests are found in more disturbed areas and the areas described above (Wongsiri *et al.*, 1996).

Both species have a nest comprised of a single opened comb, typically on a single branch of shrubs and small trees (fig 2.3 and 2.4). Like the other social bees, inside the comb has brood cell, drone cell, honey and pollen storage cells. Often, both species apply sticky resin called “sticky band”, on nesting branches and all the other objects connected their nest to the outside which aids in defense against ants (fig. 2.4) (Seeley and Seeley, 1982; Wongsiri *et al.*, 1996).



Figure 2.4 The *A. andreniformis* nest. Photo By S. Wongvilas.

A. mellifera

A. mellifera, the most common commercial species, is native to Africa, Europe and portions of Western Asia (Ruttner, 1988). It is also called the Western honeybee. They are usually found in tree hollows and build three or more parallel combs affixed to the roof of the cavity (Oldroyd and Wongsiri, 2006). There are about 25 named

sub-species (Ruttner, 1992 cited in Sheppard and Meixner, 2003) that have adapted to the environment of their geographic and climatic areas. They can adapt to living in cavities in manmade structure including the made hives (fig 2.5). It tends to be slightly larger than the Asian hive species. In similarity in characteristics, *A. mellifera* is considered to be in the same phylogenetic group (fig. 2.8) with other Asian cavity nesting honey bees (Michener, 1974). The biological status of *A. mellifera* is similar to that of the Asian cavity nesting bees, *A. cerana* Ruttner and Maul (1983) demonstrated that the hybrid created by artificial insemination between *A. mellifera* and *A. cerana* are not viable. This species is well-known for its economic value and it has also been disseminated around the world by humans (Ruttner, 1975).



Figure 2.5 The *A. mellifera* colony. Photo by A. Rattanawanee.

2.3 The Phylogenetic Relationship of Ants and Bees

The first critical cladistically based study of the phylogeny among the Aculeata (wasps, ants and bees) was conducted by Brothers (1975, 1999). One of the major conclusions was that the previous seven superfamilies should be reduced to three: Chrysidoidea (parasitoid or cleptoparasitic wasps), Apoidea (sphecoid wasps and their descendants, the bees) and Vespoidea (wasps and ants). By reviewing the work of himself and others, Brothers (1999) had composited a new cladogram

showing relationships among taxa of Aculeata which should be recognized at the family level (fig. 2.6).

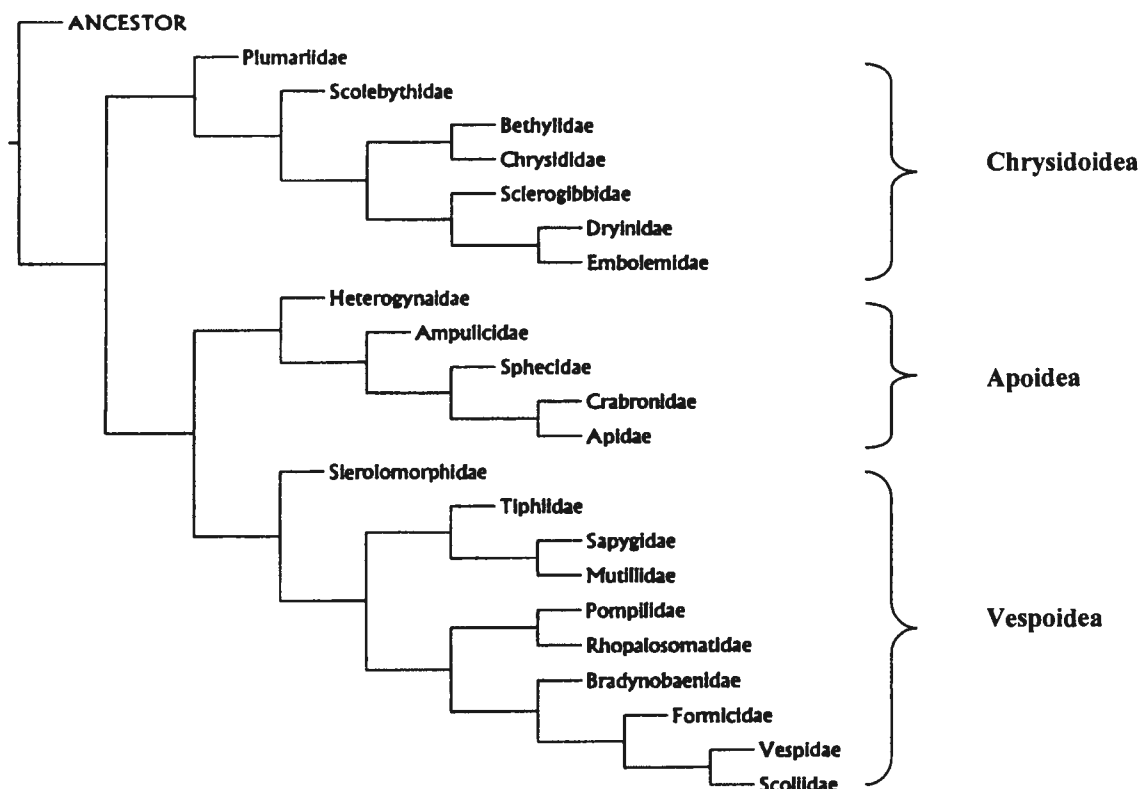


Figure 2.6 Composite cladogram showing relationships among taxa of Aculeata which should be recognized at the family level (Brothers, 1999).

The superfamily Vespoidea consists of ten families including the ant taxa which we studied that are categorized into Formicidae. On the other hand, social bees are categorized into the family Apidae of the superfamily Apoidea (fig: 2.6).

The Formicidae comprises of a large and distinct group. Much work has been done to clarify the relationships among various subfamilies and tribes, but there is still considerable uncertainty about the true situation. In particular, the discovery of enigmatic species with unusual combinations of characters (e.g. by Ward, 1994) has caused problems. Bolton (1995) recognized the modern subfamilies as Aenictinae, Aenictogitoninae, Aneuretinae, Apomyrminae, Cerapachyinae, Dolichoderinae, Dorylinae, Ecitoninae, Formicinae, Leptanillinae, Leptanilloidinae, Myrmeciinae, Myrmicinae, Nothomyrmeciinae, Ponerinae and Pseudomyrmecinae and the fossil subfamilies as Armaniinae, Formiciinae, Palaeosminthurinae and Sphecomyrminae. No detail about relationships was given, but they are apparently those described in the

paper of Baroni Urbani *et al.* (1992) in which two large clades were recognized. Later, Wilson and Hölldobler (2005) presented a version which interpret to be the consensus among recent published reconstructions (fig 2.7).

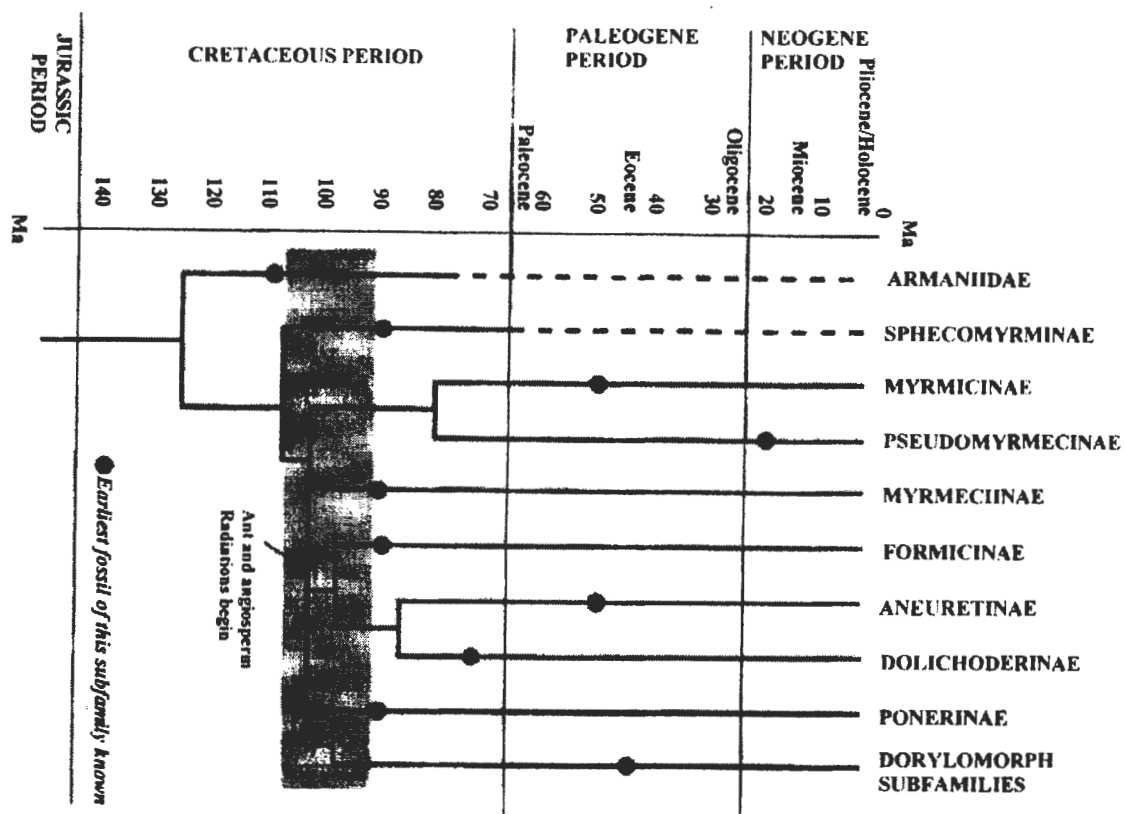


Figure 2.7 A schematic of the evolution of ants (Formicidae) at the subfamily level (Wilson and Hölldobler, 2005)

Until recently, the search of the ancestry of the ants always ended in frustration. In 1967, Wilson *et al.* obtained the first ant, known as *Sphecomyrma*, remains of Cretaceous age which had primitive wasp-like traits: mandibles very short and with only two teeth, gaster unconstricted, sting extruded and middle and hind legs furnished with double tibial spurs. On the other hand, the ant also possesses distinctively ant-like traits: thorax reduced in size and wingless and petiole pinched down posteriorly at its juncture with the rest of abdomen, primitive in form compared to the later ants. There is an important character which is the key diagnostic trait of modern ants, an apparent metapleural gland. Within the subfamily level, the

representative of Formicinae, Myrmicinae and Dolichoderinae were found in the amber of Mid-Eocene age from Arkansas (Wilson, 1985).

The weaver ants, *Oecophylla*, prospered especially during the Oligocene and Miocene (20-30 million years ago). The common ancestor of extant *Oecophylla* species (Wilson and Taylor, 1967) is the Miocene specimens discovered in Sicily, Italy (Bolton 1995). The recent species were established in the middle or late Miocene. Later, during the Pliocene cooling, a desert expanded over North Africa to the Middle East (Morley, 2000) and completely isolated *O. smaragdina* from *O. longinoda* (Azuma *et al.*, 2006).

Wheeler (1914) reported that not fewer than 24 genera represented in Baltic amber fossils of the early Oligocene, still survive and are widespread including currently species such as *Ponera*, *Tetraoponera*, *Aplaenogaster*, *Monomorium*, *Iridomyrmex*, *Lasius* and *Formica* (Hölldobler and Wilson, 1990). This suggested that the ancestor of *Formica* was presented at least in early Oligocene.

The Apoidea is a holophyletic group (Brothers, 1975). The superfamily itself comprises two monophyletic groups, sphecid wasps and bees. The sphecids have often been considered to be a single family, particularly (Bohart and Menke, 1976), whereas the bees have been regarded by all bee specialists as comprising several families. However, according to the recommendation of Melo (1997), some families were dismembered and reduced to a subfamily level. This resulted in that the Apoidea consisted of five families (Brothers, 1999).

The bees divers as a monophyletic clade from the sphecid wasps in the early Cretaceous period 120-130 million years ago (Winston, 1987; Engel, 2001; Oldroyd and Wongsiri, 2006). Around 90-100 million years ago, before the break-up of Gondwana, a new way of packing and carrying pollen emerged among bees. This led to the clades of corbiculate, bee's pollen-holding organ, Apidae. The four modern tribes of bees are considered as corbiculate Apidae (Oldroyd and Wongsiri, 2006), including the orchid bees (Euglossini), the stingless bees (Meliponini), the bumble bees (Bombini) and the honey bees (Apini).

The Meliponini (stingless bees) are considered to be the first in evolutionary lineage (Winston and Michener, 1977). The oldest known specimen is a fossil of female stingless bee found in an 80 million year old amber (Michener and Grimaldi, 1988; Crane, 1999). These bees have recently distributed in tropical area of Africa, Asia, Australia and South and Central America. One or two species extend into subtropical region in Australia and Brazil (Oldroyd and Wongsiri, 2006).

Two main hypotheses about the ancestor of honeybees are put forward; first supports a close relationship between honeybees and bumble bees and second a closer relationship between honeybees and stingless bees. There is no satisfactory explanation which of these two hypotheses is correct. If the first hypothesis is true, the ancestor of honeybees is probably a solitary ancestor related to the orchid bees. However if the honeybee and stingless bees are sister taxa, suggested that the common ancestor might have been an ancient bumble bee, with a colony dominated by a queen but still lacking the strongly divergent queen and worker caste (Wilson, 1971). Therefore, two possible origins of honeybees have been suggested; the honeybees either arose from the common ancestor of orchid bees (the time is unclear) or stingless bees (more than 70 million years old) (Oldroyd and Wongsiri, 2006).

The extinct of ancient honeybee and stingless bee species in the global cooling at the middle of Miocene, but persist in tropical Asia, indicated that honey bees originated with open-nesting, single comb (Ruttner, 1988). The modern open-nesting species were suggested to appear first in Southeast Asia 6-10 million years ago (Engel, 1999). Cavity nesting species emerged later, possibly 6 million years ago in Himalayan region (Ruttner, 1988; Oldroyd and Wongsiri, 2006). Then it radiated into the temperate regions of Europe and Asia. The expansion of the desert areas in the Middle East caused the isolation of these two lineages. The European lineage evolved to modern *A. mellifera* whereas the Asian lineage evolved to *A. cerana* and the other related species (Oldroyd and Wongsiri, 2006).

Within the genus *Apis*, the open nesting species, *A. florea* and *A. andreniformis*, were suggested as species in the basal clade in phylogeny cluster which gave by the most like topology in the author's opinion (Engel and Schultz, 1997; Raffiudin and Crozer, 2000, Arias and Sheppard, 2005; Oldroyd and Wongsiri, 2006). The giant species, *A. laboriosa* and *A. dorsata*, occupied later on the clade.

Then, cavity nesting bees were clustered on the tip of phylogenetic tree. The molecular evidence suggested that *A. mellifera* and *A. koschevnikovi* (Arias *et al.*, 1996) had diverted earlier than those other species, *A. cerana*, *A. nigrocincta* and *A. nuluensis* (fig. 2.8)(Oldroyd and Wongsiri, 2006).

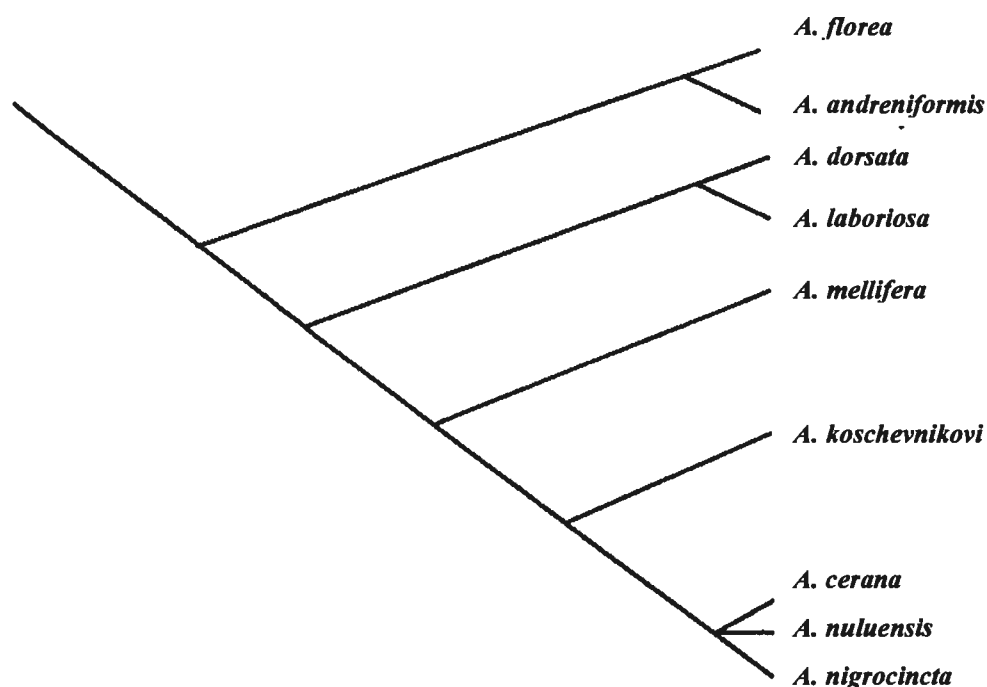


Figure 2.8 Phylogeny of the genus *Apis* (Oldroyd and Wongsiri, 2006).

2.4 Chemical Ecology of the Defenses of Plants and Insects against ants

In response to ant predation, social insects play various defensive behaviors as fleeing, abandoning the nest, encountering and using physical and chemical barriers to protect their nests (Skutch, 1971; Michener, 1974; West-Eberhard, 1989; Wenzel and Pickering; 1991; Kojima, 1993; Wcislo and Schatz, 2003, Nakamura and Seeley, 2006).

The highly oxidized diterpenoids, neo-clerodane diterpenoids, from the leaves of *Cornutia grandifolia* are significant repellents of the leafcutter ant, *Acromyrmex octospinosus* (Chen and Wiemer, 1992). The sesquiterpenoid, lasidiol angelate, from *Lasiantheae fruticosa* (Compositae) had been reported as a potent leafcutting ant repellent. In many insects, defensive compounds that are effective against ants have been found in integument of some larvae (Nick-isch-Rosenegk and Wink, 1993), in the hemolymph and a secretion from specialized gland (Deml and Dettner, 1995;

Osborn and Jaffe, 1998). The extracts of the spines and neck glands of two nymphalid butterfly larvae, *Dione junio* and *Abananote hylonome*, were repellent to ants, *Camponotus rufipes* (Osborn and Jaffe, 1998). The fecal shields of larvae of the tortoise beetle, *Eurypedus nigrosignata*, had a deterrent effect on feeding in the *Myrmica rubra* ant. The larval compounds displayed the same terpene pattern as the host-plant leaves, namely β -terpinene, α -pinene and sabinene (Gomez *et al.*, 1999). The larvae of *Caliroa* Costa species are covered by a slimy secretion helped to repel predator (Dathe, 2003, Betz and Kolsch, 2004). To protect the larvae from the ants predation, members of family Vespidae, *Parischnogaster* spp. use the secretion from Dufour's gland to construct sticky defensive barrier (Hermann and Blum, 1981; Betz and Kolsch, 2004).

2.5 Collection of the Plant Resin: Defense Material in Bees

Bees collect resins and use them for many purposes. Some species in the genus *Passaloecus* (Hymenoptera: Sphecidae) form the adhesion rings of resin around their nest entrances. It probably functions by chemical active ingredients even obviously solely mechanically (Hermann and Blum, 1981). *Plebia* species make large deposits of resin inside their nests. *Lestrimelitta* bees also use resins to trap and glue their enemies (Patricio *et al.*, 2002).

A. florea and *A. andreniformis* applied the resin on the substrate of nesting branches. These bands are 2.8 ± 2.1 cm. wide with 0.5-10.05 cm in range. Any small animals attempted to cross would stick on, hence the named "sticky band". These bands serve as barriers against small, crawling animals especially ants (Seeley and Seeley, 1982). There was no evidence about how the dwarf honeybee possessed the sticky band using resin from plants. However, the chemical compounds of plant resin deter to the enemies in sticky material may be defense (Harborne, 1988).

Another group of honeybees, Western cavity nesting, *A. mellifera* collects the resin to form propolis from the wounds or cracks in the bark of trees and leaf buds. This resin is masticated, salivary enzymes added and the partially digested material and mix with the beeswax to use in the hive (Ghisalberti, 1978; Marcucci, 1995; Burdock, 1998, Nakamura and Seeley, 2006). It is generally composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Cirasino *et al.*, 1987; Monti *et al.*,

1983; Burdock; 1998). In precisely, the composition of raw propolis varies with the source related to the flora and mineral variation. The bees use propolis to seal holes in the nest cavity and their honey comb, protect the nest against intruders (Burdock, 1998), embalm the carcasses of invaders which they have killed but cannot carry outside and build a smooth, clean coating over the nest cavity's walls (Seeley, 1985). Propolis also serves in colonial defense against fungi, bacteria viruses (Seeley and Seeley, 1982; Seeley, 1985; Popova *et al.*, 2005) and protozoa (Machado *et al.*, 2007).

In contrast to honeybees, by which resinous material is applied only to the nest periphery, stingless bees, excluding *Hypotrigona* (s. lat.), build nests not by pure wax but by cerumen, wax admixed with abundant resins (Sakagami, 1982). It is used in lining thin or virtually absent on the intact inner walls of cavity and closing all the opened cracks. Lining is especially thick near the entrance, where the surface is often sticky by constant addition of fresh resins. The stingless bees in the genera *Trigona*, *Plebeia*, *Tetragona* and others plaster the resins, gum and sometimes mixed with wax on the body of an invader. Moreover, stingless bees employed wax mixed with vegetable gum and significant component of resin as allowance that only few bees go in and out and entrance blockages to defense across predator (Maidl, 1934; Hermann and Blum, 1981). Bees also quickly prepared resinous network at the entrance tube to refuse further invasion (Sakagami *et al.*, 1983).

2.6 Chemical Activities and Compositions of Bee Material

The propolis from *A. mellifera* has been applied as a remedy by man since ancient time (Popova *et al.*, 2003). In the Balkan states, propolis was applied for treatment of wounds and burns, sorethroat, stomach ulcer and etc., as an antibacterial substances (Wollenweber *et al.*, 1990). Also numerous reports describe the antimicrobial properties of bee glue (Marcucci, 1995; Bankova *et al.*, 2000; Banskota *et al.*, 2001). Some resin collecting bee species from about 1000 species have been investigated (Pereira *et al.*, 2003). However, most research has concentrated on *A. mellifera* propolis. Mainly active components have been also identified which are phenolic acids, flavonoids and derivatives (Patricio *et al.*, 2002; Popova *et al.*, 2003; Pereira *et al.*, 2003; Teixeira *et al.*, 2005; Pisco *et al.*, 2006). The chemistry of propolis depends on the location of the host plant which bees collect propolis from (Teixeira *et al.*, 2005). Pereira and collaborators (2003) compared the chemistry of

propolis from European honeybees, *A. mellifera*, and stingless bees, *Tetragonisca angustula* and found that propolis from both bees are quite similar in chemical compositions and antimicrobial activity, except some amino acids and erythrose. Velikova *et al.* (2000) analyzed the resinous material from 12 different species of stingless bees by GC-MS and found that several chemicals in resins could be grouped to the prevailing types like “gallic acid”, “diterpenic” and “triterpenic”. The chemical activities and medical properties in resinous material from Brazilian stingless bee have been also investigated (Pereira *et al.*, 2003). Eisner *et al.* (1986) reported that the ant repellent activities of stingless bee propolis came from some monoterpenes.

By far the largest group of compounds in the resin/propolis is flavonoid pigments. The series of isolated flavonoids correlate with those of the plants from which the honeybees collect the propolis. It has also been suggested that some of the flavones are modified by an enzyme in the honey bees (Burdock, 1998). The simple aromatic compounds have also been reported in propolis (Ghisalberti, 1978; Burdock, 1998). Moreover, the hydroquinone (0.1%, Greenaway *et al.*, 1991; Burdock, 1998), caffeic acid and its esters (2-20%, Bankova *et al.*, 1995) and quercetin (<0.1-0.7%, Greenaway *et al.*, 1991; Burdock 1998) in propolis have also been detected. Shrestha *et al.* (2007) reported more two new dalbergiones and a new flavonone from methanolic extract from propolis collected from Chitwan, Nepal. The several papers reported over hundred compositions in resinous material from honey bees and stingless bees (table 2.1).

Although several publications have reported about the biological and medical properties of resinous material in bee nests of *A. mellifera*, the choice of nest resins from *A. florea*, *A. andreniformis* and some *Trigona* spp. and chemistry useful as repellents of natural enemies has not been studied.

Table 2.1 Compounds analyzed from resinous material collected from nests of honeybee, *A. mellifera* and stingless bee, *Frieseomelitta* spp., and of different regions.

Component	Literature source								
	a	b	c	d	e	f	g	h	i
Acacetin						+			
Acetic acid				+					
Alanine				+					
β -Amyrin				+					
α -Amyrin				+					
Apigenin (4',5,7-trihydroxyflavone)		+	+						
Benzoic acid									+
Benzyl cinnamate									+
Benzyl <i>p</i> -coumarate									+
Benzyl ferulate									+
α -Bergamotene				+					
Borneol				+					
Bornyl acetate				+					
Cadina-1,4-diene				+					
γ -Cadinene				+					
δ -Cadinene				+					
Caffeic acid (Phenolic acids)	+		+				+		+
Camphene				+					
3-Carene				+					
β -Caryophyllene				+					
Chrysin (5,7-dihydroxyflavone)	+	+	+			+		+	
Chrysin 7-methyl ether								+	
Cinnamic acid								+	+
Cynnamil caffeate									+
Cinnamyl cinnamate									+
α -Copaene				+					
<i>o</i> -Coumaric acid (Phenolic acids)			+						+
<i>p</i> -Coumaric acid (Phenolic acids)			+				+		
Coumaroyl glycerol									+
α -Cubebene				+					
β -Cubebene				+					
Chrysin									+
1,1-Dimethyl- allylcaffeic acid								+	+
2, 2 Dimethyl-6-carboxyethenyl-2H-1-benzopyran							+		
3,4-Dihydroxy-Cinnamic acid						+			

Table 2.1 Compounds analyzed from nests of honeybee, *A. mellifera* and stingless bee, *Frieseomelitta* spp., and of different regions (continued).

Component	Literature source								
	a	b	c	d	e	f	g	h	i
Palmitic acid				+					
2-Pentadecanone				+					
6-propenoic-2,2-dimethyl-8-prenyl-2H-1-benzopyranic acid							+		
Pentacosane				+					
α -Phellandrene				+					
Phenylethyl caffeate									+
Pilloyl(3',5-dihydroxy,4',7-dimethoxyflavone)		+							
Pinobanksin 3-acetate						+			+
Pinobanksin						+		+	+
Pinocembrin	+	+				+		+	+
Pinocembrin 7-methyl ethert								+	
Pinostrobin chalcone		+							
α -Pinene				+					
β -Pinene				+					
Quercetin (Flavones)			+			+			
Sabinene				+					
Sakuranetin						+			
Simularene				+					
Spathulenol				+					
Sugiol				+					
Tectochrysin (5-hydroxy,8-methoxyflavone)		+				+			
Terpinen-4-ol				+					
α -Terpinene				+					
γ -Terpinene				+					
α -Terpineol				+					
2-Tridecanone				+					
Totarol				+					
2-Undecanol				+					
Vanillin									+
Ylangene				+					
α -Ylangene				+					

a: Kosalec *et al.*, 2003, **b:** Maciejewicz, 2001, **c:** Medic-Saric *et al.*, 2004, **d:** Patricio *et al.*, 2002, **e:** Pereira *et al.*, 2003, **f:** Pietta *et al.*, 2002, **g:** Sawaya *et al.*, 2004, **h:** Markham *et al.*, 1996, **i:** Popova *et al.*, 2005.