CHAPTER 2

LITERATURE REVIEW

2.1 *Tropilaelaps clareae* Delfinado and Baker (Acari: Laelapidae)

T. clareae was first discovered and originally described by Delfinado and Baker in 1961 from a collection of dead *A. mellifera* and from field rats nesting near a beehive in the Philippines (Delfinado and Baker, 1961). Subsequently, the same mite was found in *A. dorsata* colonies in the Philippines (Laigo and Morse, 1968) and in India (Bharadwaj, 1968). *T. clareae* has since been found infesting different species of honeybees in Asia. Delfinado-Baker and Baker (1982) reported *T. clareae* infestation in brood combs of *A. cerana indica* in Pakistan and Burma. However, *T. clareae* was unable to complete its life cycle in sealed brood of *A. cerana* in Thailand (Wongsiri et al., 1989). In India, *T. clareae* was found associated with five species of honeybees: *A. dorsata, A. laboriosa, A. mellifera, A. florea* and *A. cerana* (Aggarwal, 1988).

2.1.1 Distributions and infestations of *T. clareae*

The geographical distribution of *T. clareae* seems to be limited in tropical Asia and coincides with the indigenous areas of *A. dorsata* (Table 2.1) (Crane, 1968; Burgett et al., 1983; Burgett and Akratanakul, 1985; Delfinado-Baker and Aggarwal, 1987; Delfinado-Baker et al., 1989). However, infestation of *T. clareae* in *A. mellifera* brood was observed in Afghanistan and South Korea, which are outside the range of *A. dorsata* (Figure 2.1) (Woyke, 1984; Woo and Lee, 2001).

Table 2.1 Distributions of *T. clareae* and the infested honey bees from countries in Asia.

| Countries	Bee species	Authors
Thailand	*A. mellifera*	Akratanakul (1979)
	A. dorsata	Burgett and Kitprasert (1989)
	A. cerana	Wongsiri et al. (1989)
Burma	*A. cerana*	Delfinado and Baker (1982)
	A. mellifera, A. dorsata	Nixon (1983)
Hong Kong	*A. mellifera*	Delfinado (1963)
India	*A. dorsata*	Bharadwaj (1968)
	A. mellifera	Stephen (1968)
Indonesia	*A. cerana*	Delfinado (1963)
Malaysia	*A. cerana*	Delfinado (1963)
	A. dorsata	Koeniger and Koeniger (1980)
Pakistan	*A. cerana*	Delfinado and Baker (1982)
Afghanistan	*A. mellifera*	Woyke (1984)
Vietnam	*A. mellifera*	Woyke (1985a)
South Korea	*A. mellifera*	Woo and Lee (2001)
Taiwan	*A. mellifera*	McDonald (1971)
Philippines	*A. mellifera*	Delfinado and Baker (1961)
	A. dorsata	Laigo and Morse (1968)
China	*A. mellifera*	Chen (1993)
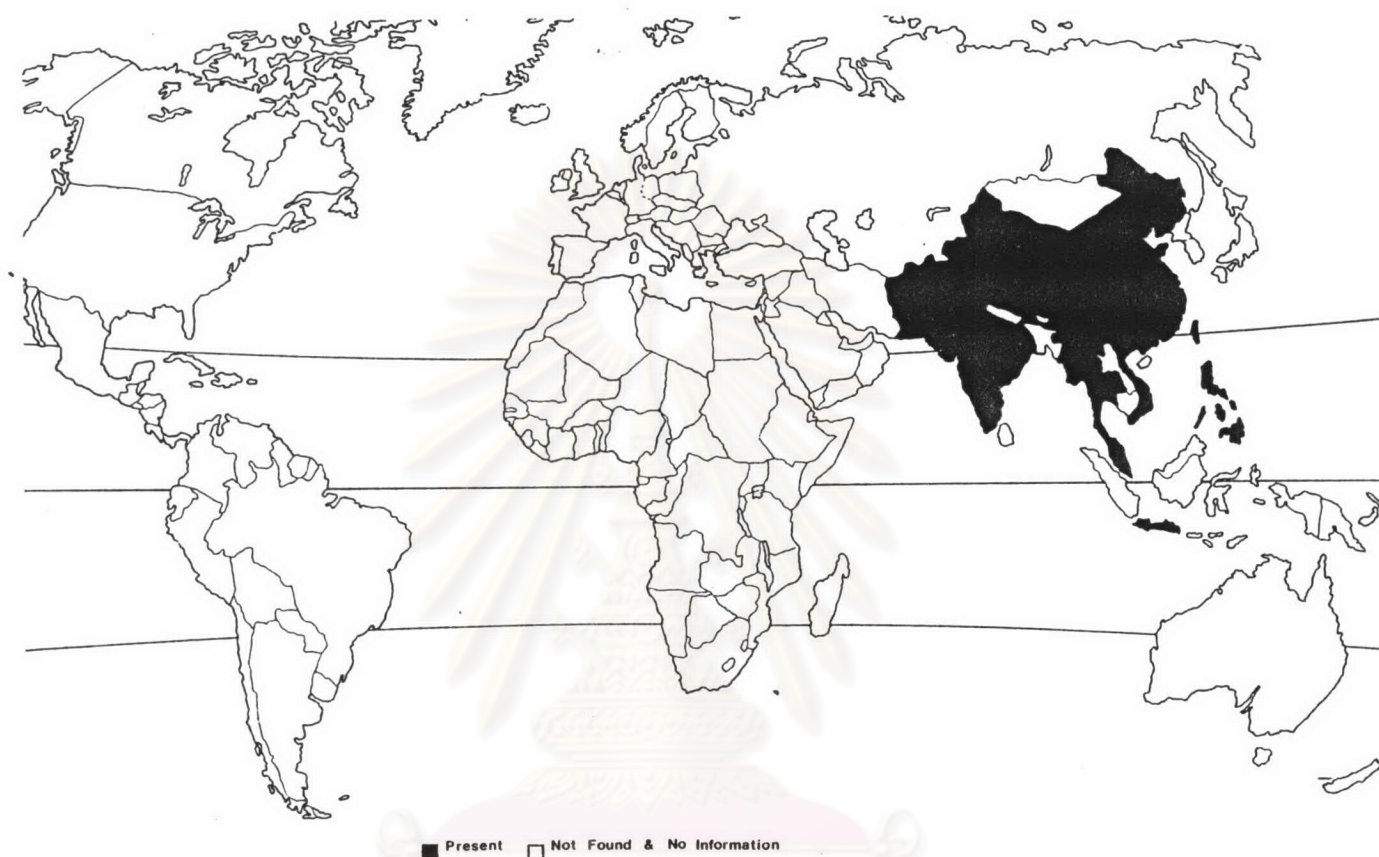
Figure 2.1 Worldwide distribution of *Tropilaelaps* (Nixon, 1983).

The levels of *T. clareae* infestation vary with honeybee species, brood gender and location of the colonies. In *A. dorsata* colonies, brood infestation of 5-30 mites/colony was observed in India (Bharadwaj, 1968). High infestations of *T. clareae* were observed from March to May in this area (Aggarwal and Kapil, 1986). In the Philippines, Laigo and Morse (1968) found mite infestations in seven out of eight *A. dorsata* nests examined. Brood infestations less than 10% were reported from Nepal (3 to 6%) and Thailand (0.2 to 9%) (Underwood, 1986; Burgett and Kitprasert, 1989). *T. clareae* infestation in *A. dorsata* worker brood (4.3%) was higher than that of drone brood (1.2 %) (Underwood, 1986; Burgett and Kitprasert, 1989). In Malaysia, Koeniger et al. (2002) reported that the

percentage of infested worker (20.0%) and drone (20.8%) brood cells did not differ, nor did the number of mites per cell (6 in worker brood and 6.1 in drone brood). On adult bees, Thapa (1998) found that the mite infestation rates on *A. dorsata* adults ranged from 3.10 to 11.12%. Infestation of *T. clareae* on adult workers of *A. mellifera* (0.6-3.4%) was less than in brood (16.6-48.0%) because *T. clareae* adults stay outside sealed brood cells only for short periods of time (Woyke, 1984).

2.1.2 Morphology of *T. clareae*

T. clareae is smaller than *Varroa* but can be seen without magnification. The mites can walk rapidly on the comb surfaces and are difficult to collect. On average, *T. clareae* adult males are 0.92 mm long and 0.49 mm wide while females are about 0.97 mm long and 0.49 mm wide (Woyke, 1987a). The color of adult female mites is light reddish-brown. Females are distinguished from males by their horseshoe-shaped anal plates. The anal plates of males appear to be pear-shaped (Delfinado-Baker and Baker, 1982). The female mite's entire body is covered with short setae. Through a strong magnifying glass, a red streak running logitudinally on the ventral surface of the adult female, the fusion of her epgynial and anal shields, can be seen (Figure 2.2) (Akratanakul, 1987).

T. *clareae* has been morphologically classified as follows; (Krantz, 1978).

Phylum	Arthropoda
Class	Arachnida
Subclass	Acari
Order	Parasitiformes
Suborder	Gamasida
Family	Laelapidae
Genus	*Tropilaelaps*
species	*clareae*

Figure 2.2 Adult females and males of *T. clareae* (Photo by Boonmee Kavinseksan).

(a) ventral view of *T. clareae* female   (b) dorsal view of *T. clareae* female

(c) ventral view of *T. clareae* male   (d) dorsal view of *T. clareae* male

2.1.3 Life history of *T. clareae*

The life cycle of *T. clareae* is similar to that of *V. destructor* (formally, *V. jacobsoni*) in *A. mellifera* brood cells (Atwal and Goyal, 1971; Burgett et al., 1983). However, a detailed life history of *T. clareae*, especially in the male mite has yet to be published. The life cycle of *T. clareae* is well synchronized with that of the host bee (Figure 2.3). In all its immature stages, the mites live within the brood cells of the bees, feeding on the brood's haemolymph. One or several female mites enter an open brood cell containing a late instar larva of *A. mellifera*. Both worker and drone brood serve as hosts. Oviposition frequently occurs on the cuticle of the host larva (Burgett and Krantz, 1984). The stages of development of the mite are as follows: egg, six-legged larva, protonymph, deutonymph, adult (Figure 2.4).

Studying worker cells of *A. mellifera* in Thailand, Ritter and Schneider-Ritter (1988) suggested that the mite laid eggs in the late L-5 stage (shortly after the sealing of the brood) or PP-stage (Figure 2.5) of bee brood. Woyke (1987b) found that eggs were not laid immediately after the cells were sealed because the first *T. clareae* eggs and larvae were detected in sealed cells containing spinning larvae (LS-stage), i.e., on brood 9 or 10 days of age (from egg laying). The highest percentage of bee brood with mite eggs occurred among cells containing prepupae (PP-stage or 11 and 12 days after egg-laying).

The first protonymphs of *T. clareae* were found on prepupae (PP-stage) (Woyke, 1987a). Ritter and Schneider-Ritter (1988) found 39% of protonymphs in the PP-stage. The first deutonymphs appeared in the Pw-stage (13 days after egg laying) (Woyke, 1987; Ritter and Schneider-Ritter, 1988). The first young adults were found in Pd-stage (16 days from egg-laying) (Figure 2.5) and the first adult males and females of the offspring were found in the Pr/Pd-stage (Ritter and Schneider-Ritter, 1988).

Woyke (1987a) reported the last stages of the mite development on honeybee pupae; the last eggs on Pp-stage (14 days from egg-laying) (Figure 2.5), the last larvae

on Pr-stage (15 days from egg-laying), the last protonymphs on Pd-stage and the last deutonymphs on Pdd-stage (19 days old). Only mite adults were present on brood older than 19 days. Ritter and Schneider-Ritter (1988) reported that 99% of nymphs developed to be adult mites before the emergence of the adult bees. The average developmental period from eggs to adult mites was 8.8 days under laboratory conditions (Kitprasert, 1984) while Woyke (1987a) reported a development period of 6 days in worker brood cells in bee colony conditions (Table 2.2).

Table 2.2 Duration (days) of developmental stages of *T. clareae* in *A. mellifera* colonies.

Places	Duration (days) of developmental stages				Total (days)	Authors
	Egg	Larva	Protonymph	Deutonymph		
Afghanistan*	0.35	0.78	2.34	2.53	6.00	Woyke (1987a)
Vietnam*	0.47	0.35	1.98	3.20	6.00	Woyke (1987a)
Thailand**	1.05	1.85	2.11	3.75	8.76	Kitprasert (1984)

* Bee colony conditions

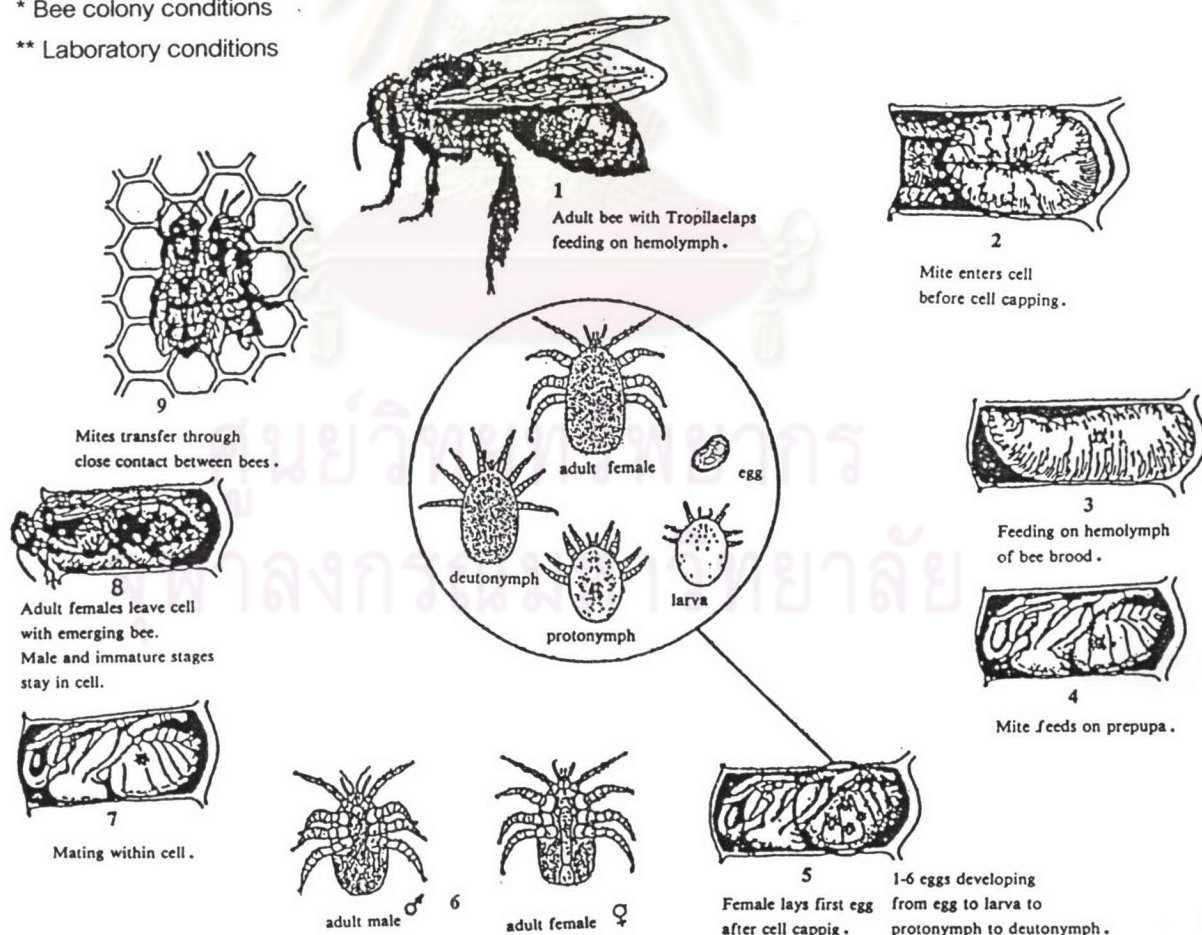** Laboratory conditions

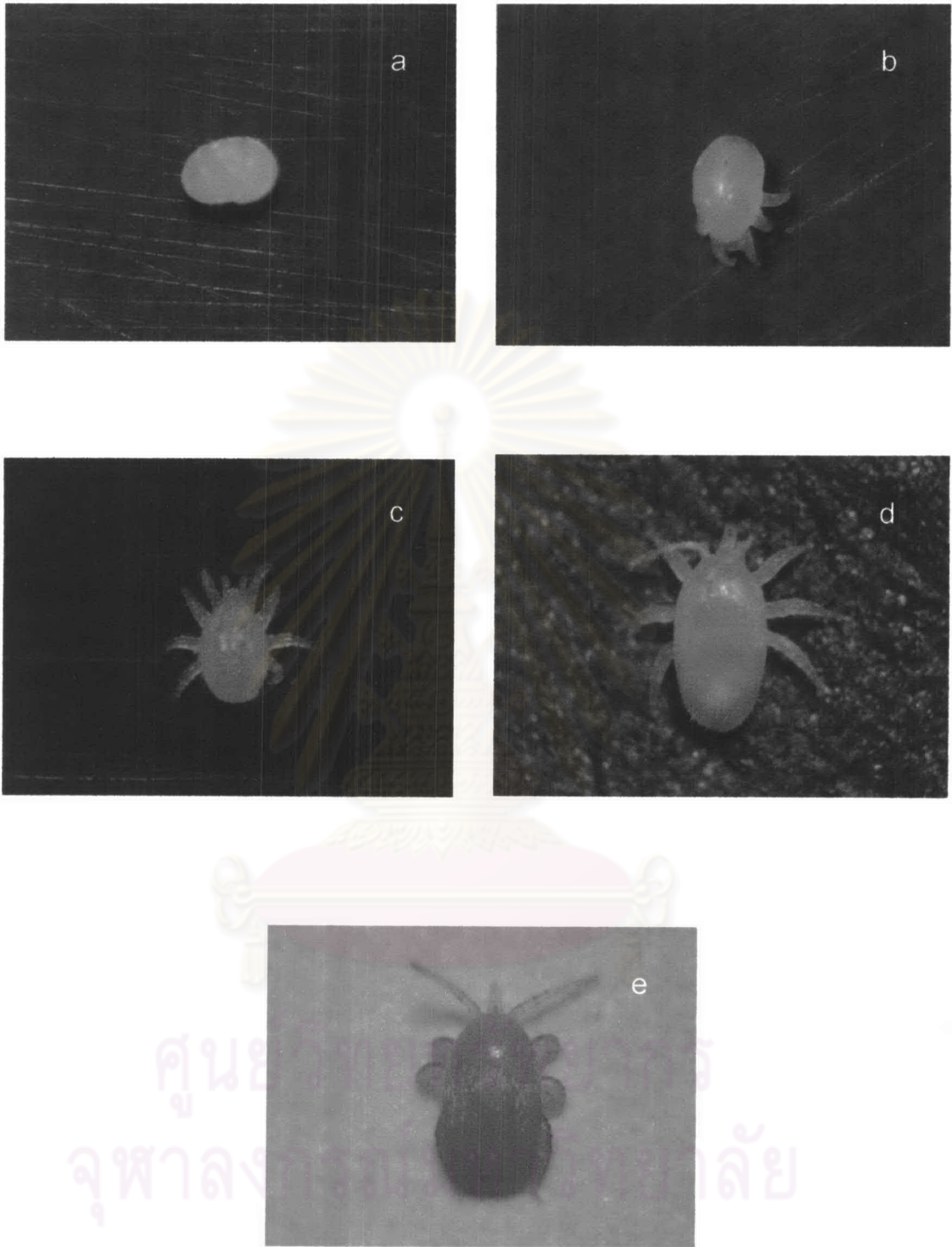Figure 2.3 Life cycle of *T. clareae* (Wongsiri et al., 1987).

Figure 2.4 Stages in the developmental of *T. clareae*

(a) egg   (b) larva   (c) protonymph   (d) deutonymph   (e) adult female

(Photo by Boonmee Kavinseksan).

T. clareae mates by podospermy both inside and outside of the brood cells. Multiple mating is observed in males whereas such a circumstance was not found in females (Rath et al., 1991). The first eggs are usually females, while the second are males (Rath et al.,1991; Ritter and Schneider-Ritter, 1988). Adult males of Tropilaelaps do not feed because their chelicerae (the organs originally used for piercing the bees' integument) have been modified for the sperm-transfer function (Akratanakul, 1987).

The maximum number of offspring produced by a single T. clareae female was found to be four (Woyke, 1987a). In Thailand, Ritter and Schneider-Ritter (1988) reported that 64% of the females of Tropilaelaps had produced 1, 33% had produced 2 and 3% had produced 3 offspring.

2.1.4 Sex ratios of T. clareae

The ratio of male to female T. clareae varies considerably. Under laboratory conditions, the ratio of male to female T. clareae was nearly 1:5 (Rath et al., 1991). While Ritter and Schneider-Ritter (1988) reported that the numbers of male and female eggs laid and male and female offspring appeared in relatively equal numbers in worker cells of A. mellifera in Thailand. In debris of A. mellifera colonies, a sex ratio of 1:4 (male: female) was reported by Woyke (1990) in Afghanistan. Rath et al. (1991) estimated a similar ratio (1:3) in hive debris of A. mellifera in Thailand. A ratio of 1:7.6 was observed in A. dorsata brood in Thailand (Burgett and Kitprasert, 1989). A higher estimate of 1:29 was reported in India in A. dorsata brood (Aggarwal and Kapil, 1986). Seasonal variations of sex ratio either in A. mellifera or A. dorsata have not been established.
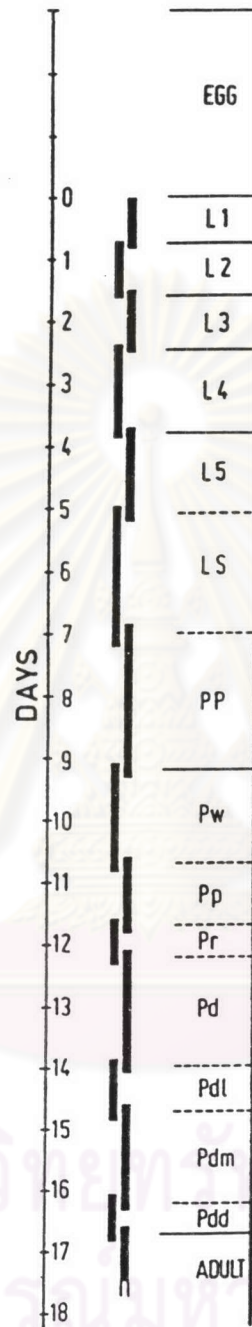
Figure 2.5 Duration of larval and pupal stages in *A. mellifera* workers.

L1-L4=1st-4th larval instar; L5=5th larval instar before sealing; LS=5th larva instar after sealing; PP=prepupa; P=pupa (w=white eyes; p=pink eyes; r=red eyes; d=dark brown eyes; dl=dark brown eyes, light pigmented thorax; dm=dark brown eyes, medium colored thorax; dd=dark brown eyes, dark thorax) (Adapted from Rembold et al., 1980).

2.1.5 Symptoms and Injuries

The damage caused to colonies by *T. clareae* infestation is similar to that brought by *Varroa,* and the injuries on individual bees and bee brood are essentially the same. The abdomen of *A. mellifera* adult bees surviving mite attacks is reduced in size. Surviving adult bees from mite infestation in pupal stages are often malformed and frequently have stubby wings and a shorter life-span than healthy bees (Akratanakul, 1987). Bees with deformed wings can be observed on the comb surfaces and at the vicinity of the hive entrance. Infested brood cells were opened by the worker bees. The pupa in the opened cells was either undamaged or eaten so that only the abdomen or parts of the pupa remained in the cells, and 94% of the opened cells showed a heavy infestation with *T. clareae.* Infested *A. mellifera* pupae show darkly colored spots mainly on their extremities (Ritter and Schneider-Ritter, 1988). Crane (1990) reported that *Tropilaelaps* can kill untreated colonies of *A. mellifera* within a few months.

2.2 *T. clareae* controls

The life cycle of *T. clareae* is believed to be similar to that of *V. jacobsoni.* Therefore, several methods for controlling the two species of mites are similar. However, some methods that were effective in decreasing *Varroa* populations were not effective in regulating *T. clareae* populations in *A. mellifera* colonies (Atwal and Goyal, 1971).

2.2.1 Chemical control

In *A. mellifera* colonies, *Tropilaelaps* is usually controlled by using chemicals since chemicals are simple to use, readily available and oftentimes highly effective (Table 2.3). On the other hand, some chemicals are expensive, can kill honey bees when used improperly and contaminate bee products. In Thailand, Mitac® and a mixture powder of sulphur and naphthalene (inexpensive) were found effective for *T. clareae* control (Wongsiri et al., 1987a; Ritter and Schneider-Ritter, 1988). However, resistance to Mitac® by *T. clareae* had already been detected in Thailand (Wongsiri et al., 1987a).

In India, Rajesh et al. (1984) controlled *T. clareae* successfully by continuous fumigation of 65% formic acid (5 cm^3 per day) for 3 weeks. Other chemicals such as Perizin® and Asuntol® are found not to be effective in controlling the mite (Wongsiri et al., 1987a).

The ineffectiveness of chemical treatment is due to the short phoretic period of adult mites and the restriction of reproductive mites in the brood cells. *T. clareae* adults stay outside sealed brood cells about 2-3 days. Thus, most mites have re-entered the cells before the next treatment is applied. Hence, the use of chemicals with prolonged continuous action is suggested to effectively control *T. clareae* (Woyke, 1987b).

Table 2.3 Chemicals and applications for controlling *T. clareae* in *A. mellifera* colonies.

Trade names	Active ingredients	Types of Products	Application methods	Authors
Fluvalinate	Fluvalinate	Plastic strip	Plastic strip	Lensky et al.(2001)
Apitol	Cymiazol	Aqueous solution	Trickled on	Ritter and Schneider-Ritter (1988)
Perizin	Coumaphos	Aqueous solution	Trickled on	Wongsiri et al.(1987a)
Folbex-VA	Bromopropylate	Fumigation strip Aqueous solution	Fumigation Spray	Akratanakul (1987)
Formic acid	Formic acid	Aqueous solution	Evaporation	Rajesh et al. (1984) Hoppe et al.(1989)
Sulphur with Naphthalene	Sulphur with naphthalene	Powder	Evaporation	Wongsiri et al.(1987a)
Asuntol	Organophosphate	Aqueous solution	Spray	Wongsiri et al.(1987a)
Mitac	Diphenyl compound	Aqueous solution	Spray	Wongsiri et al.(1987a)

2.2.2 Integrated control

The use of chemicals can be minimized by using them in combination with biotechnical methods (Ritter, 1993). In Thailand, Tangkanasing et al. (1988) reported that 95% of *Tropilaelaps* populations in *A. mellifera* colonies were decreased when coumaphos (Asuntol®) (0.8 gm/l of water) was applied twice in 3 days while the queens were caged for 9-12 days. In Burma, Neyin and Zmarlicki (1982) caged the queens for at least one complete brood cycle (21 days), uncapping the dead brood to facilitate removal by worker bees and the inclusion of a fumigation regimen (in this case phenothiazine). However, the population of *T. clareae* was not reduced to an economically satisfactory level and the methods were labor-intensive. For another technique, beekeepers prefer to combine chemical treatment with the brood-deprivation technique. In this approach, all sealed brood is removed from the infested colonies, which are then fumigated. The most adult mites, having no capped brood cells in which to hide, are killed by the fumigant. Only one chemical treatment is required in this technique instead of three or four (Akratanakul, 1987). However, an integrated control seems to be complicated, time-consuming, and bee products can still be contaminated by the chemicals (Ritter, 1993).

2.2.3 Non-chemical control

Since *T. clareae* survives on adult bees for about 2-3 days, Woyke (1984) developed a non-chemical method to control the mite by caging queens for 3 weeks until all brood emerges. Removal of all brood without caging the queen was also suggested to deprive the mites from their food (sealed and unsealed brood). In both conditions, the last mites died within 3 days after all the bees emerged from the brood combs. However, colonies will suffer due to a significant decline in bee populations when using these methods and queen cells must be removed from colonies on at least one and perhaps two occasions.

2.2.4 Natural control

A selection of strains of A. mellifera having resistance may be a solution to the Tropilaelaps problem in A. mellifera, which would be of great value to beekeepers throughout the world (Wongsiri et al., 1989). At present, no studies have been done to determine the potential resistance of A. mellifera to T. clareae. However, A. mellifera stocks resistant to V. destructor and A. woodi have been documented by several researchers. The following stocks are shown to have some degree of resistance to Varroa mites: hybrids of A. mellifera monticola in Kenya and A. mellifera ligustica in Sweden (Thrybom and Fries, 1991), A. mellifera carnica or native Austrian bee (Ruttner and Hanel, 1992), A. mellifera carnica from Yugoslavia (ARS-Y-C-1) (de Guzman et al., 1996) and A. mellifera of Far-Eastern Russia or ARS Primorsky honey bees (Rinderer et al., 1999, 2000, 2001). The Primorsky honey bees were also proven to be resistant to A. woodi (de Guzman et al., 2001) and are now commercially available for beekeepers (Danka et al., 1995; Rinderer et al., 1997, 1999, 2000).

2.3 Defense mechanisms of honey bees to T. clareae

The growth and development of mites in colonies of A. mellifera and A. dorsata depend on several factors. These factors include: non-reproduction (Harbo and Harris, 1999), colony migration (Wongsiri et al., 1989; Koeniger et al., 1993), grooming (Wongsiri et al., 1989; Rath and Delfinado, 1990; Koeniger et al., 2002) and hygienic behaviors (Spivak and Reuter, 1998; De Guzman et al., 2001) of the bee hosts. The influence of genotypes of honeybees on mite populations has not been investigated.

2.3.1 Non-reproduction of T. clareae

Studies on non-reproduction of T. clareae as a mechanism of resistance are very limited. Woyke (1990) reported about 18.3 % non-reproduction of T. clareae in lightly infested colonies of A. mellifera in Vietnam. A lower percentage of non-reproduction (7.3 %) was observed in highly infested colonies of A. mellifera in Afghanistan (Woyke, 1990).

In Thailand, *Tropilaelaps* mites did not reproduce at a rate of about 27%, and no cells were found in which the offspring could not have become adult up to the emerging of the adult bees (Ritter and Schneider-Ritter, 1988).

2.3.2 Migrations of honey bees

T. clareae is not considered to be a serious pest of *A. dorsata* (as compared to *A. mellifera*) because of the bees' migratory nature. Morse and Laigo (1969) believe that *A. dorsata* colonies abscond due to mite and/or moth infestations. When mite populations build up beyond the grooming capacity, colonies may start migrating in order to decrease the mite populations (Wongsiri et al., 1989). Koeniger and Koeniger (1993) found *Tropilaelaps* mites in capped cells, which contained dead or living pupae of deserted combs of *A. dorsata*. They found a rate of infestation of 63% and there were 25 to 28 mites per cell in several cells of a deserted comb in Malaysia. The bees of this colony had left more than 1,060 *T. clareae* behind when they absconded. However, Thapa (1998) contradicted this hypothesis and suggested that *T. clareae* may not have a significant impact on colony migrations of *A. dorsata*. Thapa based his claim on 7 mites found out of 22 cells examined from a deserted comb of *A. dorsata*.

2.3.3 Broodless periods of honey bees

T. clareae feeds mainly on brood and can survive on adult bees of *A. mellifera* no longer than 48 hours (Woyke, 1985b). *T. clareae* cannot survive more than 3 days without feeding on bee brood because their chelicerae (mouthparts) are not specialized for feeding on adult bees (Kitprasert,1984; Woyke, 1984; Koeniger and Muzaffar, 1988; Tangkanasing et al., 1988; Delfinado-Baker et al., 1992; Rinderer et al., 1994). Consequently, *T. clareae* is thought to be limited to the tropical zones because it cannot survive long broodless periods in *A. mellifera* colonies that happen during winter in the temperate zones (Woyke, 1985a). Broodless periods occur also in *A. dorsata* colonies. Laigo and Morse (1968) reported that *T. clareae* was not found in an *A. dorsata* colony that had swarmed several weeks earlier. Thapa (1998) found no *T. clareae* in brood cells

of new (1 to 2 month-old) *A. dorsata* colonies while old (6 to 7 month-old) colonies were infested by the mite. In Thailand, *T. clareae* was not found in 5 new colonies (swarms or colonies with the first generation of brood) of *A. dorsata*. While 5 established colonies (with more than the first generation of brood) had 5-16 mites in the sampled sealed brood, and 9 mites were found from adult bees in one of the 5 established colonies (Kavinseksan et al., 2003). These observations suggest that broodless periods during the swarming events of *A. dorsata* interrupted the mite's life cycle, and the mite populations gradually build up probably due to reinvasion of *T. clareae* from other *Apis* species or infested *A. dorsata* nests present in the area. *T. clareae* is known to be phoretic on adult bees of *A. dorsata*, *A. laboriosa*, *A. mellifera*, *A. florea* and *A. cerana* (Aggarwal, 1988). Thus foraging of different bee species in the same flower or robbing must be considered as a source of *T. clareae* infestation for uninfested colonies.

2.3.4 Grooming behavior of honey bees

A. dorsata effectively groom mites from their bodies (Koeniger and Koeniger, 1980; Wongsiri et al.,1989). Buchler et al. (1992) reported that *A. dorsata* has autogrooming behavior in response to infestation by *T. clareae*. This bee species not only removes the mites from the bee's body, but also kills them (Wongsiri et al., 1989; Rath and Delfinado-Baker, 1990). The high numbers of injured mites from debris of *A. dorsata* colonies were reported by Rath and Delfinado-Baker (1990) in Thailand (73-76%) and Koeniger et al. (2002) in Malaysia (84%). *A. dorsata* removed *T. clareae* from their bodies in a few seconds to several minutes, but the cleaning behavior was not as frequent as that observed in *A. cerana* colonies (Wongsiri et al., 1989). From cage tests, Koeniger and Koeniger (1993) found that *A. dorsata* workers apparently were able to injure *T. clareae* and many mites were mutilated. *A. dorsata* actively grooms and hunts for mites. The mites found under the cages of *A. mellifera* were not mutilated. In *A. mellifera* colonies, only a small percentage (8.5%) of damaged *T. clareae* was recorded (Wongsiri et al., 1989). *T. clerae* prefers adult drones over worker bees in *A. mellifera* (Ritter and Schneider-Ritter, 1988). Thus, the mites evade bees' grooming activities by remaining on the drones, which do not perform social grooming (Rath, 1991). The

grooming behavior of different stocks of *A. mellifera* towards *T. clareae* has not been studied.

2.3.5 Hygienic behavior of honey bees

Hygienic behavior is a defense mechanism of honey bees against mites (Peng et al., 1987; Boecking and Drescher, 1991). This behavior is an important mechanism for mite resistance in *A. cerana*. Workers of *A. cerana* were able to detect and remove *T. clareae* encountered in brood cells during nursing activities. The bees removed mites from the hives or chewed them with their mandibles (Wongsiri et al., 1989). *A. dorsata* workers can also detect *T. clareae* in sealed brood cells and remove the infested brood with the mite from the cells. This behavior would limit the growth of mites in the colonies (Koeniger and Muzaffar, 1988; Rath and Delfinado-Baker, 1990). Although it is not the main mechanism of resistance against parasitic mites in *A. mellifera*, it can limit the reproduction and population growth of mites to some degree (Spivak and Reuter, 1998). The cells infested with *T. clareae* (especially in the pupal stages) are recognized by the worker bees of *A. mellifera*. They attempt to remove the parasitized brood and the mites. In Thailand, a large number of infested cells with *T. clareae* in heavily infested colonies was opened by the worker bees (Ritter and Schneider-Ritter, 1988). The hygienic trait was also observed in the Primorsky honey bees, which are known for their resistance to both *A. woodi* and *V. destructor*. Whether or not Primorsky honey bees can detect and remove *T. clareae* or *V. destructor*-infested brood has yet to be established.

2.4 *A. dorsata* Fabricius, 1793 and ARS Primorsky honey bee (*A. mellifera* L.)

2.4.1 Taxonomy of *A. dorsata* and *A. mellifera*

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Hymenoptera
Family	Apidae
Genus	*Apis*
species	*dorsata*
	mellifera

2.4.2 The giant honey bee (*A. dorsata*)

The giant honey bee or *A. dorsata* is the largest bee species in terms of nest and body size in its genus (Ruttner, 1988). The average number of *A. dorsata* worker bees in a colony ranges from 5,000-70,000 individuals (Seeley et al., 1982; Moritz et al., 1995; Wongsiri et al., 1996). This bee species is a native honey bee species of South-East Asia (Wongsiri et al., 1989). *A. dorsata* lives in the open, frequently in exposed positions, in single comb nests, hanging on cliff, tree branches or eaves of buildings (Figure 2.6).

The shape of the comb is more or less semicircular or cuneiform. The honey is stored in the uppermost corner of the comb, close to the attachment (Morse and Laigo, 1969). A typical nest of *A. dorsata* holds 1.8-4.0 Kg of honey (Kallapur, 1950; Morse and Laigo, 1969). In Thailand, *A. dorsata* colonies produce no more than 2-17 Kg of honey during the main honey flow season of *Dimocarpus longan* (Sapindaceae) (Wongsiri et al., 1998).

There are no specific drone size cells, but a uniform cell type is constructed for brood rearing. Drones are reared in the same cells as workers, but the capping are slightly elevated (Morse and Laigo, 1969; Thapa, 1988). The uniform diameter of the cells is 5.35-5.64 mm. The depth of brood cells is 16 mm. (Muttoo, 1956; Thakar and Tonapi, 1961). *A. dorsata* constructs queen cells like other *Apis* species at the lower edge of the comb 4-6 cells per colony (Thakar and Tonapi, 1961; Morse and Laigo, 1969).

The development times of the individual castes of *A. dorsata* is shorter than *A. mellifera*. Development periods of queen, worker and drone of *A. dorsata* are 13-13.5, 16-20 and 20-23.5 days, respectively (Qayyum and Nabi, 1968). However, the life span of individual bees of *A. dorsata* after emerging has not been observed.

The temperature of the *dorsata* brood comb fluctuated between 30 and 32 $^{\circ}$C, while the ambient temperature varied from 20 to 28 $^{\circ}$C. The protective curtain is certainly essential for maintaining a fairly constant brood temperature (Morse and Laigo, 1969).

A. dorsata is very aggressive and the most ferocious stinging insect in the world. Usually 10-5,000 bees attack nest intruders within a few seconds after being disturbed. During nest defense, worker bees spray the alarm pheromone "iso-pentyl acetate", but no 2-heptanone (Morse et al., 1969) and they can pursue intruders for long distances (Lindauer, 1961).

(a)

(b)

Figure 2.6 Colonies of *A. dorsata* (a) hanging on a tree branch

(b) hanging on eaves of a building (Photo by Boonmee Kavinseksan).

The maximum foraging range of *A. dorsata* is around 10 km (Seeley et al., 1982). *A. dorsata* is unique among *Apis* in that it can also forage and dance in the moonlight (Diwan and Salvi, 1965: Dyer, 1985). They were observed flying between 18:00-19:00 hours. The workers were collected in the light traps with pollen on their pollen baskets between 20:00-21:00 hours in durian orchards in Chanthaburi (Thailand).

The distribution of *A. dorsata* covers almost all of far-western Asia and the Indo-Malayan region including India, Borneo, Cambodia, Laos, Nepal, Palawan, Vietnam and Thailand. To the east of the wallace line, *A. dorsata* is found in all of the Philippine islands and as far as the Kei islands east of Timor. To the further west, *A. dorsata* is extended to the Indus river and almost to the Xerotherm coasts of the Persian Gulf (Figure 2.7) (Maa, 1953; Sakagami et al., 1980). Colonies of *A. dorsata* usually occur at altitudes of up to 1500 m, but may occur seasonally at up to 2000 m in different regions (Muttoo, 1956; Gautam, 1984). In Thailand, *A. dorsata* is found from 10-1600 m from the sea level (Wongsiri et al., 1996).

A. dorsata migrates seasonally over long distances and stops brood rearing while preparing for migrations (Ruttner, 1988). The migration distance in Sri Lanka is 150-200 Km. Koeniger and Koeniger (1980) reported that *A. dorsata* swarms fly in several stages and often rest between stages of the flight. Swarms rested 1 to 3 days without building any comb but nectar gathering took place and spent more than one month on their migration. When an *A. dorsata* swarm starts a nest, comb construction is rather rapid. During one night a swarm constructed a good portion of comb (Lindauer; 1956; Koeniger and Koeniger, 1980). Thapa (1998) reported that when the ambient temperature dropped below 16 $^\circ$C, *A. dorsata* in the north of Thailand started to migrate due to their inability to maintain their optimum brood nest temperature. Wind speed (>29 km/h) also induced colony migration by dislodging their nests. Similarly predators such as human and birds (bee-eaters, *Pernis ptilorhyncus* and *Merops orientalis*) caused colony migration (Thapa and Wongsiri, 2003) whereas parasite pressure did not.

T. clareae do not appear to be a significant impact on colony migration of *A. dorsata* because the mite infestation rate was low (Thapa, 1998). However, when the mite population increases beyond the grooming capacity, then colonies might migrate in order to minimize the mite populations by abandoning their nests leaving the mites behind (Wongsiri et al., 1989).

Figure 2.7 Distributions of *A. dorsata* in Asia (Adapted from Ruttner, 1980).

2.4.3 ARS Primorsky honeybee (*A. mellifera* L.)

A. mellifera or the European honeybee make multi-comb nests in cavities. This honeybee species produces valuable bee products and sustains commercial beekeeping in Thailand and other parts of the world (Ruttner, 1988; Wongsiri et al., 1987b). *A. mellifera* is generally believed to have evolved in Africa (Wilson, 1971). This bee species has been introduced throughout the world over the past 350 years.

Modern beekeeping in Thailand started in the early 1940s. European honeybees in movable-frame hives were introduced at Chulalongkorn university, but they did not survive. The second introduction of *A. mellifera ligustica* was made by Saman Watanakit in 1953, at Kasetsart university, and subsequent introductions did not succeed commercially until the early 1970s (Wongsiri and Chen, 1995). The two ectoparasitic mites of honey bees, *Varroa destructor* and *T. clareae*, are considered to be perhaps the most important limiting factors to the development and expansion of *A. mellifera* beekeeping in Thailand and tropical Asia (Morse and Laigo, 1969; De Jong et al., 1982; Neyin and Zmarlicki, 1982).

In Thailand, there was a boom in *A. mellifera* beekeeping after 1980, due to low honey production from native bees and the high price of Thai honey in local markets. Since then, the beekeeping industry with *A. mellifera* has been expanding very rapidly, reaching at present almost 500,000 colonies in the country. *A. mellifera* is preferred by large commercial beekeepers, mainly for producing honey and royal jelly, and it is also used for pollination of longan flowers (*Euphoria longan*) in the northern part of Thailand (Wongsiri and Chen, 1995).

Primorsky honey bees were moved by settlers from European Russia to the Primorsky territory in Asian Far-Eastern Russia in the mid 1800's (Crane, 1978). The bees are, for the most part, dark bees (Figure 2.8) that is some regard are similar to Carniolian honey bees (Rinderer et al., 2000). They can coexist with *V. jacobsoni*, which is the natural parasite of *A. cerana* in this region, and they have developed genetic resistance

natural parasite of *A. cerana* in this region, and they have developed genetic resistance to the mite (Danka et al., 1995). In 1997, Primorsky queens were brought to the USA in order to make Primorsky colonies (Rinderer et al., 1997). Then these colonies were tested to *V. jacobsoni* resistance. The result showed that they did have resistance to the mite. After that, some queens of these colonies were chosen for breeding program. Some of the daughter colonies were compared for *Varroa* resistance and honey production with domestic colonies, which were considered an excellent honey producer by many beekeepers in the USA. It was found that the Primorsky and domestic (an *A. mellifera* stock in the USA) colonies had very similar average honey production, but the Primorsky colonies had lower rates of *Varroa* infestation in worker brood than that of the domestic colonies (Rinderer et al., 1999). Nowadays, the Primorsky bees are used in industrial beekeeping by many beekeepers in the USA. (Rinderer et al., 2000).

Figure 2.8 **ARS** Primorsky honey bees (Photo by Boonmee Kavinseksan).