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Title: Parasitism by the Brood Mite, *Euvarroa sinhai* Delfinado and Baker (Acari: Varroidae) on the Dwarf Honey Bee, *Apis florea* F. (Hymenoptera: Apidae) in Thailand

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The relationship of the parasitic brood mite, *Euvarroa sinhai* Delfinado and Baker to the dwarf honey bee, *Apis florea* F. was investigated in northern Thailand. The population density of this mite on adult bees from various stages of *A. florea* colony development were determined. The mite density from queenright colonies following swarming, was higher than colonies examined pre-swarming and other stages of colony development. Swarming tends to effect the density of *Euvarroa* mites within a colony by leaving most of the mites in the mother colonies, thus reducing mite load in the swarm. This behavior is one of the effective defense mechanisms of *A. florea* against parasites.

Normally, prevalence infestation rate of *Euvarroa* mites in drone brood from queenright *A. florea* colonies was low and varied among colonies. The distribution of this mite on drone brood is aggregated. A truncated negative binomial distribution suggests a mite-induced mortality in older pupal drone brood (5-7 days old after cell capping). There is a possibility that the worker bees have the ability to remove pupae with high mite loads from the cells because no dead drone pupae (by mite infestation) were observed directly within the capped drone cells. This evidence was supported by the observation of *Euvarroa* mite populations in the debris from *A. florea* colonies. A positive correlation was found between the number of dead drone pupae and female

mites in the debris. Most of the dead drone pupae were purple-eyed and older stages of development. Males and immature stages of the mites were also found in the debris.

Euvarroa mites associate not only with drone brood but also with adult bees of *A. florea*. It appears that the mites have the ability to feed on adult *A. florea* worker bees and survive longer than on adult workers of *A. cerana* and *A. mellifera*. This mite also displayed a phoretic preference to adult drones over adult workers.

The reproduction of *Euvarroa* mites on its natural host was also determined from the infested drone brood of *A. florea* colonies. The average number of *Euvarroa* mite offspring from a single parent invasive female mite was 3.3 mites per host and the maximum number of progeny was seven.

In queenless *A. florea* colonies, the population density of *Euvarroa* mites on adult bees and prevalence infestation rate in drone brood were higher than in queenright colonies. Mite distribution on drone brood also fits a negative binomial and a truncation of the negative binomial indicates mite-induced mortality in drone brood pupae. Dead drone pupae were observed in brood cells of queenless colonies suggested that the worker bees in queenless colonies have a reduced capacity to remove infested drone pupae.

Parasitism by the Brood Mite, *Euvarroa sinhai* Delfinado and Baker (Acari: Varroidae) on the Dwarf Honey Bee, *Apis florea* F. (Hymenoptera: Apidae) in Thailand

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CHAPTER I
INTRODUCTION

Six species of mites are reported as brood parasites of Asian honey bee species: *Varroa jacobsoni* Oudemans, *V. underwoodi* n. sp., *Euvarroa sinhai* Delfinado and Baker, *E. wongsirii* n. sp., *Tropilaelaps clareae* Delfinado and Baker and *T. koenigerum* n. sp. *Varroa jacobsoni* and *V. underwoodi* originally were associated with the Eastern honey bee, *Apis cerana* F. (De Jong *et al.* 1982; Delfinado-Baker and Aggarwal 1987). *Euvarroa sinhai* was found associated with the dwarf honey bee, *A. florea* F. (Delfinado and Baker 1974; Akratanakul and Burgett 1976), while *E. wongsirii*, a new species, was recently found associated with *A. andreniformis* Smith in Thailand (Lekprayoon and Tangkanasing 1991). The original host of both species of *Tropilaelaps* is the giant honey bee, *A. dorsata* F. (Delfinado-Baker and Baker 1982; Burgett *et al.* 1983). Reproduction of these parasitic mites occurs inside the brood cells of the honey bee host colony. Nymphal and adult female mites feed on the haemolymph of bee larvae and pupae. No pathological consequences have been reported from these parasitic mites on their native hosts.

Varroa jacobsoni, *E. sinhai* and *T. clareae* became associated with the European honey bee, *A. mellifera* L., after it was introduced to Asia around 1950 (Crane 1979). *Varroa jacobsoni* and *T. clareae* have caused severe damage to European honey bees (Laigo and Morse 1969; Burgett and Krantz 1984; Burgett and Akratanakul 1985). However, on the Asian honey bees, these mites have little effect on colony productivity (Koeniger *et al.* 1983; Underwood 1986), probably mechanisms exist that control the balance between the bee hosts and their mite parasites.

Peng *et al.* (1987) reported that *A. cerana* has a high success rate of phoretic mite removal from adult bees by auto- and allogrooming. Additionally, mite infested

brood are preferentially removed from the brood nest. These behaviors are important components in the resistance mechanisms that maintain a low level of mite infestation in *A. cerana* colonies.

Other factors that might affect the population dynamics of *V. jacobsoni* in *A. cerana* colonies include swarming and migrating behaviors, which help colonies to leave diseased brood and parasites in the combs of abandoned colonies (Woyke 1976). Royce *et al.* (1991) proposed that the swarming of *A. mellifera* colonies can reduced the density of the endoparasitic mite, *Acarapis woodi* (Rennie), within a colony. The authors believed that this behavior interrupts the population of young worker bees, which are the preferred hosts for *Acarapis* infestation. However, the role of swarming, absconding, migrating and drifting of Asian honey bees on parasitic mite dispersal and population dynamics needs to be studied further. Studies of the relationships between the natural hosts and their parasites will be important in understanding the unbalanced host-parasite relationships between *A. mellifera,* an alternative nonadapted host, and parasitic Asian honey bee brood mites.

Review of Literature

Asian honey bees and their parasitic brood mites

Apis cerana - Varroa jacobsoni

Varroa jacobsoni was first described in 1904 as an ectoparasite of the Indian honey bee, *A. cerana* from Sumatra (Delfinado 1963). After the European honey bee, *A. mellifera*, was introduced into many countries of Asia in 1950 (Crane 1979), *V. jacobsoni* readily adapted to this new host (De Jong *et al.* 1982). In the early 1960s, *Varroa* was discovered in the Philippines and recognized as a parasite of *A. mellifera* (Delfinado 1963; De Jong *et al.* 1982). Presently, *V. jacobsoni* has spread rapidly throughout most of the beekeeping regions of the world except for Australia and New Zealand (Matheson 1993).

Female mites of *V. jacobsoni* reproduce in worker and drone brood cells by entering the brood cell of 5 day-old larva before the cell is capped and lay eggs in the brood cell. The mites complete their development inside the sealed brood cell (De Jong *et al.* 1982). The development period from egg to adult is 7.5 days for females and 5.5 days for male mites (Ifantidis 1983). Mated female mites leave the cell with the emerging bee while males and incompletely developed offspring die in the cell (De Jong *et al.* 1982). Adult female mites can live and feed on haemolymph of adult worker and drone bees. They are phoretic on adult bees through the long winter broodless period in the northern temperate zone. The life span of adult female mites on adult bees is two months in summer and about five months in winter (Shabanov *et al.* 1978). However, the mites cannot reproduce without feeding on larval haemolymph (Hänel 1983).

Koeniger *et al.* (1983) studied the relationship between the parasitic mite, *V. jacobsoni* and its natural host, *A. cerana* in Sri Lanka. They reported that

V. jacobsoni prefers drone brood over worker brood and reproduces only in drone brood of *A. cerana*. In contrast, with *A. mellifera*, the mites reproduce in both worker and drone brood. This partitioning of brood by gender keeps a *Varroa* infestation level in *A. cerana* lower than in *A. mellifera* and maintains the balance of *Varroa* mite and its natural host, *A. cerana* (Koeniger *et al.* 1981, 1983). However, De Jong (1988) reported that *V. jacobsoni* can reproduce on worker brood of *A. cerana* in South Korea but there is still a preference for drone brood. The natural reproduction of *V. jacobsoni* in *A. cerana* colonies was found to be restricted to drone brood and to the springtime (Tewarson *et al.* 1992). They also observed the potential for reproduction in worker brood by artificially induced infections.

Peng *et al.* (1987) were the first to investigate the resistance mechanisms of *A. cerana* to *V. jacobsoni*. They reported that *A. cerana* worker bees perform cleaning behaviors that effectively remove the mites from the adult bees (allogrooming) and infested brood cells. This behavior occurs with *A. mellifera* infested with *Varroa* but at frequency so low that it is not successful in removing enough mites from both the adult bees and the brood to seriously impact the mite population. Rath and Drescher (1990) also described a differential hygienic behavior of *A. cerana* workers that removes more mites from infested worker than from drone brood.

Apis dorsata* - *Tropilaelaps clareae

Tropilaelaps clareae was first reported as a brood parasite of the European honey bee, *A. mellifera* in the Philippines (Delfinado and Baker 1961). It was later reported as a parasite of the giant honey bee, *A. dorsata* in India (Bharadwaj 1968) and the Philippines (Laigo and Morse 1968). *Apis dorsata* is considered the native host for *T. clareae* (Burgett and Krantz 1984). When *A. mellifera* was introduced to Southeast Asia around 1950 (Crane 1979), *T. clareae* became a serious problem for *A. mellifera* beekeeping in this region (Burgett *et al.* 1983; Burgett and Krantz 1984). Woyke

(1985b) reported *T. clareae* on *A. mellifera* in Afghanistan, which is outside the range of *A. dorsata*, however its occurrence is most probably anthropogenic in origin. Underwood (1986) found *T. clareae* from colonies of *A. laboriosa* in Nepal. A recent report from Aggarwal (1988) indicated that *T. clareae* is found associated with five honey bee species: *A. mellifera, A. dorsata, A. cerana, A. florea* and *A. laboriosa.*

The life cycle of *T. clareae* is similar to that of *V. jacobsoni* (De Jong *et al.* 1982). The average developmental period of the mites was 8.8 days under laboratory conditions (Kitprasert 1984). Ritter and Schneider-Ritter (1988) reported a development period of 9 days and suggested that the mites lay eggs before the cell is sealed. However, Woyke (1987c) reported a development period of 6 days. A sex ratio of *T. clareae* on *A. mellifera* brood of 4:1 (females/males) was reported by Woyke (1987d). This is similar to the observation of Underwood (1986) on *A. dorsata.* Burgett and Kitprasert (1989) reported a female biased sex ratio of 7.6:1 (females/males) from *A. dorsata* brood from Thailand.

The observation on the incidence of *T. clareae* from *A. dorsata* colonies in India showed that male mites appeared in combs in October, November, January and March to April (Aggarwal and Kapil 1986). Male and immature stages of the mite were found in *dorsata* brood in March, April and May, indicating that the mites reproduce during this period. A sex ratio of 29:1 (females/males) was observed from brood samples. There are some reports suggesting no preference of *T. clareae* for drone over worker brood. Woyke (1987d) showed the ratio of mite infestation from *A. mellifera* colonies for drone brood compared with worker brood, was 1:0.7. Underwood (1986) reported *T. clareae* infestation on *A. dorsata* drone brood of 1.2%, which was less than worker brood (4.3%), and is similar to the observations of Burgett and Kitprasert (1989).

Most of the papers reporting on the infestation of *T. clareae* have concentrated on *A. mellifera* (a nonadapted host to the parasite). Atwal and Goyal (1971) reported brood infestation in *A. mellifera* colonies of up to 50%. Burgett *et al.* (1983) reported

10-90% of *A. mellifera* worker brood parasitized by *T. clareae*. Woyke (1985a, 1987a) showed that the infestation rate of adult bees by *T. clareae* was very low, although the brood infestation was high. The consequence of this evidence is that *T. clareae* stays outside sealed brood cells for only a short time. According to Kitprasert (1984), the longevity of *T. clareae*, phoretic on *A. mellifera* worker bees under laboratory conditions, was 2-3 days and the mite's longevity was only 1-2 days when there was no provision of adult bees. Woyke (1987b) reported that most *T. clareae* mites did not remain away from host brood longer than 2 days before entering a new brood cell. The life span of *T. clareae* on other species of honey bee was studied by Koeniger and Muzaffar (1988). They reported that *T. clareae* survived in cages with adult worker bees of *A. mellifera* up to 25 hrs.; with *A. cerana* up to 27 hrs., and with *A. dorsata*, 57 hrs.

The rate of mite infestation in the brood of *A. dorsata* is lower than *A. mellifera*. Underwood (1986) reported the brood infestation rates of mites from three *A. dorsata* colonies in Nepal, providing a range from 3 to 6%. Burgett and Kitprasert (1989) observed the parasitism of the mite on *A. dorsata* colonies in Thailand and reported infestation rates ranging from 0.2 to 8.8%. In one longer term study of *A. dorsata*, Aggarwal and Kapil (1986) reported on the seasonal population dynamics of *T. clareae* in *A. dorsata* colonies over 38 months and showed the population levels of mites were high from March to May.

Burgett *et al.* (1990) observed that the distribution of *T. clareae* in *A. dorsata* brood is aggregated and proposed a model of a control mechanism. It appears that *A. dorsata* has a specific behavior to remove larvae infested with *T. clareae* from the brood cells. The defense mechanism of the giant honey bee against the parasitic mite, *T. clareae* was also observed by Rath and Delfinado-Baker (1990). The high numbers of injured *T. clareae* adults found in the debris of *A. dorsata* suggests a defense behavior of *A. dorsata* against their parasite similar to that of *A. cerana* e.g., allogrooming by adult

worker bees. Büchler *et al.* (1992) reported that *A. dorsata* has autogrooming behavior in response to infestations by *T. clareae.*

Apis florea* - *Euvarroa sinhai

Biology of *Apis florea* - the natural host

Apis florea, the dwarf honey bee, is distributed from Oman and Iran through the Indian subcontinent to the east of Indonesia (Free 1981). The primary habitat of *A. florea* is the Indian subcontinent, Southeast Asia as far west as Palawan in the Philippines in altitudes up to 500 m. (Ruttner 1987). In many parts of its distribution, *A. florea* is sympatric with *A. cerana* and *A. dorsata* (Free 1981).

The nest of *A. florea* consists of a single comb, varying in size from 25 to 35 cm. in width, 15 to 27 cm. in length and 16 to 19.8 mm. in depth (Free 1981). The comb is attached to a thin branch of a small tree or a bush, generally 3 to 5 m. above ground. Because of the narrow support on the branch, the comb structure of *A. florea* differs from the other species of *Apis*. Bees start to construct a comb with the hexagonal cell pattern and place the cells around the branch (Ruttner 1987). *Apis florea* has an open-nest which is protected by a curtain of worker bees (3-6 layers) over the comb (Seeley *et al.* 1982). The upper part of the comb is used for storing honey and pollen, the lower part is primarily for brood production. When the colonies are mature, drone and queen cells are built along the lower edge of the comb. The size of brood cells are 2.7 to 3.1 mm. diameter and 6.9 to 8.2 mm. deep for worker cells; 4.2 to 4.8 mm. in diameter and 8.9 to 12.0 mm. deep for drone cells and 13.5 to 14.0 mm. long , 8.5 to 10.0 mm. wide for queen cells (Free 1981). The immature developmental period of *A. florea* brood is similar to that of *A. mellifera*: 20.6 days for workers and 22.5 days for drones (Ruttner 1987).

Brood rearing in *A. florea* colonies starts to increase when the food sources (nectar and pollen) and environmental temperatures are suitable. Normally, most brood is reared in March, April and May (Free 1981). Drone cell construction begins in the swarming season (March to June) when colony population is near their peak (Akratanakul 1977). The colony starts queen rearing when a mature stage of colony is achieved. The number of queen cells is from 12-16 cells. About half of the bee population leaves the colony with the first swarm. Secondary and tertiary swarms become smaller until an only little group of bees is left on the parent comb (Ruttner1987). In queenless or laying worker colonies, brood cells are irregular (Akratanakul 1977). Almost all of the worker cells are enlarged to accommodate the drone brood and are irregular in shape. Workers lay several eggs in those cells but most eggs are destroyed before they hatch (Free 1981).

Colony absconding may occur at any time of the year. Therefore, the residence of an *A. florea* colony on any given site is limited (Ruttner 1987). Seeley *et al.* (1982) reported that the mean residence time of *A. florea* colonies in Thailand was 2 months in the dry season (November-February) and 5 months in the wet season (April-September).

Biology of *Euvarroa sinhai* - the parasite

Euvarroa sinhai was first reported on the dwarf honey bee in India and placed in the family Varroidae (Delfinado and Baker 1974). This mite was later indentified as an ectoparasite on drone brood of *A. florea* (Akratanakul and Burgett 1976). It has also been reported on *A. florea* in Sri Lanka (Koeniger *et al.* 1983) and Iran (Mossadegh and Birjandi 1986).

Euvarroa sinhai is a large setaceous mite (length of idiosoma 1040 μm., width 1000 μm.), brown and broadly pear-shaped (Delfinado and Baker1974). The taxonomy and morphology of all life stages of *E. sinhai* have been described and compared with

those of *V. jacobsoni* (Delfinado and Baker 1974; Akratanakul 1976; Delfinado-Baker 1987).

Developmental changes occurring in the protonymphal, deutonymphal and adult stages were discussed by Delfinado-Baker (1987). The morphological characters include the absence of fixed cheliceral digit, the movable digit being a pointed piercing structure in female and nymphal stages but modified for sperm transfer in the male; the reduction in hypostomal and palpal chaetotaxy in both the adult and nymphal stages; the complete absence of ambulacral claws in all stages of the development; the presence of looped peritremes in female and the hypertrichy of the dorsal body surface. Males resemble the nymphal stage and are very weakly sclerotized.

The life history stages of *E. sinhai* comprise the egg, larva, protonymph, deutonymph and the adult. The larva is inactive and molts within the egg, becoming a protonymph (Akratanakul and Burgett 1976). The adult female, protonymph and deutonymph stages of both sexes are haemophagic. All stages in the development of *E. sinhai* take place inside the capped drone brood cell; only the adult female mites leave the cells with host bee emergence (Akratanakul 1976). Males, nymphs and eggs are found only inside the sealed drone brood cells. Male mites remain in the sealed brood cell and presumably die after mating. The chelicerae of the male mite are modified for sperm transfer and it is not able to feed as an adult.

There are few published studies on the relationship between *E. sinhai* and its natural host, *A. florea*. Akratanakul and Burgett (1976) reported that only adult female mites were found in association with adult drones. However, Koeniger *et al.* (1983) found adult female mites on adult worker bee, and Mossadegh and Birjandi (1986) observed adult female mites on both adult worker and drone bees of *A. florea*. The level of *Euvarroa* infestation on adult *A. florea* workers is high in March, April and September and the mites appear to reproduce during these months (Kapil and Aggarwal 1987, 1988). Mossadegh (1991) reported that the infestation on adult worker bees was

high when there was no drone brood in colonies. Mite infestation of drones was high at the time of their emergence, but was reduced within 24 hrs. He also observed that the adult female mites are phoretic on adult workers and feed on bees when brood rearing has ceased.

This mite also has been reported in debris samples from *A. mellifera* colonies but in low numbers (Sihag 1987). Burgett and Sukumalanand (pers. comm.) also found *E. sinhai* females associated with *A. dorsata* colony debris samples. Mossadegh (1990a, 1990b) observed the development of *E. sinhai* on *A. mellifera* worker and drone brood under laboratory conditions and found that the mite was able to feed and reproduce on *A. mellifera* brood. The developmental period from egg to adult was 5 and 6-7 days for males and females, respectively. Fertile females produced 1-8 offspring per cell on worker brood and 1-9 offspring per cell on drone brood.

Objectives

The relationship between the parasitic brood mite, *Euvarroa sinhai* and its natural host, *Apis florea* requires further study to understand how they live together and to further elucidate the host parasite relationship. Therefore, the specific objectives of this study are:

A. to determine the distribution pattern of *Euvarroa sinhai* on the brood of its host, *Apis florea.*

B. to determine whether or not the adult *Apis florea* worker bees remove mite-infested brood from the capped cells.

C. to examine the transmission of *Euvarroa sinhai* between host colonies during broodless periods of its adapted host, *Apis florea.*

D. to determine whether or not *Euvarroa sinhai* has the ability to survive and feed on the adult of its natural host, *Apis florea*, and also to discover if other *Apis* species can serve as hosts for *E. sinhai* phoresy.

E. to construct the initial life tables of *Euvarroa sinhai* during the reproductive period while infested on the brood of its host, *Apis florea.*

CHAPTER II
POPULATION DENSITY OF THE PARASITIC MITE, *EUVARROA SINHAI* DELFINADO AND BAKER IN VARIOUS STAGES OF THE DWARF HONEY BEE, *APIS FLOREA* F. COLONY DEVELOPMENT

Abstract

Colonies of *Apis florea,* in various stages of development, were collected from the field during February-July 1992-93. Adult workers and drones from each colony were examined for the presence of *Euvarroa sinhai* mites. The population density of mites (number of female mites per 100 adult bees) from the various stages of *A. florea* colony development differed under the conditions of swarming and the presence or absence of queens. The mean mite density was highest (0.89 mites per 100 bees) from colonies after drone brood rearing (collected post-swarming) and the lowest mean mite density was from colonies sampled during the drone brood rearing (0.14 mites per 100 bees). The mean mite density from swarms was 0.35 mites per 100 bees and from queenless colonies it was 1.58 mites per 100 bees.

Introduction

The dwarf honey bee, *Apis. florea*, is distributed throughout Southeast Asia (Ruttner 1987). Usually it inhabits the plains, but can survive up to 1500 m. (Free 1981). Populations of worker bees in *A. florea* colonies vary greatly. Seeley *et al.* (1982) reported the number of bees present in *A. florea* colonies from Thailand, averaged 6271 workers with a standard deviation of 4927. Normally brood rearing occurs in late February until May. In India, a newly established colony may achieve full size in 6 to 8 weeks when forage is abundant (Free 1981). Drone and queen rearing, normally associated with swarming, takes place from March to June (Akratanakul 1977), when colony populations are maximum. The primary swarm to leave the mother colony contains about half of the worker bee population. In the case of queenless colonies, some workers develop ovaries and will lay eggs. The worker cells are enlarged to

accommodate the drone brood; therefore brood cells from queenless colony are irregular in shape (Akratanakul 1977).

The parasitic mite, *Euvarroa sinhai* Delfinado and Baker, was found associated with the dwarf honey bee, *A. florea* F. (Delfinado and Baker 1974). Akratanakul and Burgett (1976) reported that *Euvarroa* parasitizes only the drone brood of the dwarf honey bee. All stages in the development of this mite take place inside the capped drone cells and only adult female mites leave the cells with emerging drone adults. Akratanakul (1976) also reported the mite as phoretic only on adult drones; however, *Euvarroa* females were found on adult worker bees in Sri-Lanka (Koeniger *et al.* 1983). Mossadegh and Birjandi (1986) observed adult female mites on both adult worker and drone bees of *A. florea*. The level of *Euvarroa* infestation on adult worker bees was high in March, April and September (Kapil and Aggarwal 1988). Mossadegh (1991) reported that the number of *Euvarroa* females on worker bees was low in the period April-June when drone brood rearing is at a peak. He also observed that the adult female mites are phoretic on adult worker bees and feed on bees when drone brood rearing has ceased.

This study reports on the population density of *Euvarroa* mites in *A. florea* colonies during the swarming and drone brood rearing periods. Queenright and queenless colonies were observed to determine whether or not there is any difference in the density of mites between various stages of *A. florea* colony development.

Materials and Methods

Study area and *A. florea* colonies in Northern Thailand

The study was conducted in the northern part of Thailand during February-July 1992-93. Chiang Mai Province, the location of this study, is located at 16°north latitude and 99°east longitude and 310 meters above sea level. Chiang Mai is surrounded by numerous mountain ranges. The cool season begins in December and ends in February, a low temperature of 6°C has been recorded. The hot season occurs from March to May; April as the hottest month with a maximum temperature of 41°C. The rainy season extends from May to October with the highest rainfall in August and September.

Brood rearing in *A. florea* colonies increases rapidly from March until May when the nectar and pollen sources are abundant. At this time, drone brood is also present and the colonies begin swarm preparations. During the rainy season, brood is reduced but it is still present through out the year.

Six stages of *A. florea* colony development were considered for this study: Stage I, a swarm-the group of bees departing the mother colony with the old queen in order to establish a new colony at a new nest location. Because only a few swarms were found, newly established colonies (about 1-2 weeks old and before first adult worker bees emerge) were included in this stage of colony development. Stage II, colonies before drone brood rearing. Stage III, colonies during drone brood rearing. Stage IV, colonies after drone brood rearing but collected before the first swarm left the mother colony. Stage V, colonies after drone brood rearing collected after the first swarm left the mother colony and stage VI, queenless colonies.

Twenty-five swarms: thirty-three queenright colonies with and without drone brood, and nineteen queenless colonies, were collected from the field. Whole colonies were cut from substrate branches and placed in plastic bags for transport to the laboratory. Bee samples were collected by taking the layer of bees covering the comb or

cutting only the bottom part of the colony and placing it in a plastic bag. All the combs with adult bees were frozen overnight. Adult bees were soaked in 70% ethyl alcohol for 2-3 hours. Then the container was shaken for 10 minutes. The number of bees and mites in the solution were counted. To examine drone brood infestation, sealed drone brood cells from each colony were sampled; mite prevalence and mite load were recorded.

Results

Seventy-seven colonies of *A. florea* were collected for this study. *Euvarroa* mites were found in 84% (n=25) of the swarms and newly established colonies (stage I); 90% (n=10) of the colonies before drone brood rearing (stage II); 83% (n=6) of colonies during drone brood rearing (stage III); 89% (n=9) of colonies collected pre-swarming (stage IV); 100% (n=8) of colonies collected post-swarming (stage V), and 89% (n=19) of queenless colonies (stage VI).

Mite loads on adult bees were generally low and varied with the different stages of colony development (Tables II.1. - Table II.5.). Only female mites were found associated with adult bees. Mite loads on adult bees (number of mites per 100 bees) were lowest during drone brood rearing when mites were reproducing in sealed drone brood cells. The mean mite load for this stage (III) was 0.14 (range from 0.00-0.34, Table II.3.). The mean mite load from swarms and newly established colonies (stage I) was 0.35 (range from 0.00-1.55, Table II.1.); from the colonies before drone brood rearing (stage II) the average was 0.31 (range from 0.00-1.72, Table II.2.), and from colonies after drone brood rearing collected pre-swarming (stage IV), it was 0.30 (range from 0.00-1.35, Table II.4.). The highest mite load experienced in queenright colonies was on adult bees following swarming with a mean mite load of 0.89 (range from 0.03-2.22, Table II.4.). Queenless colonies had the highest mite load with an average of 1.58 (range from 0.00-12.65, Table II.5.).

Mean parasite densities for adult bees were compared (LSD test) between the various stages of queenright colonies. There was a significant difference between the mean mite density after swarming and other colony developmental stages (P=0.036). The mean mite density from the colonies after drone brood rearing (collected post-swarming) was the highest, 0.89 mites per 100 bees and was higher than the mean from swarms, or colonies before drone brood, during drone brood and after drone brood collected pre-swarming (Table II.6.). The mean mite density was similar for colonies before drone brood rearing and swarms (0.31 and 0.35 mites per 100 bees, respectively).

During drone brood rearing, the density of mites on the adult bees was low and the percentage of brood infestation varied from 6.7-39.0%. In queenless colonies, the level of mite infestation was high (13.0-63.5% brood infestation) and the mean mite density was 1.58 mites per 100 bees.

For each colony developmental stage the correlations of the number of mites, workers and drones were compared (Table II.7.). There was a positive correlation between the number of mites and workers and the number of mites and drones in a swarm ($r=0.64$, $P=0.0005$; $r=0.94$, $P<0.0001$ respectively). In the colonies collected before drone brood rearing the correlation of mites, workers and drones was negative. Summary of the number of worker and drone bees from various stages of *Apis florea* colony development compared with the number of *Euvarroa sinhai* showed in Table II.8.

Mite loads on adult bees was compared between the different stages of *A. florea* colony development (Fig. II.1.). Most queenless colonies had higher mite loads than queenright colonies (>1 mites per 100 bees). Among queenright colonies, mite loads ranged from 0-0.14 mites per 100 bees before swarming. After swarming mite loads on the mother colonies increased to 0.5-0.99 mites per 100 bees.

Discussion

Euvarroa mite density in swarms, in colonies before drone brood, in colonies during drone brood and in colonies after drone brood (pre-swarming) were very similar. Mite load on adult bees decreases during drone brood rearing because most female mites enter drone brood cells to reproduce. Mite loads on adult bees increase as drone rearing decreases and female mites leave cells with emerging drones. However, mite density from colonies after drone brood rearing, pre-swarming did not differ significantly from colonies before drone brood suggesting that honey bee colonies have the ability to stabilize mite population at low level throughout the year.

The colonies collected after swarming had the highest number of mites and significantly differed from swarms and other stages of colony development. This indicates that most of *Euvarroa* mites remained in the mother colonies and fewer mites travel with swarms. Swarming tends to reduce mite population density within a colony. Dustmann (1993) suggested that swarming is one of the effective defense mechanisms of honey bee against diseases and parasites. This behavior interrupts the infection chain of the disease and also reduces the parasite load on their hosts by leaving the diseases brood or parasites in the mother colonies. Royce *et al.* (1991) also proposed that swarming by the European honey bee, *A. mellifera* can reduce the density of tracheal mites, *Acarapis woodi* within a colony. The evidence from my studies shows a similar effect of swarming on the population density of the parasitic mite, *E. sinhai.*

The numbers of workers, drones and mites that collected from *A. florea* swarms were related. Mite density seems to increase as the number of adult bees in the colonies increases. Female mites must be able to stay on the adult workers or drones of *A. florea* for 3-4 months. This indicates that *Euvarroa* most likely feeds on adult bees during broodless period. The transmission of *Euvarroa* from mother colonies to offspring colonies occurs during swarming. Mossadegh (1991) reported that *E. sinhai* spent about 6 months on adult worker bees in *A. florea* colonies in Iran.

The samples from queenless colonies indicate that there were more *Euvarroa* mites per adult bee than in queenright colonies. Mite densities in queenless colonies were high whether there was drone brood rearing or not. Later stage queenless colonies will possess laying workers, which will ultimately result in colonies possessing only drone brood thereby providing *Euvarroa* with ideal reproductive conditions.

Table II. 1. Number of adult female *Euvarroa sinhai* on adult workers and drones of *Apis florea* from swarms and newly established colonies

Colony	Date collected	No. of workers	No. of drones	No. of mites	Average mites per 100 bees	Remarks
1A	2/27/92	715	54	0	0.00	sample
2B	4/23/92	12,436	93	21	0.17	whole colony
3B	5/13/92	3510	0	2	0.06	whole colony
4B	5/17/92	614	10	0	0.00	sample
5B	6/3/92	1846	0	5	0.27	whole colony
6A	3/11/93	818	0	6	0.73	whole colony
7B	3/25/93	2826	11	4	0.14	whole colony
8B	3/31/93	2532	0	3	0.12	whole colony
9B	4/1/93	1833	5	7	0.38	whole colony
10B	4/3/93	4465	143	64	1.39	whole colony
11B	4/3/93	3008	72	16	0.52	whole colony
12A	4/7/93	2538	0	2	0.08	whole colony
13B	4/18/93	2212	0	9	0.41	whole colony
14B	4/18/93	1453	0	2	0.14	whole colony
15A	4/24/93	2258	1	5	0.22	whole colony
16B	4/25/93	1277	0	1	0.08	whole colony
17B	5/1/93	10,988	137	61	0.55	whole colony
18A	6/22/93	2563	0	0	0.00	whole colony
19B	6/24/93	4786	29	12	0.25	whole colony
20A	6/24/93	1390	25	2	0.14	whole colony
21B	6/29/93	13,812	422	221	1.55	whole colony
22B	6/29/93	4208	10	34	0.81	whole colony
23A	7/5/93	9011	2	30	0.33	whole colony
24B	7/23/93	11,886	0	45	0.38	whole colony
25B	8/4/93	10,280	22	0	0.00	whole colony

A: swarm

B: newly established colony (1-2 wks.)

Table II. 2. Number of adult female *Euvarroa sinhai* on adult workers and drones of *Apis florea* before drone brood rearing

Colony	Date collected	No. of workers	No. of drones	No. of mites	Average mites per 100 bees	Remarks
1	3/9/92	3746	0	1	0.03	whole colony
2	3/9/92	3075	0	0	0.00	whole colony
3	3/31/93	3964	0	16	0.40	whole colony
4	4/3/93	3585	11	9	0.25	whole colony
5	4/18/93	2212	0	9	0.41	whole colony
6	5/8/93	4674	0	8	0.17	sample
7	7/23/93	6672	0	2	0.03	whole colony
8	7/23/93	2618	0	45	1.72	whole colony
9	8/18/93	5550	0	3	0.05	whole colony
10	8/21/93	7485	0	4	0.05	whole colony

Table II. 3. Number of adult female *Euvarroa sinhai* on adult workers and drones of *Apis florea* during drone brood rearing

Colony	Date collected	No. of workers	No. of drones	No. of mites	Average mites per 100 bees	%Brood infestation	Remarks
1A	6/25/92	5426	27	4	0.07	20.7	whole colony
2A	3/15/93	3479	33	12	0.34	39.0	whole colony
3B	5/3/93	12,133	0	16	0.13	6.7	whole colony
4B	5/4/93	3670	0	11	0.30	10.3	sample
5B	5/5/93	5852	0	1	0.02	8.0	sample
6B	5/31/93	6270	0	0	0.00	0.0	sample

A: after some drones emerged
B: before drones emerge

Table II. 4. Number of adult female *Euvarroa sinhai* on adult workers and drones of *Apis florea* after drone brood rearing

Colony	Date collected	No. of workers	No. of drones	No. of mites	Average mites per 100 bees	Remarks
Before swarm						
1	5/19/93	4722	0	3	0.06	sample
2	6/25/93	25,080	107	46	0.18	whole colony
3	7/2/93	4270	230	15	0.33	sample
4	7/7/93	4304	52	21	0.48	sample
5	7/7/93	6708	34	91	1.35	sample
6	8/13/93	9107	7	4	0.04	sample
7	8/13/93	7303	145	11	0.15	sample
8	8/21/93	8038	0	0	0.00	sample
9	8/21/93	8372	13	6	0.07	sample
After swarm						
1	7/9/92	2670	1	48	1.80	whole colony
2	7/16/92	6443	50	58	0.89	whole colony
3	7/20/92	5351	216	53	0.95	whole colony
4	7/20/92	7224	0	2	0.03	whole colony
5	4/26/93	7014	14	21	0.30	whole colony
6	5/21/93	3080	347	76	2.22	whole colony
7	6/25/93	8830	78	60	0.67	whole colony
8	7/5/93	4617	70	11	0.23	whole colony

Table II. 5. Number of adult female *Euvarroa sinhai* on adult workers and drones of queenless *Apis florea* colonies

Colony	Date collected	No. of workers	No. of drones	No. of mites	Average mites per 100 bees	%Brood infestation	Remarks
1	3/9/92	2169	68	283	12.65	58.7	whole colony
2	4/12/92	2758	41	81	2.89	54.5	whole colony
3	5/9/92	3890	12	31	0.79	60.0	whole colony
4	5/13/92	734	11	1	0.13	13.0	whole colony
5	5/13/92	364	35	1	0.25	no brood	whole colony
6	6/3/92	993	0	0	0.00	no brood	whole colony
7	7/9/92	2682	65	159	5.79	45.9	whole colony
8	3/14/93	900	30	13	1.40	39.6	whole colony
9	3/14/93	205	0	2	0.98	no brood	whole colony
10	3/17/93	2235	7	0	0.00	no brood	whole colony
11	3/17/93	4423	65	15	0.33	no brood	whole colony
12	3/31/93	1156	0	4	0.35	no brood	whole colony
13	4/3/93	1526	0	4	0.26	no brood	whole colony
14	4/20/93	10,702	48	148	1.38	63.5	whole colony
15	5/1/93	3444	0	2	0.06	no brood	whole colony
16	5/16/93	5200	95	18	0.34	no brood	whole colony
17	5/16/93	798	1	2	0.25	no brood	whole colony
18	5/31/93	3995	277	50	1.17	no brood	whole colony
19	7/5/93	765	47	8	0.99	no brood	whole colony

Table II. 6. Mean parasite density (mites per 100 adult bees) and multiple range analysis (LSD test) for various stages of *Apis florea* colony development

Colony	Mean (SE)
During drone brood (n=6)	0.14 (0.06) a
After drone brood (collected pre-swarming) (n=9)	0.30 (0.14) a
Before drone brood (n=10)	0.31 (0.16) a
Swarm and newly established colony (n=25)	0.35 (0.08) a
After drone brood (collected post-swarming) (n=8)	0.89 (0.27) b

Means with the same letter do not possess a significance differrent in mean parasite density (P=0.05)

Table II. 7. The correlations for the number of *Euvarroa* mites, workers and drones from various stages of *Apis florea* colony development

Colony	mite vs. worker		mite vs. drone	
Swarm	r = 0.64	P = 0.0005	r = 0.94	P < 0.0001
Before drone brood	r = -0.42	P = 0.2212	r = -0.02	P = 0.9596
During drone brood	r = 0.33	P = 0.5285	r = 0.12	P = 0.8135
After drone brood (collected pre-swarming)	r = 0.26	P = 0.4977	r = 0.07	P = 0.8503
After drone brood (collected post-swarming)	r = -0.25	P = 0.5516	r = 0.64	P = 0.0855
Queenless	r = 0.37	P = 0.1168	r = 0.26	P = 0.2728

r = correlation coefficient , P = significance level

Table II. 8. Summary of the number of worker and drone bees from various stages of *Apis florea* colony development compared with the number of *Euvarroa sinhai*

	Swarm (n=25)	Before drone brood (n=10)	During drone brood (n=6)	After drone brood (pre-swarming) (n=9)	After drone brood (post-swarming) (n=8)	Queenless (n=19)
Parasite load (mites/100 bees)						
mean	0.35	0.31	0.14	0.30	0.89	1.58
min.	0.00	0.00	0.00	0.00	0.03	0.00
max	1.55	1.72	0.34	1.35	2.22	12.65
No. of workers						
mean	4530.6	4358.1	6138.3	8656	5653.6	2575.7
min.	818	2212	3479	4270*	2670	205
max.	13812	7485	12133	25080	8830	10702
No. of drones						
mean	41.4	1.1	10.0	65.3	97.0	42.2
min.	0	0	0	0	0	0
max.	422	11	33	230*	347	277

* from sample

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CHAPTER III
THE DISTRIBUTION OF THE PARASITIC MITE, *EUVARROA SINHAI* DELFINADO AND BAKER IN THE BROOD OF *APIS FLOREA* F.

Abstract

The distribution of *Euvarroa sinhai* Delfinado and Baker on drone brood from queenright and queenless colonies of *Apis florea* F. is aggregated. A truncated negative binomial distribution was applied to indicate mite-induced mortality in drone brood pupae. Differences between the observed and predicted values occurred at the truncation point in 7 days old pupae. Because dead drone pupae (by mite infestation) were not observed directly within capped drone cells, it is suggested that the worker bees probably have the ability to remove pupae with high mite loads from the cells.

Introduction

Euvarroa sinhai Delfinado and Baker is a parasitic brood mite associated with the dwarf honey bee, *Apis florea* F. (Akratanakul and Burgett 1976; Koeniger et al. 1983; Mossadegh 1991). Most past studies have concentrated on the life history and systematic of this mite.

Euvarroa sinhai is listed in the family Varroidae and the taxonomy and morphology of all life stages have been described by Delfinado and Baker (1974). The reproducing female mite enters the brood cell of a late larval host before the cell is capped and lays eggs inside the brood cell. All stages of the mite live inside the capped drone brood cell and only adult female mites leave the cell when adult bee eclosion occurs (Akratanakul 1976).

Little is known about the interactions of *E. sinhai* and its natural host, *A. florea*. Normally, the mite populations in *A. florea* colonies are low, because only drone brood is infested by this mite and the amount of drone brood is temporally limited to a small proportion of the brood nest (Mossadegh 1991).

However, other factors could restrict mite populations in *A. florea* colonies. Studies illustrating the defense behavior of the Asian honey bees (*A. cerana* and *A. dorsata*) against parasitic brood mites have been demonstrated by Peng *et al.* 1987 (*Apis cerana* against *Varroa jacobsoni*) and Burgett *et al.* 1990 (*Apis dorsata* against *Tropilaelaps clareae*). They concluded that the Asian honey bees have the ability to recognize and remove parasitic brood mites from the brood cells.

The distribution of parasites on their hosts is a method that provides some understanding of the relationship between brood mites and bees. This study determined the pattern of *Euvarroa* mite distribution in *A. florea* drone brood and examined whether or not parasite-induced host mortality occurs in the population by using the truncated negative binomial (Crofton 1971; Lanciani and Boyett 1980).

Materials and Methods

Twenty-nine colonies of *Apis florea* (24 queenright and 5 queenless colonies) were collected from the field or purchased from the markets in the northern provinces of Thailand during March-June 1992-93. Most of drone brood of *A. florea* was constructed at the bottom of the comb. The number of drone brood cells varied from ca. 50 to 350 brood cells per comb side. Sealed drone brood cells (prepupal and pupal stages) were sampled from each colony. Four categories (stages) of pupal development were noted: I, prepupae (2-3 days old after cell-capping); II, white-eyed pupae (4 days old after cell-capping); III, pink-eyed pupae (5-6 days old after cell-capping); IV, purple-eyed pupae (7 days old after cell-capping). Individual drone cells were decapped and drone pupae were removed from each cell. The numbers of *Euvarroa* mites from infested cells were recorded. During the period from prepupae to purple-eyed pupae, off-spring from parental female mites have not completed their development in drone brood cells of *A. mellifera* (Mossadegh 1990).

The observed distribution of female mites on the drone bee pupae was compared to an expected negative binomial distribution. The expected frequencies for the negative binomial distribution were calculated by using the BASIC program NEGBINOM (Ludwig and Reynolds 1988). The number of female mites per drone brood cell is summarized as a frequency distribution (the number of drone pupae with 0, 1, 2, ..., x mites) and the probability of finding x mites in drone pupae is

$$P(x)=[\mu/(\mu + k)]^x \{(k + x - 1)!/[x! (k - 1)]!\}[1 + (\mu/k)]^{-k}$$

The parameter μ is the mean number of mites per drone pupa estimated from the sample mean (m) and the parameter k is the degree of clumping estimated by using the iterative equation:

$$\log_{10} (N/N_0) = k \log_{10} [1+(m/k)]$$

where N is the total number of drone pupae and N_0 is the number of drone pupae with 0 mites.

The truncated negative binomial distribution was applied to the collected data from each developmental stage of drone pupae and from three combinations of the developmental stages: I,prepupae and white-eyed pupae; II, prepupae, white-eyed and pink-eyed pupae, and III, prepupae to purple-eyed pupae. If pupal mortality occurs, fewer hosts will be observed than expected in the parasite load classes (numbers of female mites per drone cell) at the truncation point. The calculation of the truncated negative binomial distribution was described in Crofton 1971; Lanciani and Boyett 1980; Royce and Rossignol 1990 (computer programs are available in BASIC upon request from L. A. Royce, Dept. of Ent., OSU).

Results

Prevalence infestation rates of *Euvarroa* in drone brood from *A. florea* colonies ranged from 2.0-38.0%, in 15 queenright colonies and 58.7-63.0%, in 5 queenless colonies. The data for drone pupal infestation in queenright and queenless colonies are given in Tables III.1. - Table III.8. The negative binomial model was applied to these data. The ratio of the variance (s^2) to mean (m) was proposed as the index of dispersion (ID). When variance>mean, the negative binomial was recommended as a model for clumped dispersions. The parameter k was measured as the degree of aggregation (tends toward zero at maximum clumping) and was affected by the total number of sampling units and the number of individuals in the samples. The sample sizes from this experiment were not equal; thus the k value could not be compared from each developmental stage of drone pupae.

The truncation of the negative binomial distribution was determined whether or not mite-associated mortality occurred with the mite load. Expected numbers from the negative binomial and truncated negative binomial distribution were compared with the observed numbers from each stage of pupal development: prepupae, white-eyed, pink-eyed and purple-eyed pupae (Table III.1. - Table III.4.). The negative binomial distribution of mites on the early pupae (prepupae and white-eye stage, Table III.1. and Table III.2.) fitted the observed data very well (chi-square goodness of fit test, P>0.75), but truncation did not improve the fit in the lowest 3 mite load classes (using the chi-square value of the first three mite load classes for measuring the goodness of fit test). There was no difference in the value of total hosts between observed and predicted numbers when the truncation was applied. At the older pupae (pink-eye and purple-eye) stage, (Table III.3. and Table III.4.), the negative binomial distribution fitted the observed data moderately well (chi-square goodness of fit test, P>0.25). As truncation was applied, there were approximately 0.3-1.0 % fewer total pupae in the observed than predicted. When truncation occurred at mite load class 3, the total number of pupae

parasitized by three or more mites was predicted to be 3.8 (pink-eyed pupae) and 5.2 (purple-eyed pupae) but the observed values were 1 and 3 respectively. The differences between observed and truncated values suggest parasite-induced mortality (Crofton 1971; Lanciani and Boyett 1980). Dead pupae were never observed directly from the drone brood cell of *A. florea* queenright colonies, indicating that the adult bees probably remove mite-infested pupae from the cells.

The samples from each pupal development stage were combined to increase the number of drone pupae. The negative binomial distribution of mites fit the observed data (Table III.5. - Table III.7.) very well (chi-square goodness of fit test, $P>0.75$). When truncation was applied at the level of 4 mites load class, there were approximately 0.4-1.3 % fewer pupae in the observed than predicted.

Because of the small sample size from queenless colonies, the drone brood samples were not separated for each stage of development. Brood samples were combined from prepupae, white-eyed and pink-eyed pupae. The negative binomial distribution fit the observed data moderately well (Table III.8.; chi-square goodness of fit test, $P>0.25$). Density of the parasites (number of mites/brood cell) and the value of k parameter were higher than in queenright colonies. There were differences between observed and truncated values when the truncation was applied. Dead drone pupae were observed in brood cells in queenless colonies but the number was not recorded and was not counted with the normal pupae.

Discussion

The distribution of *Euvarroa* mites on drone brood of *Apis florea* queenright colonies is demonstrated to be aggregated and to fit a negative binomial distribution. A truncated negative binomial distribution suggests a mite-induced mortality of drone brood pupae in pink-eyed and purple-eyed stages of pupal development. Because dead infested pupae were never directly observed from the capped brood cells, the ability of worker bees to remove infested pupae from the brood nest possibly occurs in *A. florea* colonies. This evidence is supported by the field observation of dead drone bee pupae and mites collected from debris under *A. florea* colonies (see Chapter IV). There was a positive correlation between the number of *Euvarroa* mites and drone pupae from the debris. Most of dead drone pupae were purple-eyed and older stages.

A resistance mechanism for Asian honey bees to parasitic mites was first reported for *A. cerana* to *Varroa jacobsoni* (Peng *et al.* 1987). Their observations showed that *A. cerana* has a high frequency of cleaning behavior that removes the mites from adult bees and from sealed brood cells. According to Burgett *et al.* (1990) the giant honey bee, *A. dorsata*, possesses the ability to recognize and remove larvae or pupae infested with the brood mite, *Tropilaelaps clareae*. This behavior indicates that *A. dorsata* has evolved a specific defense against parasitic brood mites, probably *A. florea* also has a similar mechanism and removes both pupae and mites from infested cells.

Mite distribution in queenless *A. florea* colonies also fits a negative binomial. The density of mites in queenless colonies was higher than in queenright colonies with higher mite loads (mites per cell) and prevalence (percentage of cells infested). Truncation of the negative binomial indicates missing drone pupae (the missing infested-pupae from these queenless colonies could be the result of not counting dead drone pupae, although dead pupae were observed in capped cells), possibly the worker bees in queenless colonies have a reduced capacity to remove infested drone pupae. Cell-

cleaning activities may be performed by bees of a younger age than most bees found in queenless colonies.

The distribution pattern of *Euvarroa* mite on *A. florea* helps us to understand the relationship between the parasitic mite and its natural host. The ability of worker bees to remove drone pupae infested with mites from the cells indicates that *A. florea* has a specific defense behavior against its parasite and is capable of limiting parasite density. This equilibrium between host and parasite occurs in normal (queenright) colonies of *A. florea*. In queenless colonies, the parasite density increases. The dwarf honey bee, appears to have a stable relationship with its brood parasite, *E. sinhai*. This study supports a general hypothesis that Asian honey bee species have evolved defense mechanisms against haemophagic brood mite species.

Table III. 1. Observed and expected numbers of female *Euvarroa sinhai* in each mite load class from drone brood (prepupae) of queenright *Apis florea* colonies [Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2 and 3)]

No. of female mites per cell	No. of drone cells Observed	Expected	Truncation 3	2
0	420	419.98	420.84	421.16
1	28	28.09	28.66	28.16
2	4	4.07	4.46	*4.56
3	1	0.70	*0.82	0.88
>4	0	0.16	0.21	0.24
Total	453	453.00	454.99	455
Mean (m)	0.09	0.09	0.09	0.09
Variance (s^2)	0.11			
Variance/Mean (ID)	1.27			
k		0.30	0.28	0.26
C		0.00	0.06	0.07

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

Table III. 2. Observed and expected numbers of female *Euvarroa sinhai* in each mite class from drone brood (white-eyed pupae) of queenright *Apis florea* colonies [Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2 and 3)]

No. of female mites per cell	No. of drone cells Observed	Expected	Truncation 3	2
0	183	182.98	183.92	184.60
1	21	21.43	21.11	21.07
2	6	5.75	6.26	*6.01
3	2	1.84	*2.23	2.06
4	1	0.63	0.86	0.76
>5	0	0.36	0.59	0.48
Total	213	212.99	214.99	214.98
Mean (*m*)	0.20	0.20	0.22	0.21
Variance (s^2)	0.33			
Variance/Mean (ID)	1.64			
k		0.28	0.24	0.25
C		0.02	0.02	0.01

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

Table III. 3. Observed and expected numbers of female *Euvarroa sinhai* in each mite load class from drone brood (pink-eyed pupae) of queenright *Apis florea* colonies [Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2 and 3)]

No. of female mites per cell	No. of drone cells		Truncation	
	Observed	Expected	3	2
0	169	168.99	166.56	167.75
1	16	17.79	18.77	18.02
2	7	4.35	5.84	*5.58
3	1	1.27	*2.21	2.10
>4	0	0.60	1.58	1.51
Total	193	193.00	194.96	194.96
Mean (m)	0.17	0.17	0.23	0.22
Variance (s^2)	0.25			
Variance/Mean (ID)	1.44			
k		0.27	0.22	0.21
C		1.79	0.67	0.60

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

Table III. 4. Observed and expected numbers of female *Euvarroa sinhai* in each mite load class from drone brood (purple-eyed pupae) of queenright *Apis florea* colonies [Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2 and 3)]

No. of female mites per cell	No. of drone cells Observed	Expected	Truncation 3	2
0	1695	1694.81	1699.29	1693.83
1	64	64.52	62.23	63.04
2	13	11.91	13.26	*13.14
3	2	2.78	*3.61	3.49
>4	1	0.99	1.60	1.49
Total	1775	1775.01	1779.99	1774.99
Mean (*m*)	0.06	0.06	0.06	0.06
Variance (s^2)	0.09			
Variance/Mean (ID)	1.52			
k		0.11	0.094	0.098
C		0.10	0.07	0.01

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

Table III. 5. Observed and expected numbers of female *Euvarroa sinhai* in each mite load class from drone brood (prepupae - white-eyed pupae) of queenright *Apis florea* colonies [Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2,3 and 4)]

No.of female mites per cell	No. of drone cells Observed	Expected	Truncation 4	3	2
0	603	602.99	604.94	604.94	603.88
1	49	49.07	49.43	49.43	47.48
2	10	10.32	11.20	11.20	*10.05
3	3	2.61	3.08	*3.08	2.57
4	1	0.72	*0.92	0.92	0.72
>5	0	0.29	0.42	0.42	0.30
Total	666	666.00	669.99	669.99	665
Mean (*m*)	0.12	0.12	0.13	0.13	0.12
Variance (s^2)	0.18				
Variance/Mean (ID)	1.49				
k		0.24	0.22	0.22	0.23
C		0.01	0.14	0.14	0.05

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

Table III. 6. Observed and expected numbers of female *Euvarroa sinhai* in each mite load class from drone brood (prepupae - pink-eyed pupae) of queenright *Apis florea* colonies [Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2, 3 and 4)]

No. of female mites per cell	No. of drone cells Observed	Expected	Truncation 4	3	2
0	772	771.98	773.51	772.43	772.1
1	65	66.83	68.76	67.59	65.46
2	17	14.70	18.33	17.04	*15.68
3	4	3.89	5.98	*5.23	4.57
4	1	1.11	*2.12	1.75	1.45
>5	0	0.49	1.27	0.95	0.72
Total	859	859.00	869.97	864.99	859.98
Mean (*m*)	0.13	0.13	0.16	0.15	0.14
Variance (s^2)	0.20				
Variance/Mean (ID)	1.48				
k		0.24	0.20	0.21	0.21
C		0.41	0.30	0.10	0.11

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

Table III. 7. Observed and expected numbers of female *Euvarroa sinhai* in each mite load class from drone brood (prepupae - purple-eyed pupae) of queenright *Apis florea* colonies [Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2, 3 and 4)]

No. of female mites per cell	No. of drone cells Observed	Expected	Truncation 4	3	2
0	2467	2466.80	2470.44	2470.73	2466.96
1	129	131.40	130.97	130.56	129.08
2	30	26.52	30.38	30.40	*30.39
3	6	6.67	8.86	*8.90	9.01
4	1	1.84	*2.85	2.87	2.95
5	1	0.54	0.97	0.98	1.02
>6	0	0.23	0.51	0.52	0.55
Total	2634	2634.00	2644.98	2644.96	2639.96
Mean (*m*)	0.08	0.08	0.09	0.09	0.09
Variance (s^2)	0.12				
Variance/Mean (ID)	1.51				
k		0.152	0.129	0.128	0.125
C		0.50	0.039	0.03	0.005

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

Table III. 8. Observed and expected numbers of female *Euvarroa sinhai* in each mite load class from drone brood of queenless *Apis florea* colonies
[Expected numbers are predicted by nontruncated and truncated negative binomial distributions (at mite load 2,3 and 4)]

No. of female mites per cell	No. of drone cells Observed	Expected	Truncation 4	3	2
0	60	60.00	58.28	56.70	58.28
1	33	41.78	42.47	41.33	42.47
2	35	25.36	28.94	28.15	*28.94
3	16	14.64	19.26	*18.73	19.26
4	6	8.23	*12.66	12.32	12.66
5	6	4.56	8.26	8.04	8.26
6	3	2.49	5.37	5.22	5.37
>7	1	2.93	5.72	5.56	5.72
Total	160	159.99	180.96	176.05	180.96
Mean (*m*)	1.44	1.44	1.99	1.99	1.99
Variance (s^2)	2.52				
Variance/Mean (ID)	1.75				
k		1.34	1.15	1.15	1.15
C		5.51	3.43	3.54	3.43

C is the sum of chi-square statistic of the first 3 mite load classes for measuring goodness of fit to the negative binomial

* Highest mite load class considered in fitting the truncated negative binomial distribution

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CHAPTER IV
OBSERVATIONS OF THE PARASITIC MITE, *EUVARROA SINHAI* DELFINADO AND BAKER POPULATIONS FROM COLONY DEBRIS OF THE DWARF HONEY BEE, *APIS FLOREA F.*

Abstract

The number of *Euvarroa* mites was observed from the debris of *Apis florea* colonies. The number of debris associated mites varied greatly among the colonies; however a positive correlation was found between the number of dead drone pupae and mites in the debris. The number of damaged mites found in the debris was low, ranging from 4.7 to 21.1%. Male and immature stages of the mite were also found in the debris. The data provide supporting evidence that *A. florea* workers remove mite-infested sealed drone brood from the colony brood nests. This specific behavior is one of defense mechanisms of *A. florea* against its parasitic mite, *Euvarroa sinhai.*

Introduction

Euvarroa sinhai Delfinado and Baker is a brood mite which parasitizes the drone brood of the dwarf honey bee, *Apis florea* F. (Delfinado and Baker 1974; Akratanakul and Burgett 1976). All the developmental stages of the mite take place inside sealed drone brood cells; only the adult female mites leave the brood cells following host bee emergence (Akratanakul 1976). The relationship between *Euvarroa* mites and its natural host, *A. florea* has not been well studied. However, this mite appears to do little harm to its host.

The number of adult female mites phoretic on adult *A. florea* workers is highest in March, April and September and the mites appear to reproduce during these months (Kapil and Aggarwal 1988).

Previous studies on the other species of Asian honey bees, *A. cerana* and *A. dorsata* and their parasitic mites, *Varroa jacobsoni* and *Tropilaelaps clareae* showed that Asian honey bees possess specific defense mechanisms against these brood mite

parasites (Peng *et al.* 1987; Rath and Drescher 1990; Büchler *et al.* 1992). Grooming behavior (both auto- and allogrooming) and removal of mite-infested sealed brood are suggested to be the effective mechanisms to rid mites from infested colonies.

The objective of this study was to determine whether or not *A. florea* has the ability to remove mite-infested sealed brood from the honey bee colonies.

Materials and Methods

The field experiments were conducted in northern Thailand during February through July 1993. Amphoe Mae Rim was the study site, which is approximately 30 kilometers from the city of Chiang Mai and is characterized by numerous flowering plants and shrubs.

Ten colonies of *A. florea* were observed in this study. Colonies of *A. florea* were found by searching the surrounding habitat. The average colony size was about 16 cm. wide and 15 cm. in length with approximately 5000 bees. Colonies were transferred from their natural sites to the study area by cutting the nests gently from their substrate and placing them on branches of longan (*Dimocarpus longan* Lour.) trees in the study area. The nests were cut early in the morning or late in the evening so that the maximum number of bees remained with the colony. The positions of the transferred nests for the observations were ca. 2-2.5 meters above the ground. The selected branches of the trees where the nests were tied were shaded and free from predators such as ants. The bees on transferred colonies appeared undisturbed by the replacement.

For collecting colony debris, white plastic sheets covered with vaseline as a trap for the mites, were placed on trays (30 X 45 cm.) and raised by bamboo poles ca. 30 cm. below *florea* nests. The sheets were replaced daily and the number of mites and bee pupae on the sheets were recorded. A sample of dead mites from the debris was also examined for any morphological damage. The collections started when the bees began to construct drone cells and lasted until all adult drones emerged from the brood nest.

Whole colonies of *A. florea* were sacrificed at the end of the experiments and the remaining number of mites in the colonies were recorded.

Results

Adult and immature *Euvarroa* mites were found with dead drone pupae in the debris during the drone brood rearing period. The number of mites and pupae varied greatly among the colonies (Table IV.1.). The maximum number of female mites collected was 149 mites during a 42 day period and the minimum number was 0 mites during a 30 day period. Some mites were morphologically damaged which was typically expressed by lost appendages. Some 4.7-21.1% of the mites from the debris were damaged, most probably by bees biting the mites (Fig. IV.4.).

The sex ratio of *Euvarroa* mites from the debris varied from 1:1.5 to 1:19. The number of female mites increased with the number of dead drone pupae (Fig. IV.1., pooled data from 10 colonies) and gave a positive correlation for each colony (Table IV.2.) and for pooled data ($r=0.68$, $P<0.0001$). Dead worker pupae were also found in the debris but there was only a weak correlation between the number of mites and dead worker pupae (Table IV.2.).

Most of the dead drone pupae from the debris were in the purple-eyed and older development stage. Figure IV.2. showed the number of dead drone pupae in various stages of development from the debris (pooled data from 10 colonies); 383 (59.56%) of purple-eyed pupae were found in the debris, 121 (18.82%) were dark brown eyes with pigmented thorax and 134 (20.84%) had complete body pigmentation.

The average number of mites (female, male and immature) per purple-eyed drone pupae in the debris was 0.7, (total number of mites was 129 with 173 dead drone pupae from 4 colonies). These mites and dead drone pupae (purple-eyed stage) were positively correlated in three of the observation colonies (colony #1: $r=0.93$, colony #3: $r=0.97$ and colony #7: $r=1$) and negatively correlated from one colony (colony #4: $r=-0.53$). Figure

IV.3. showed the number of mites (female, male and immature) and the number of dead drone pupae (purple-eyed stage) in the debris from each colony. The average number of mites per drone pupa (purple-eyed stage) from the brood of *A. florea* colonies (12 colonies) was 3.9 (the data from Chapter VI); the total number of mites was 316 from 80 infested drone brood cells.

Female *Euvarroa* mites were also found from adult bees following drone brood rearing (Table IV.3.). The average mite density (number of mites per 100 adult bees) in the colonies collected pre-swarming (colony no. 3, 6, 7, 8 and 9) was 0.42 (ranging from 0.04-1.35 mites per 100 bees) and 0.85 in the colonies collected post-swarming (colony no. 1, 4, 5 and 10, ranging from 0.23-2.22 mites per 100 bees).

Discussion

The number of dead drone pupae, worker pupae and *Euvarroa* mites found in the debris beneath *A. florea* colonies during drone brood rearing provided positive correlations between the number of female mites and the number of dead drone pupae. However, the number of female mites showed weak correlation with the number of dead worker pupae. This evidence suggests the possibility of the bees removing mites from the infested drone cells. The removal behavior of infested sealed infested brood by honey bees was studied in another species of Asian honey bee, *A. cerana* by Peng *et al.* (1987) and Rath & Drescher (1990). They showed that artificially infested worker brood was removed by the worker bees. However, this behavior could not be investigated directly from *A. florea* colonies by artificially infesting brood cells. *Apis florea* has a different nest structure from *A. cerana*. It has an open-nest which is protected by 3-6 layers of worker bees. To inoculate mites into brood cells, the bees would have to be removed from the comb. The colony would likely abscond if the bees were disturbed in this manner.

Males and immatures of *Euvarroa* were also found in the debris. Because males and immature stages occur only inside the drone brood cells, this indicates that these mites were removed from the sealed drone brood cells.

During the observation of 28 colonies of *A. florea*, 7000 worker brood cells were examined for *Euvarroa* infestation. No *Euvarroa* mites were found in any of this large sample of worker brood. The mechanism whereby *Euvarroa* mites select drone brood is unknown and deserves further investigation.

Most of the removed drone pupae were purple-eyed pupae and pupae with body pigmentation which supports the truncated negative binomial distribution of *Euvarroa* mites on *A. florea* drone brood (see Chapter III). The average number of mites (female, male and immature) per drone pupa (purple-eyed stage) in the debris was less than the average of mites per drone pupa from samples drone brood. There is a possibility that worker bees may remove sealed drone brood randomly. All removed drone pupae were not infested with the mites. However, when the bees removed infested sealed brood, some adult mites could drop to the comb and remain with the colony. Also some of immature mite stages might still remain within the cells.

The number of mites on the adult bees from the mother colonies, after swarming, had an average mite density greater than the colonies collected pre-swarming. This evidence suggests that most of the mites remain on the adult bees of the mother colonies. Swarming would be one of the mechanisms to reduce mite density for the new colonies.

Previous study on the parasitic mite population from the debris of Asian honey bee colonies was reported by Rath and Delfinado-Baker (1990). They observed *Tropilaelaps clareae* from the debris of *A. dorsata* and suggest that adult *A. dorsata* have a specific defense behavior against this parasitic mite. Nearly three quarters of all adult *T. clareae* from the debris were injured by the bees' biting. This study of *A. florea* revealed that only 4.7-21.1% of mites were damaged. *Apis florea* is very effective in

removing mite-infested drone brood *via* brood nest hygiene. This behavior, combined with auto- and allogrooming, maintains *Euvarroa* infestations at manageable levels.

Removal of mite-infested sealed brood is one part of the hygienic nest cleaning behavior of honey bees (Boecking *et al.* 1993). This behavior in *A. florea* would impact the population of *Euvarroa* by interrupting the development of this mite in the drone brood. The ability of *A. florea* workers to remove both worker and drone brood cells is contrast to that of *A. cerana.* Observations suggest that *A. cerana* workers are able to identify and remove infested sealed worker brood more effective than sealed drone brood (Rath and Drescher 1990). The different structure of the drone cell cap, which is thicker than in worker cell cap, might influence this removal ability by *A. cerana* (Rath 1992). This phenomenon may account for the mites ability to reproduce only in drone brood of *A. cerana* better than in worker brood cells.

Table IV. 1. Number of *Euvarroa sinhai* and pupae of *Apis florea* from the debris beneath the colonies

Colony	Period of collecting (days)	No. of *E. sinhai*			No. of damaged mites	Sex ratio	No. of drone pupae	No. of worker pupae
		female	male	nymphs				
1	3/11-4/21 (42)	149	54	22	7 (4.7%)	1:2.8	160	3
2	3/16-4/21 (37)	33	8	8	0	1:4.1	49	7
3	4/6-5/15 (40)	19	1	1	4(21.1%)	1:19	48	0
4	5/1-6/24 (55)	27	3	9	2 (7.4%)	1:9	251	222
5	5/10-5/21 (11)	6	4	1	0	1:1.5	24	2
6	5/31-6/24 (25)	3	0	4	0	0	57	60
7	6/17-7/4 (18)	26	7	8	0	1:3.7	7	105
8	6/5-7/4 (30)	0	0	0	0	0	37	125
9	6/7-7/8 (32)	1	0	0	0	0	5	11
10	6/18-7/4 (17)	2	0	0	0	0	7	18

Table IV. 2. The correlations for the number of *Euvarroa* mites, dead drone pupae and dead worker pupae of *Apis florea* in colony debris

Colony	mite vs. dead drone pupa		mite vs. dead worker pupa	
1	r = 0.83	P < 0.0001	r = 0.48	P = 0.0011
2	r = 0.73	P = 0.0001	r = 0.05	P = 0.8097
3	r = 0.57	P = 0.001	no worker pupa	
4	r = 0.43	P = 0.0011	r = 0.02	P = 0.8969
5	r = 0.75	P = 0.0079	r = -0.26	P = 0.4344
6	r = 0.80	P = 0.0001	r = 0.47	P = 0.05
7	r = 0.54	P = 0.0203	r = 0.37	P = 0.1294
8	no mite			
9	r = -0.06	P = 0.7292	r = -0.04	P = 0.8023
10	r = 0.15	P = 0.5628	r = 0.06	P = 0.8117

r = correlation coefficient, P = significance level

Table IV. 3. Number of adult female *Euvarroa sinhai* on adult workers and drones of *Apis florea* after drone brood rearing

Colony	No. of workers	No. of drones	No. of mites	Average mites per 100 bees	Remarks
1B	7014	14	21	0.30	whole colony
2					abscond before collecting
3A	4722	0	3	0.06	sample
4B	8830	78	60	0.67	whole colony
5B	3080	347	76	2.22	whole colony
6A	25,080	107	46	0.18	whole colony
7A	6708	34	91	1.35	sample
8A	4304	52	21	0.48	sample
9A	9107	7	4	0.04	sample
10B	4617	70	11	0.23	whole colony

A : collected pre-swarming
B : collected post-swarming

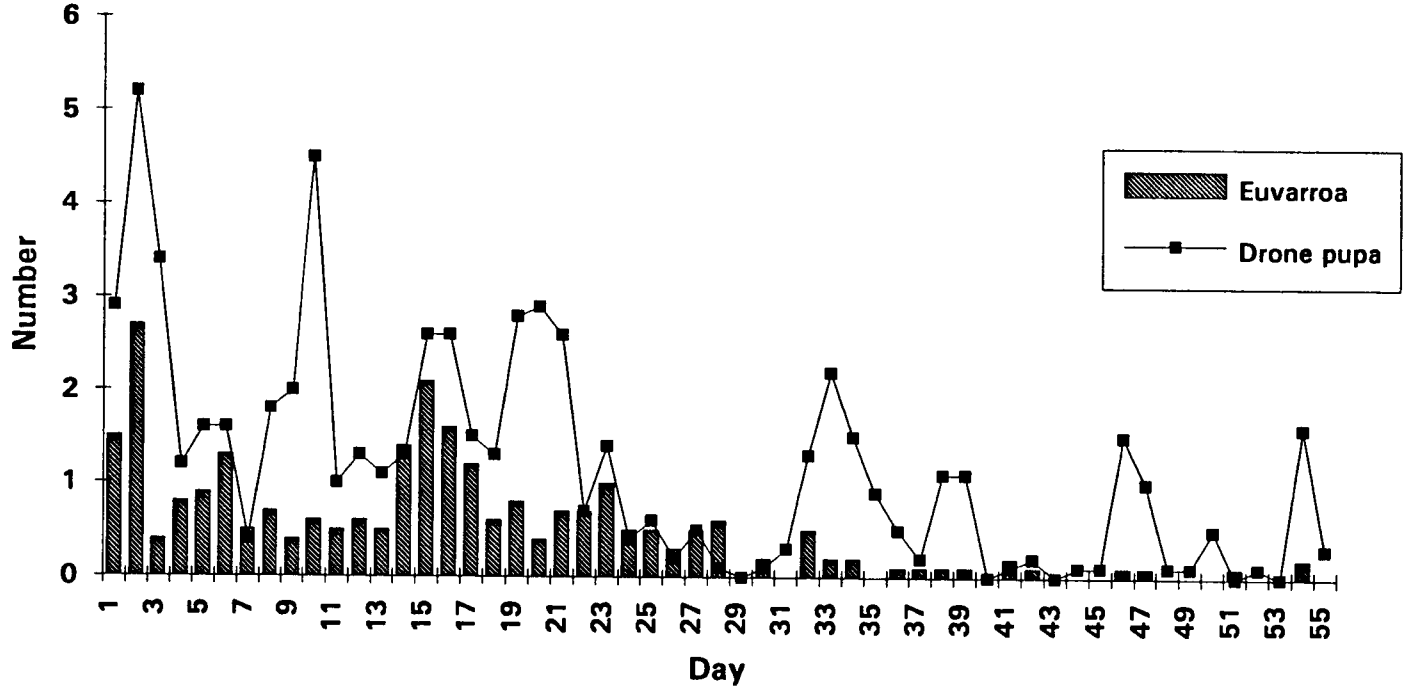
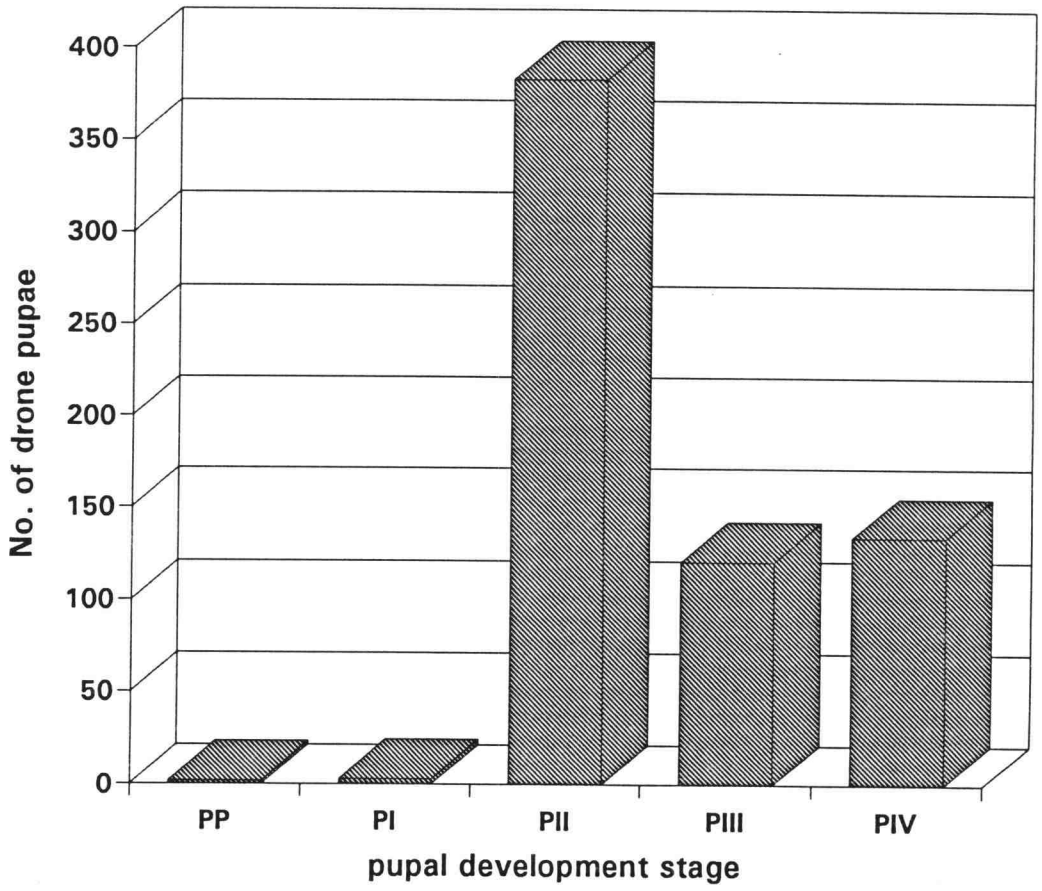


Figure IV. 1. Number of female *Euvarroa* mites (the bars) and dead drone pupae (the broken-lines) from debris of ten observation colonies of *Apis florea* (pooled data), March through July 1993



Fugure IV. 2. Number of dead drone pupae in various stages of development from debris of ten observation colonies of *Apis florea* (pooled data), March through July 1993

Developmental stages of drone brood:

PP = Prepupa

PI = Pupa I, no eye pigmentation

PII = Pupa II, light purple to purple eyes

PIII= Pupa III, dark brown eyes with thorax pigmented

PIV= Pupa IV, completed body pigmentation

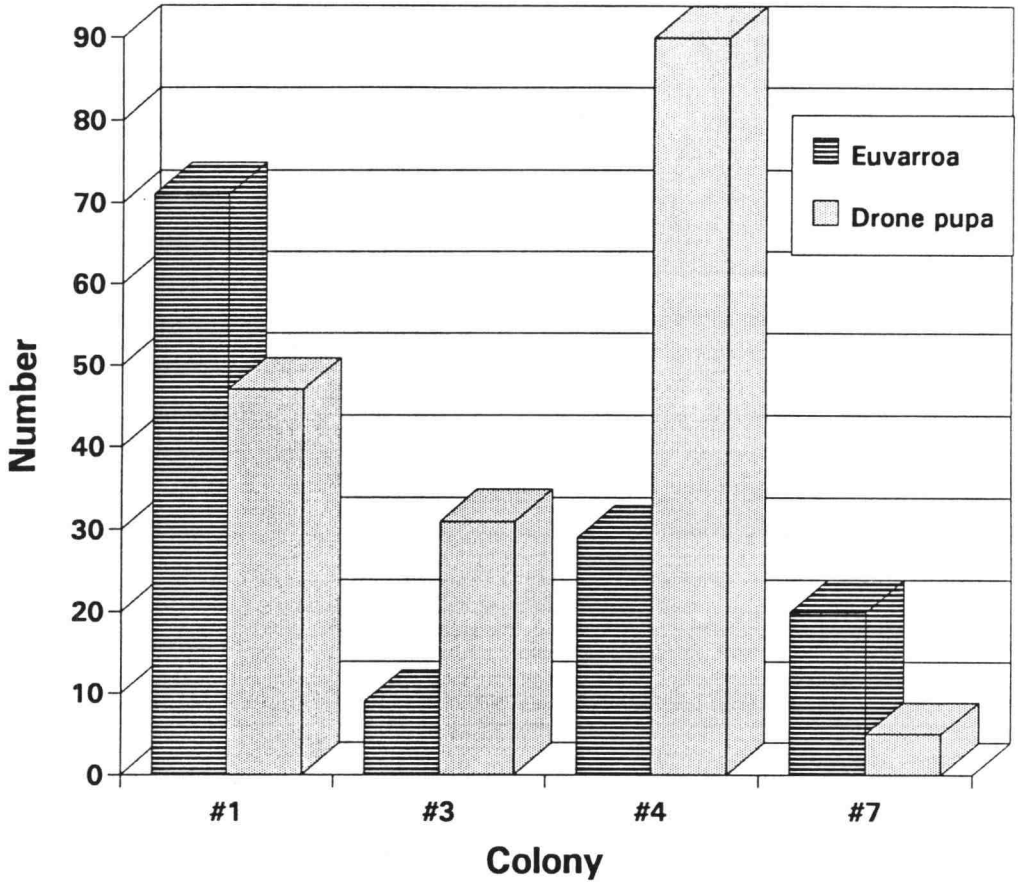


Figure IV. 3. The number of *Euvarroa* mites (female, male and immature stages) and the number of dead drone pupae (purple-eyed stage) from the debris of four observation colonies of *Apis florea*, March through July 1993

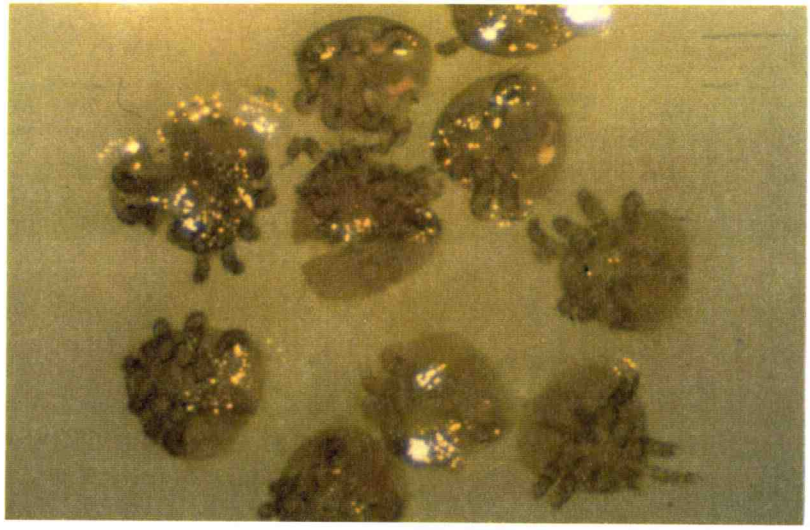


Figure IV. 4. Damaged mites (adult female *Euvarroa sinhai*) from the debris of *Apis florea* colonies

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CHAPTER V
SURVIVAL AND ABILITY OF *EUVARROA SINHAI* DELFINADO AND BAKER TO FEED ON ADULT WORKERS OF *APIS FLOREA* F., *A. CERANA* F. AND *A. MELLIFERA* L.

Abstract

The survivorship of the haemophagic brood mite *Euvarroa sinhai* on three species of adult worker bees: *Apis florea*, *A. cerana* and *A. mellifera* were observed. The mean survival time of mites on *A. florea* and *A. cerana* were significantly different from *A. mellifera*. Longest mean mite survival was on *A. florea*, its native host, 56.8 ± 9.2 hrs. and shortest mean survival was found in the control (no adult bees), 16.8 ± 1.3 hrs. The mean mite survival times on *A. cerana* and *A. mellifera* were 51.6 ± 5.4 and 30.3 ± 2.7 hrs., respectively.

Euvarroa sinhai successfully feed on adult workers of *A. florea* and *cerana*. The mean survival time on worker and drone pupae of *A. florea* was higher than from adult worker bees. The mite also showed a significant preference for adult drones over adult workers.

Introduction

Euvarroa sinhai Delfinado and Baker is a parasitic mite of drone brood of the dwarf honey bee, *Apis florea* (Delfinado and Baker 1974; Akratanakul and Burgett 1976). The reproductive phase of the mite takes place inside sealed drone brood cells (Akratanakul 1976). In addition to infesting drone brood of the dwarf honey bee, the mite also is found on adult drone and worker bees (Akratanakul and Burgett 1976; Koeniger *et al.* 1983; Mossadegh and Birjandi 1986). The adult female mites have been reported to be phoretic on adult worker bees and feed on the bees during broodless periods until the colony begins drone brood rearing whereupon the mites enter and reproduce in the drone brood cells (Mossadegh 1991).

Euvarroa sinhai has also been reported in debris from *A. mellifera* colonies (Sihag 1987), but it has not been observed to parasitize the brood of this bee species. Mossaddegh (1990a, 1990b) reported that *E. sinhai* was able to feed and reproduce on *A. mellifera* worker and drone brood under laboratory conditions.

The ability of *E. sinhai* to survive on its natural host outside of the reproductive niche of drone brood, is still not fully studied. Mossadegh (1991) observed that female mites live on the cluster of bees 4 to10.5 months when drone brood is not present. Survivorship of female mites on adult bees is one of the phenomenon necessary to understand the dispersal of *Euvarroa* mites among the colonies of *A. florea*. It is not known whether this mite can survive on adult bees of other species of honey bees. The objectives of this study were to examine the longevity of *Euvarroa* female mites and their ability to feed on adult workers of three species of honey bee, *A. florea, A. cerana* and *A. mellifera*. Also I wished to determine whether the sex of honey bee influences the host preference by adult *Euvarroa* females and, lastly, the longevity of female mites on pupae as compared to adults.

Materials and Methods

The experiments were conducted under laboratory conditions during March-June 1992 using the facilities of the Entomology Department, Chiang Mai University, Thailand. Adult worker bees of *A. florea* were collected from a feral colony by taking the curtain layer of worker bees covering the comb. *Apis mellifera* and *A. cerana* workers were taken from hive comb. The bees were placed in the test containers and given 6 hrs. to acclimate before testing.

Euvarroa sinhai females were collected from infested sealed drone brood (late pupae, emerging bees) of an *A. florea* brood comb and placed in a tube 1 hr. before being transferred to the bees.

Survival of *E. sinhai* on adult workers of three honey bee species

The test containers consisted of inverted plastic cups: 6.5 cm. diameter, 8.5 cm. height for *A. mellifera*, *A. cerana* and 5.5 cm. diameter, 7.5 cm. height for *A. florea*. The lid was fitted with a wire screen which became the bottom. The bottom of the plastic cup became the top where feeders with sugar syrup (1:1 sucrose v/v; Fig. V.1.) were inserted. The containers were placed over petri dishes. The floor of the petri dish was covered with a layer of vaseline as a trap for mites that fell from the bees.

Ten mites were inoculated on the thorax or abdomen of 20 worker bees of each honey bee species and kept in the test containers at room temperature (33 °C). Five containers were used for *A. florea* and *A. mellifera*, 3 containers were used for *A. cerana* and 4 containers were used for control (no worker bees). Mite mortality was observed on every 4 hrs. until the last mite died.

The ability of *E. sinhai* to feed on adult workers of three honey bee species

Congo Red dye was used for injection into haemocoel of worker bees (Örösi-Pál 1934; Royce 1989). The dye was dissolved in Hayes saline and filtered twice before use. Worker bees from each species were anesthetized by cold temperature. Individual bee from each species were injected with 1, 2 or 3 microlitres of a 3% Congo Red-Hayes saline solution for *florea*, *cerana* and *mellifera*, respectively through the dorsal neck membrane using a calibrated microcapillary tube (Royce 1989). *Euvarroa sinhai* were inoculated onto the thorax or abdomen of individually dyed bee and kept in the test vials (a small vial: 0.9 cm. diameter and 3.8 cm. length) at room temperature. The dead mites from the experiment were collected and examined for dye-tagged haemolymph.

Longevity of *E. sinhai* on *A. florea* brood

An ELISA plate rearing technique described by Rath (1991) was used in this experiment. Pink, and purple-eyed worker and drone *A. florea* pupae were removed from a brood comb and placed in the ELISA microtiter cells. The pupae were replaced

with fresh ones every 3-4 days. The female *Euvarroa* mites were collected from infested sealed brood (late pupae) of *A. florea* and transferred into each ELISA cell with bee pupae. The cells were sealed with plastic sheet with a central hole of 0.5 mm. diameter. The ELISA plates were kept in an incubator at 34 °C and a relative humidity of 70%. The survival time of mites was recorded every 12 hrs.

Host gender preference of *E. sinhai* on adult *A. florea*

Adult workers and drones (<48 hrs. old) were used in these experiments. Trials were performed in plastic containers: 3 cm. diameter and 2.5 cm. height. A worker, a drone and a mite were simultaneously introduced into an individual container. The trial ended after the mite attached successfully to either host, then the mite was removed. The attachment site of *Euvarroa* mite on the bee was recorded. Each pair of worker and drone was used twice after being given 30 minutes to recuperate after the first trial. Fifty pairs (trials) were performed.

Results

Survival of *E. sinhai* on adult workers of three honey bee species

The mean survival time of *E. sinhai* on worker bees of *A. florea*, *A. cerana* and *A. mellifera* were analyzed and compared. Analysis of variance showed the differences between the bee species to be significant ($P<0.0001$, Table V.1.). The longest mite survival time was observed with *A. florea*, 56.8 ± 9.2 hrs. and differed significantly from the mean survival on *A. mellifera*, 30.3 ± 2.7 hrs. and control (no worker bees), 16.8 ± 1.3 hrs. The result from *A. cerana*, 51.6 ± 5.4 hrs. was not significantly different from *A. florea*.

The differences of mite survival from three species of honey bees are shown in Fig. V.2. More than 50% of the mite mortality was observed on *A. florea* and *A. cerana* after 36 hrs.; the longest time for the last mite was 287 and 96 hrs., respectively. LT_{50}

for mite on *A. mellifera* occurred after 24 hrs. and the longest time for the last mite was 69 hrs. In the control (no worker bees), LT_{50} was recorded after 12 hrs. and the longest survival time was 39 hrs.

The ability of *E. sinhai* to feed on adult workers of three honey bee species

Euvarroa mites collected from dye injected workers of *A. florea* and *A. cerana* showed the dye in their bodies very clearly when compared with mites from undyed bees (Fig. V.3.). The dye was barely evident in the mites' bodies that had fed on *A. mellifera*. Most mites preferred to attach between the abdominal segments or between thorax and abdomen or the ventral part of the thorax.

Longevity of *E. sinhai* on *A. florea* brood

More than 50% of mites on drone and worker pupae in ELISA plates survived 5 days. The longest time for the last mite on drone pupae was ca. 14 days and on worker pupae was ca. 8 days (Table V.2.). The longevity of mites in ELISA cells without pupae was 26.1 hrs. and the longest survival for the last mite was 46 hrs.

Host gender preference of *E. sinhai* on adult *A. florea*

Euvarroa mites preferred to attach to adult drone bees more often than the adult worker bees in 32 of 50 trials. The adult drones were significantly more attractive to adult female mites (χ^2=3.92, df=1, P< 0.05).

Figure V.4. shows the preference for attachment sites of *E. sinhai* on adult worker and drone of *A. florea*. *Euvarroa* mites were most frequently found on the abdomen of worker bees, 50% of all mites being found there; 44.4% were on the thorax and 5.5% were on the wings. On adult drone hosts, 75% were found on the thorax, 15.6% on the abdomen, 6.3% on legs and 3.1% on wings.

Discussion

Mean survival time of *Euvarroa* mites on worker bees of *A. florea* and *A. cerana* were significantly different from *A. mellifera* and the control (no worker bees). Although, the mean mite survival time on *A. florea* was similar to *A. cerana*, the longest time for the last mite on *A. florea* was by ca. three times longer than on *A. cerana*. In terms of adult mite survival worker bees of *A. florea* are the best host for *Euvarroa*. For the control (no worker bees), the mites died in ca.17 hrs. with the longest survivorship for the last mite at about 40 hrs. These results suggest the possibility of the mites feeding on worker bees of both Asian honey bee species, *A. florea* and *A. cerana* but not being able to feed on *A. mellifera*.

According to Mossadegh (1991), *Euvarroa* female mites are phoretic on *A. florea* workers and he suggested that mites feed on bees during broodless periods. Another possibility is that Euvarroa can survive on the cluster of honey bee without feeding until drone brood rearing restarts in the colony then the mites start to reproduce in the drone brood. Farish and Axtell (1971) studied on phoretic behavior of the mite, *Macrocheles muscaedomesticae* on the house fly, *Musca domestica*. They reported that there is no feeding or ontogenesis during temporary attachment of the phoresy. However, the survival time of *Euvarroa* on *A. florea* drone and worker pupae were longer than on adult bees, suggesting that pupae provided a better nutritional source for the mite.

The survivorship study of another species of brood parasitic mite, *Tropilaelaps clareae* on its natural host, *A. dorsata* was reported by Koeniger and Muzaffar (1988). *Tropilaelaps clareae* with *A. dorsata* survives longer than with *A. cerana* and *A. mellifera*. They explained that *T. clareae* takes up food from adult bees of *A. dorsata* but not from *A. cerana* nor *A. mellifera*, possibly due to structural differences of the cuticle. However, they also stated that *T. clareae* does not feed on adult *A. dorsata* bees. The putative quiescent behavior of this mite species, when phoretic on its natural

host species, is suggested to result in conservation of energy allowing greater longevity than that of mites on other bee species.

The results from the dye-tagged haemolymph of adult bees support the hypothesis that *Euvarroa* mites feed during the phoretic period. The differences in the structure of the cuticle between *A. florea, A. cerana* and *A. mellifera* probably limit the ability of the mite to penetrate the cuticle of *A. mellifera* adult bees. The physiological condition of the mites used in this study should be taken into consideration. Female mites from this study were collected from newly eclosed adult bees and may have possessed a difference in feeding ability compared to the mites collected from older adult bees in the colonies.

The preference of female *Euvarroa* for drones was higher than workers. The differences in host preference between workers and drones might be caused by gender differences in response to phoresy, i.e., drones may not engage in autogrooming, or may be only infrequently allogroomed by sisters. Worker bees do attempt to remove mites on the thorax or abdomen. This indicates self-cleaning (auto-grooming) of *A. florea* workers against *Euvarroa* mites. However, this behavior was not observed with drones. Grooming behavior by other Asian honey bee species against parasitic bee mite species has been reported (Bücher *et al.* 1992). They reported that the Asian honey bee species, *A. cerana* and *A. dorsata* display autogrooming in response to the infestation with *Tropilaelaps clareae* and *Varroa jacobsoni.*

According to Royce and Rossignol (1991), the honey bee tracheal mite, *Acarapis woodi* (Rennie), an endoparasite of adult bees, displays a preference for drones over worker bees of *A. mellifera.* This gender preference behavior by the mite may either enhance or lower transmission between colonies, depending on drone longevity and rate of contact with young workers. The transmission mechanisms of *Euvarroa* between *A. florea* colonies remains unknown. However, drone preference by the mite might be an important aspect of intercolony transmission. Longevity of adult *A. florea* drones is

unknown. For *A. mellifera*, life spans of adult drones are highly variable depending on climate and weather (Currie 1987). If the adult drones of *A. florea* are long-lived, they may well be a factor, via drifting behavior, in the intercolonial movement of mites. On the other hand, if drones are short-lived, the mite transmission would be lower and might reduce mite transmission rates between *A. florea* honey bee colonies.

Table V. 1. Mean survival of *Euvarroa sinhai* on worker of three honey bee species at room temperature (33 °C)

Bee species	Mean survival time (SE) (hrs.)	Last mite longevity (hrs.)
Apis florea	56.8 (9.2) a	287
Apis cerana	51.6 (5.4) a	96
Apis mellifera	30.3 (2.7) b	69
Control (no worker bees)	16.8 (1.3) b	39

Means with the same letter do not possess a significance difference in mean survival (P=0.05, LSD test)

Table V. 2. The longevity of *Euvarroa sinhai* on *Apis florea* pupae

	Mean survival time (SE) (hrs.)	Last mite longevity (hrs.)
Drone pupae	147.8 (12.4)	336
Worker pupae	131.2 (7.2)	207
Control (no pupae)	26.1 (1.7)	46

A **B**

Figure V. 1. The test containers for *Apis cerana* and *A. mellifera* (A), *A. florea* (B)

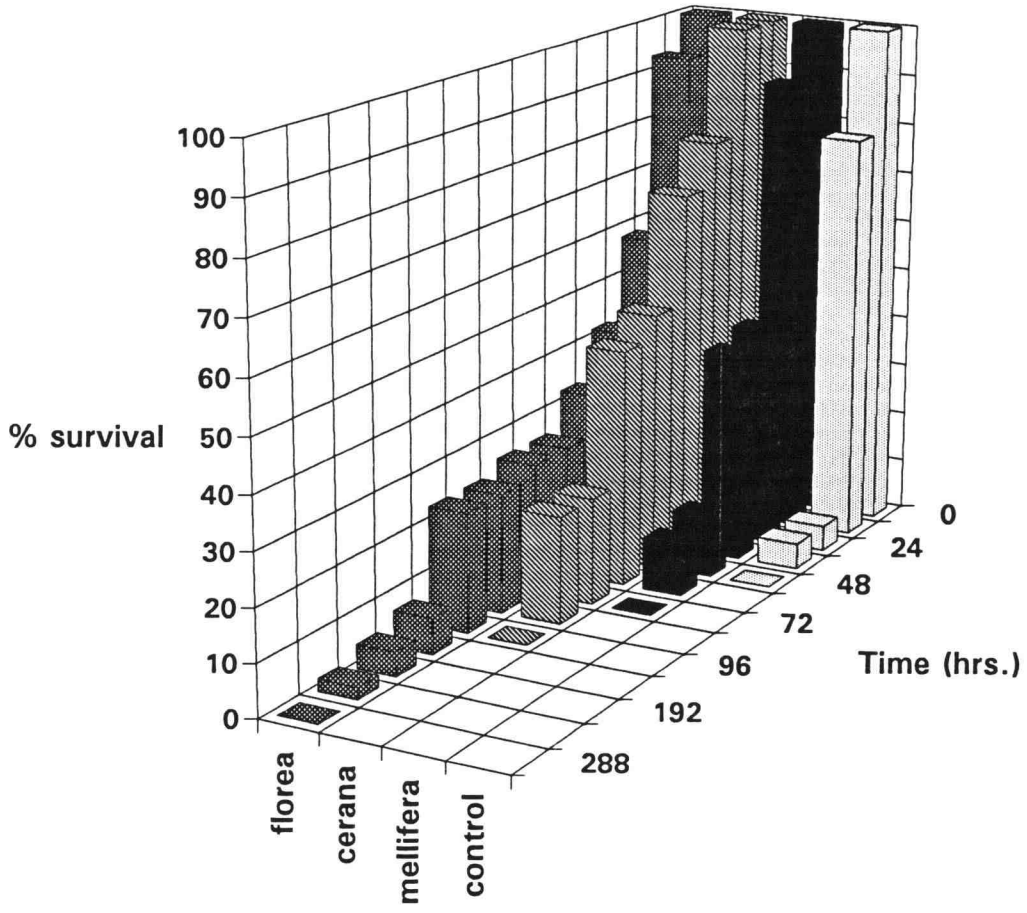
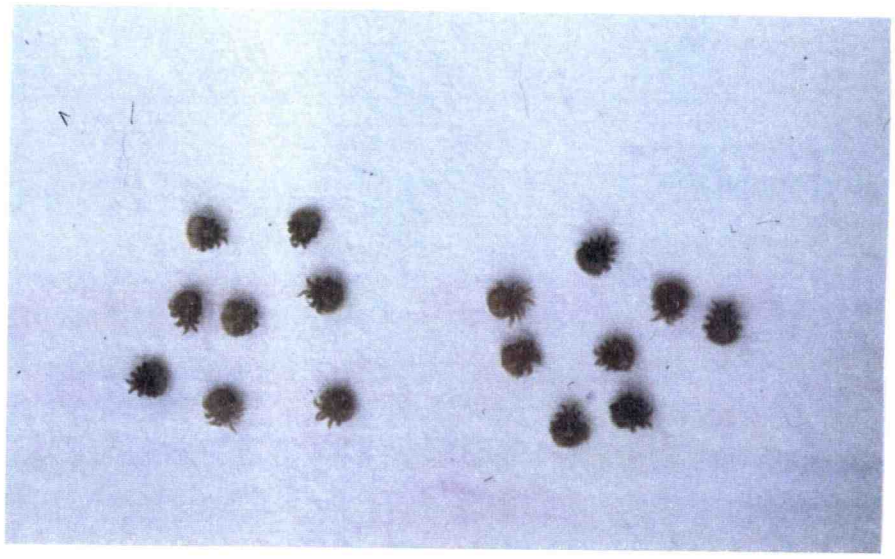


Figure V. 2. Survivorship of *Euvarroa sinhai* on three species of honey bees: *Apis florea, A. cerana* and *A. mellifera* compared with control (no worker bees)



A B

Figure V. 3. Comparison between mites from undyed bees (A) and mites from dyed bees (B)

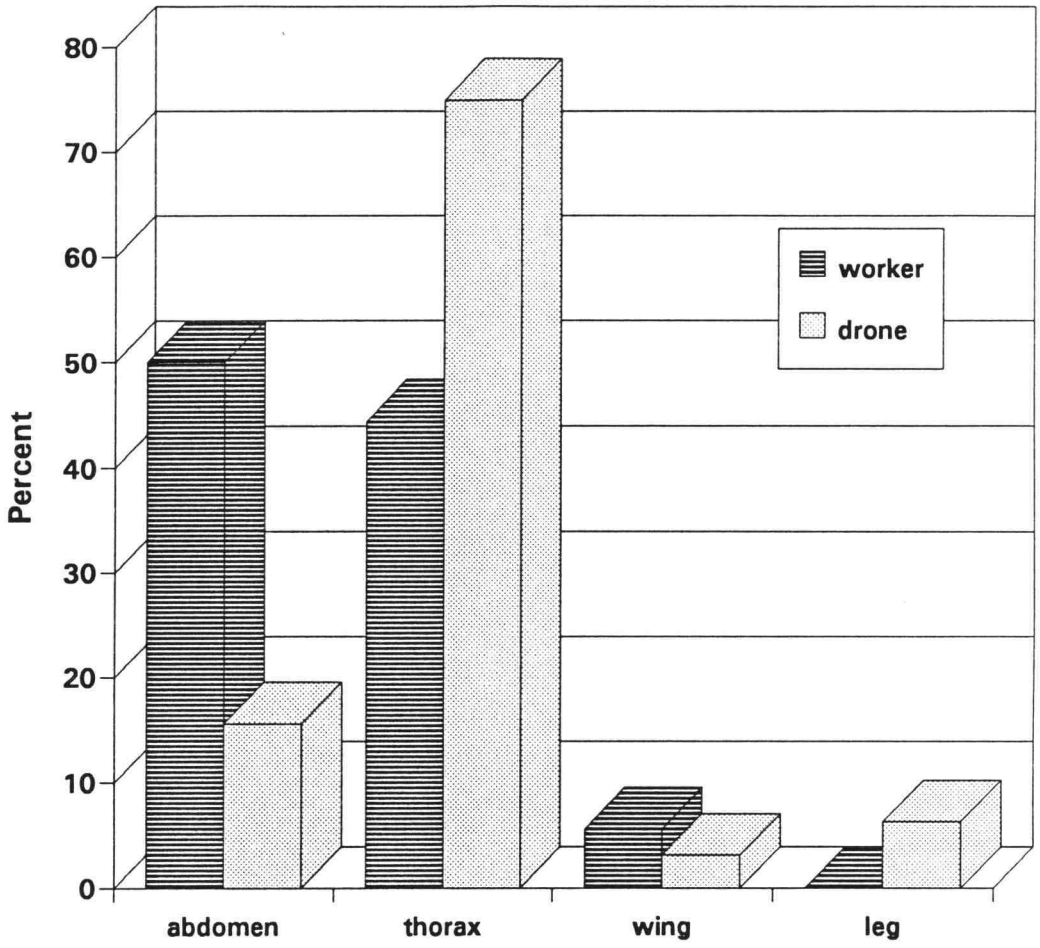


Figure V. 4. Attachment site preference of *Euvarroa sinhai* on adult *Apis florea* workers and drones

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CHAPTER VI
OBSERVATIONS ON THE REPRODUCTION OF THE PARASITIC BROOD MITE, *EUVARROA SINHAI* DELFINADO AND BAKER IN COLONIES OF THE DWARF HONEY BEE, *APIS FLOREA* F.

Abstract

The reproduction of *Euvarroa sinhai* on drone brood of *Apis florea* was examined from natural colonies in northern Thailand. Nineteen of twenty-eight colonies of *A. florea* queenright colonies were parasitized by *Euvarroa* mites with drone brood infestation rates ranging from 1.7 to 56.3%. In six queenless colonies, all colonies were mite infested and the infestations were high ranging from 13.0 to 63.5% of the drone brood. No worker brood were parasitized by *Euvarroa* mites. The average number of *Euvarroa* offspring was 3.3 mites per host in queenright colonies and 3.6 mites per host in queenless colonies. Parent female mite reproductive rates were 2.59 in queenright colonies and 3.2 in queenless colonies. In queenright colonies, 77.2% of the infested drone pupae possessed a single invasive parent female. In queenless colonies, drone pupae were infested by single parent females only 33.0% of the mites, meaning infested drone pupae in queenless colonies possessed multiple invasive females twice as often (67%) as drone pupae in queeright colonies.

Introduction

The mite *Euvarroa sinhai* was reported as an ectoparasite of the dwarf honey bee, *Apis florea* (Delfinado and Baker 1974; Akratanakul 1976). This mite has not been reported to parasitize other species of *Apis*. *Euvarroa* occurs only in Asia where *A. florea* is present, including Iran, India, and Southeast Asia (Delfinado and Baker 1974; Akratanakul and Burgett 1976; Koeniger *et al.* 1983; Mossadegh and Birjandi 1986).

Only drone brood of *A. florea* are parasitized by *E. sinhai*. The reproduction by *Euvarroa* has never been reported in worker brood cells. The life cycle of *Euvarroa*

comprises the egg, larva, protonymph, deutonymph and adult stages. Akratanakul (1976) observed that the larva molt to the protonymphal stasis occurs within the egg. The larva is an inactive stage; but the protonymph, deutonymph and adult female stages feed on haemolymph of honey bee pupae. All mite developmental stages take place inside the sealed drone brood cells with only the adult female mites leaving the cells after host bee emergence.

Ontogenesis of *E. sinhai* on *A. mellifera* drone and worker brood was observed by Mossadegh (1990a, 1990b) under laboratory conditions. He reported that *Euvarroa* is capable of feeding and reproduction on *A. mellifera*, an unadapted and unreported host. Under the conditions of his trials the developmental period from egg to adult was 5 days for males and 6-7 days for females. The developmental period for *Euvarroa* on drone brood of *A. florea*, its natural host, has not been reported.

In this study, the reproduction of *E. sinhai* on drone brood of *A. florea* was observed under natural brood infestation conditions.

Materials and Methods

The study was carried out in northern Thailand during March through June 1992-1993. Twenty-eight queenright colonies and six queenless colonies of *A. florea* were collected from the field or purchased in the markets. Sealed drone and worker brood cells (prepupal and pupal stages) of *A. florea* were sampled from the colonies.

Six categories of drone pupal development based on the degree of eye pigmentation were noted: PP, (prepupae), 2-3 days old post cell capping; PI, (pupal stage I), no eye pigmentation, 4 days post cell capping; PII, (pupal stage II), pink-eyed pupae, 5-6 days post cell capping; PIII, (pupal stage III), purple-eyed pupae, 7-8 days post cell capping; PIV (pupal stage IV), dark brown eyed with thorax pigmented, 9-10 days post cell capping, and PV (pupal stage V), complete body pigmentation, 11-13 days post cell capping.

A total of 2,896 drone brood cells, and 7,000 worker brood cells from queenright colonies and 438 drone brood cells from queenless colonies were examined for *Euvarroa* infestation. The numbers of mites in each developmental stage (egg, protonymph, deutonymph and adult) were recorded from each infested cell for each pupal category.

Results

Most of the *A. florea* colonies were infested by *Euvarroa*: 67.8% of the queenright colonies (n=28) and 100% of the queenless colonies (n=6). The intercolonial level of mite infestation varied greatly among queenright colonies. The average infestation was 13.3% (range from 1.7-56.3%, Table VI.1.). Queenless colonies had higher infestation rates. The average infestation was 48.2% (range from 13.0-63.5%, Table VI.2.). *Euvarroa* mites were not found in any of the 7,000 worker brood cells examined.

The occurrence of *E. sinhai* on drone brood of *A. florea* queenright and queenless colonies is shown in Table VI.3. and VI.4. All developmental stages of the mites were found in the sealed drone brood cells during the March-June study period. The percentage of *Euvarroa* mites and offspring in drone brood from queenright and queenless colonies are presented in Fig. VI.1. and VI.2. In queenright colonies, the percentage of eggs was highest during the prepupal stage and decreased as the pupae aged. Protonymphal stages were encountered on prepupae. The protonymph can be distinguished from a deutonymph on the basis of body size and setal characters. Adult F_1 *Euvarroa* males were first observed on purple-eyed pupae (7-8 days post cell capping). The weakly sclerotized male *Euvarroa* is smaller than a female and different in body shape. F_1 adult female offspring from parent female *Euvarroa* were found associated with pupal stage IV (9-10 days after cell capping). Young F_1 females can be distinguished from parent females on the basis of color; the parent mites have a darker color than their F_1 female offspring.

This generalized pattern of *Euvarroa* development in drone brood from queenright colonies was also observed in queenless colonies. However, with queenless colonies, mite eggs were found to occur from the prepupal stage through pupal stage IV, a longer period than in queenright colonies. The mite sex ratio (male:female) measured from males and females (F_1 females combined with parent females) on drone pupa stage V, was 1:2.9 for queenright colonies and 1:2.3 for queenless colonies.

The average number of *Euvarroa* offspring per parent female from queenright colonies was 3.3 mites measured from 33 offspring collected from ten infested drone pupae (pupal stage IV, 9-10 days old after cell capping) which had one invasive parent female mite per cell. In queenless colonies, the average number of offspring per parent female was 3.6 mites under the condition of a single invasive parent female mite per drone host (18 mites offspring collected from 5 infested drone pupa stage IV). Because of the small sample size from pupal stage IV, data from pupal stage III were added for estimating the reproductive rate of a single female mite. The actual and potential reproduction rates of *Euvarroa* females on drone brood pupae were 2.59 and 2.86, respectively in queenright colonies and 3.2 for mites in queenless colonies (Table VI.5.). The percentage of non-reproductive females in sealed drone brood in queenright colonies was 9.5% (of 74 parent female mites in sealed drone cells, 67 produced offspring).

For queenright colonies drone pupae were most often infested by single parent mite females (77.2% of the infested cells), however, in queenless colonies it was only 33%, Fig. VI.3.). In queenless colonies up to eight parent females could be found per host drone cell. The frequency distribution for the number of progeny of a single reproducing female mite is given in Fig. VI.4. In queenright colonies most of parent female mites (22.1- 27.9% 0f the infested brood) had 2-4 progeny per cell. The maximum number of progeny was seven which occurred in only 1.5% of the infested brood. In queenless colonies, 50% of the infested drone brood had three progeny per

host. The maximum number of progeny was six per host and this occurred in 10% of the infested brood.

Discussion

In *A. florea* colonies, *Euvarroa* mites were found to parasitize and reproduce only inside sealed drone brood. Although the developmental time for sealed *A. florea* drone and worker brood is quite similar (average period for capped brood is ca.12.8 days for drone and 11.2 days for worker (Ruttner, 1987)), worker brood are not parasitized by *Euvarroa*. Akratanakul (1976) suggested possible factors determining why *Euvarroa* mites prefer drone rather than worker brood. Those factors include the larger size of the brood drone pupal host when compared to that of the worker; the immature period for drone brood is longer by ca. 1.6 days, and the passive behavior of the drone. The role of kairomones from the host brood mites might well also play a role. Le Conte *et al.* (1989) reported the attraction of *Varroa jacobsoni* to the drone larvae of *A. mellifera* by kairomones. This potential host-finding mechanism should be investigated for *Euvarroa* and *A. florea.*

Mossadegh (1990a, 1990b) reported that *Euvarroa* mites reproduce on drone and worker brood of *A. mellifera* under laboratory conditions. However, this mite has not been found on parasitizing drone or worker brood from *A. mellifera* colonies present in Southeast Asia. *Euvarroa* mites are known only to successfully reproduce in drone brood of *A. florea*. The reasons for inability of *E. sinhai* to parasitize *A. mellifera* are still unknown. Although *Euvarroa* mites have been reported in the debris from *A. mellifera* colonies (Sihag 1987) and also found in *A. dorsata* colony debris (Burgett and Sukumalanand, pers. comm.), this mite has not been reported to parasitize other honey bee species besides *A. florea.*

During the observation period of March through June, the mean infestation rate of *Euvarroa* mites from queenless colonies of *A. florea* was heavy and higher than from

queenright colonies. This evidence supports the observation of Akratanakul (1976) that queenless *A. florea* colonies are usually more heavily infested than queenright. A similar phenomenon has been reported for the brood mite *T. clareae* and its native host *A. dorsata* in the Philippines (Morse and Laigo 1969). Additionally, *Euvarroa* eggs were found in later stages of pupal development in queenless colonies than queenright colonies. I also observed a greater frequency of multiple parent invasive females per pupal drone host in queenless colonies. All these data support a hypothesis that in queenless *A. florea* colonies the *Euvarroa* defensive barriers are more readily overcome.

The average number of F_1 offspring produced from infested drone pupa was 3.3 in queenright and 3.6 in queenless colonies which differs from the study of Mossadegh and Birjandi (1986). They reported that the infestation rate of *Euvarroa* mites on drone *A. florea* drone brood in Iran was 31% in both queenless and queenright colonies and the average number of immature mites per drone cell was high (6.0 in queenright and 6.9 in queenless colonies). However these data are from a single queenright colony and a single queenless colony.

Euvarroa reproduction occurred in *A. florea* drone brood throughout the study period (March through June). Non-reproducing females were found in drone cells of infested colonies but their numbers were low. Drone brood was not examined for mite reproduction from July to December because drone brood production in *A. florea* colonies decreases during that time period and was not available for examination. Kapil and Aggarwal (1988) suggest that *Euvarroa* appears to reproduce during March-April and September and there is a non-reproductive period between these two reproductive peaks. During periods when drone brood is not present in colonies female mites apparently survive phoretically on adult workers. However, in my study, non-infested colonies were found in the same period month as the infested colonies (three colonies in March, one colony in April, three colonies in May and two colonies in June).

The reproductive rate of *Euvarroa* mites was estimated by examining F_1 progeny from infested drone pupa cells. It was 2.59 in queenright and 3.2 in queenless colonies. According to Mossadegh (1990a, 1990b), the actual reproductive rate of *E. sinhai* was 3.6 in worker brood and 4.9 in drone brood of *A. mellifera* which was higher than the *A. florea* drone brood rate from my study.

The percentage of invasive female mite in queenless colonies with more than 3 was higher than in queenright colonies, but the average number of offspring per reproducing female mite was not. The reason might depend on the number of drone brood in the colonies. Queenless colonies have only drone brood albeit quantitatively less than the amount found in queenright colonies.

A quandary exists as to the absence of any reports of *Euvarroa* infestation of *Apis mellifera* colonies in Southeast Asia. *Apis mellifera* is characterized as having no specific defense mechanisms against haemophagic brood mites, as is evidenced by the ferocity with which *V. jacobsoni* and *T. clareae* ravage *A. mellifera* colonies. This conundrum is further exacerbated by Mossagh's data which show, at least under laboratory conditions, that *Euvarroa* is reproductively viable when infesting *A. mellifera* brood.

Table VI. 1. *Euvarroa sinhai* infestation on drone brood of queenright *Apis florea* colonies.

Month	n	No. of cells examined	No. of infested cells	Average % infestation	Minimum % infestation	Maximum % infestation
March 1992	4	370	27	7.3	1.7	15.6
April 1992	1	138	10	7.2	-	-
May 1992	6	788	47	6.0	2.6	12.5
June 1992	2	158	25	15.8	14.0	19.0
March 1993	1	100	38	38.0	-	-
May 1993	5	494	65	13.1	6.7	56.3

n = No. of *A. florea* colonies

Table VI. 2. *Euvarroa sinhai* infestation on drone brood of queenless *Apis florea* colonies.

Month	n	No. of cells examined	No. of infested cells	% infestation
March 1992	1	138	81	58.7
April 1992	1	121	66	54.5
May 1992	1	45	27	60.0
May 1992	1	23	3	13.0
March 1993	1	53	21	39.6
April 1993	1	52	33	63.5
Average				**48.2**

n = No. of *A. florea* colonies

Table VI. 3. The occurrence of various developmental stages of *Euvarroa sinhai* on the different stages of drone brood of queenright *Apis florea* colonies.

Colony	Date collected	No. of cells examined	No. of infested cells	Drone brood stages	Mite developmental stages				
					female	male	deuto-nymph	proto-nymph	egg
1A	3/9/92	100	3	PIII	5	0	2	1	0
2A	3/9/92	115	2	PIII	3	0	2	1	0
3A	3/9/92	122	19	PV	44	15	7	0	0
4A	3/9/92	33	3	PV	8	3	0	0	0
5B	4/6/92	33	1	PP	1	0	0	0	0
		25	2	PI	2	0	0	3	0
		80	7	PIII	8	0	7	9	3
6C	5/27/92	93	8	PIII	11	0	9	8	0
7C	5/27/92	50	1	PP	1	0	0	0	0
		10	1	PI	1	0	0	1	0
		47	8	PIII	12	6	17	5	0
8C	5/27/92	25	1	PP	1	0	0	0	1
		63	10	PIII	10	0	12	10	2
9C	5/27/92	20	1	PI	2	0	0	1	0
		55	5	PIII	5	0	3	4	1
10C	5/27/92	30	1	PP	1	0	0	0	0
		165	4	PIII	5	0	6	3	0
11C	5/27/92	230	7	PIII	9	0	13	2	0
12D	6/25/92	14	2	PP	2	0	0	0	0
		7	2	PI	3	0	0	2	0
		37	7	PIII	12	2	9	3	0
13D	6/29/92	100	14	PIII	15	0	27	22	1
14D	3/15/93	14	5	PP	7	0	0	7	4
		44	10	PI	14	0	7	18	6
		35	18	PII	26	0	34	32	9
		7	5	PIII	6	0	14	7	4
15D	5/1/93	72	14	PP	17	0	0	1	2
16D	5/3/93	75	5	PIV	6	0	15	4	0
17D	5/4/93	32	18	PIV	42	19	38	9	0
18D	5/5/93	130	5	PP	6	0	0	1	3
		20	7	PI	13	0	0	20	2
19D	5/26/93	85	3	PP	3	0	0	3	2
		37	7	PI	8	0	3	15	1
		43	6	PII	7	0	6	8	3

Table VI. 4. The occurrence of various developmental stages of *Euvarroa sinhai* on the different stages of drone brood of queenless *Apis florea* colonies.

Colony	Date collected	No. of cells examined	No. of infested cells	Drone brood stages	Mite developmental stages				
					female	male	deuto-nymph	proto-nymph	egg
1A	3/9/92	23	14	PP	33	0	0	5	0
		70	40	PIII	89	4	3	2	0
		45	27	PV	65	30	0	0	0
2D	4/13/92	31	23	PP	60	0	0	13	15
		15	6	PI	19	0	4	21	11
		30	15	PIII	46	7	63	25	7
		23	11	PIV	34	13	12	1	0
		22	11	PV	41	9	2	0	0
3D	5/9/92	20	12	PP	23	0	0	5	11
		11	7	PIII	33	5	37	33	11
		4	2	PIV	24	10	18	8	5
		10	6	PV	11	5	0	0	0
4D	5/13/92	23	3	PIII	10	3	6	1	0
5D	3/14/93	22	13	PP	27	0	0	5	5
		3	3	PI	5	0	0	9	3
		21	1	PIII	1	0	1	2	0
		7	4	PV	6	10	0	0	0
6D	4/20/93	20	11	PP	26	0	0	11	10
		14	7	PI	13	0	4	16	7
		12	11	PII	25	0	50	21	1
		6	4	PIII	10	0	22	10	0

Location:
A = Doi Tao, Chiangmai
B = Lampang
C = Nakorn Sawan
D = Mae Rim, Chiangmai

Developmental stages of drone brood:
PP = prepupa
PI = pupa I, no eye pigmentation
PII = pupa II, pink-eyed pupa
PIII = pupa III, purple-eyed pupa
PIV = pupa IV, dark brown eyed with thorax pigmented
PV = pupa V, completed body pigmentation

Table VI. 5. Reproductive rates of female *Euvarroa* mites in *Apis florea* drone brood: Queenright vs. Queenless colonies.

	Total mite progeny (a)	Parent female mites			Reproductive rate	
		Total (b)	Reproducing (c)	Non-reproducing (d)	Actual a/b	Potential a/c
Queenright	192	74	67	7	2.59	2.86
Queenless	32	10	10	0	3.2	3.2

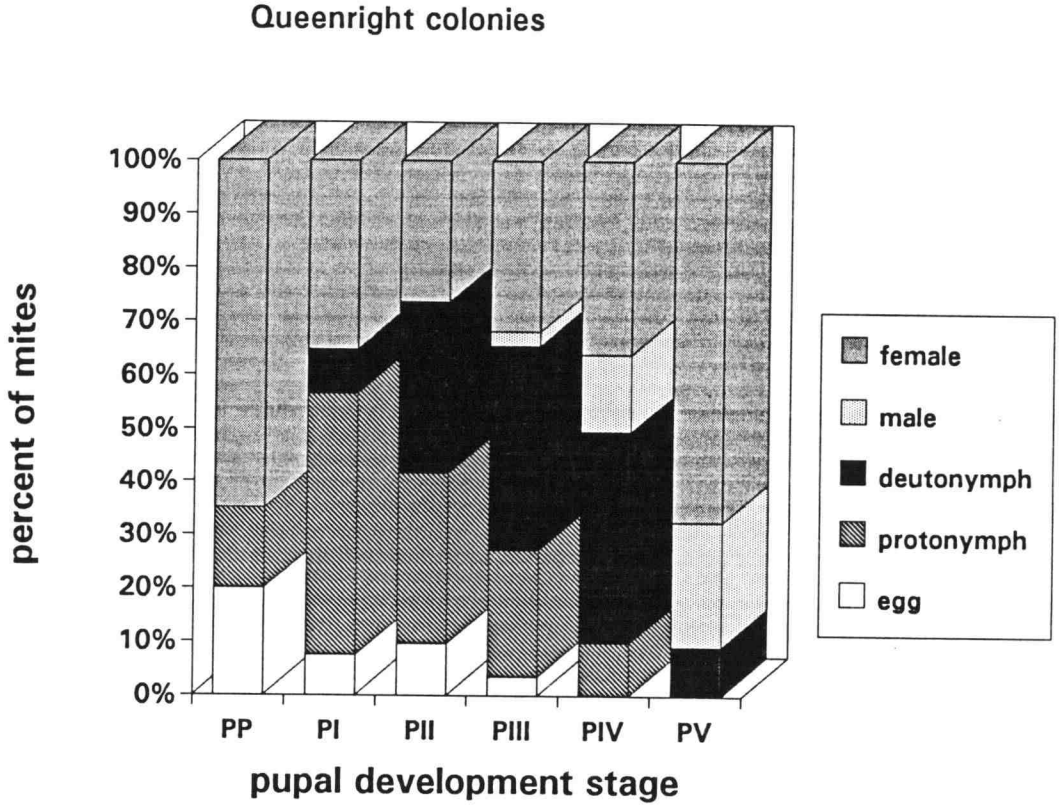

Figure VI. 1. *Euvarroa* female mites and offspring from each developmental stage of drone brood from queenright *Apis florea* colonies

Developmental stages of drone brood:
PP = Prepupa
PI = PupaI, no eye pigmentation
PII = PupaII, pink-eyed pupa
PIII= PupaIII, purple-eyed pupa
PIV= PupaIV, dark brown eyes with thorax pigmented
PV = PupaV, completed body pigmentation

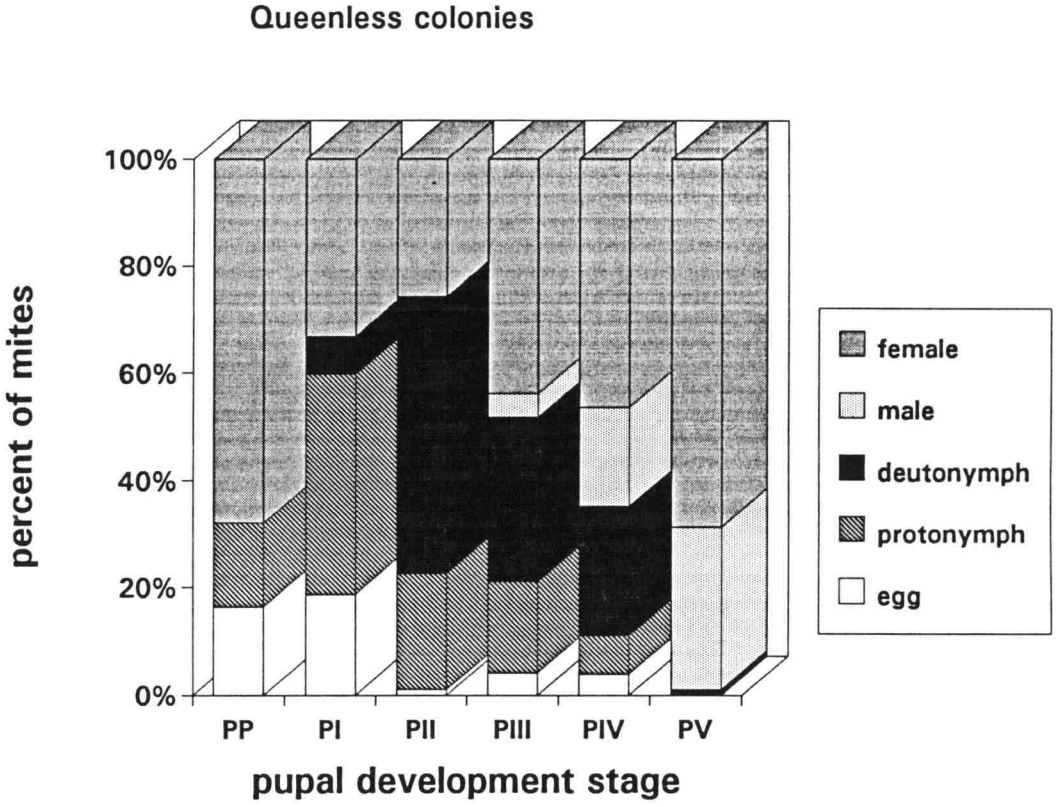

Figure VI. 2. *Euvarroa* female mites and offspring from each developmental stage of drone brood from queenless *Apis florea* colonies

Developmental stages of drone brood:
PP = Prepupa
PI = PupaI, no eye pigmentation
PII = PupaII, pink-eyed pupa
PIII= PupaIII, purple-eyed pupa
PIV= PupaIV, dark brown eyes with thorax pigmented
PV = PupaV, completed body pigmentation

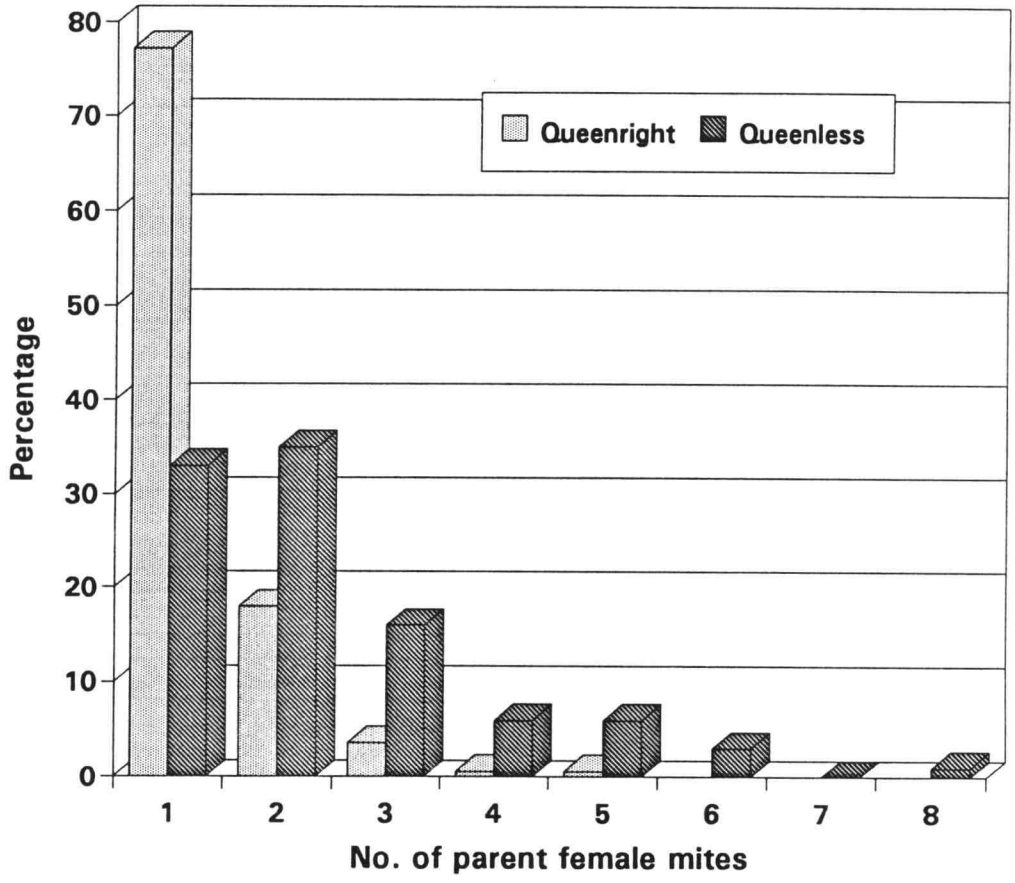

Figure VI. 3. The percentage of invasive female *Euvarroa sinhai* in drone pupa of queenright and queenless *Apis florea* colonies

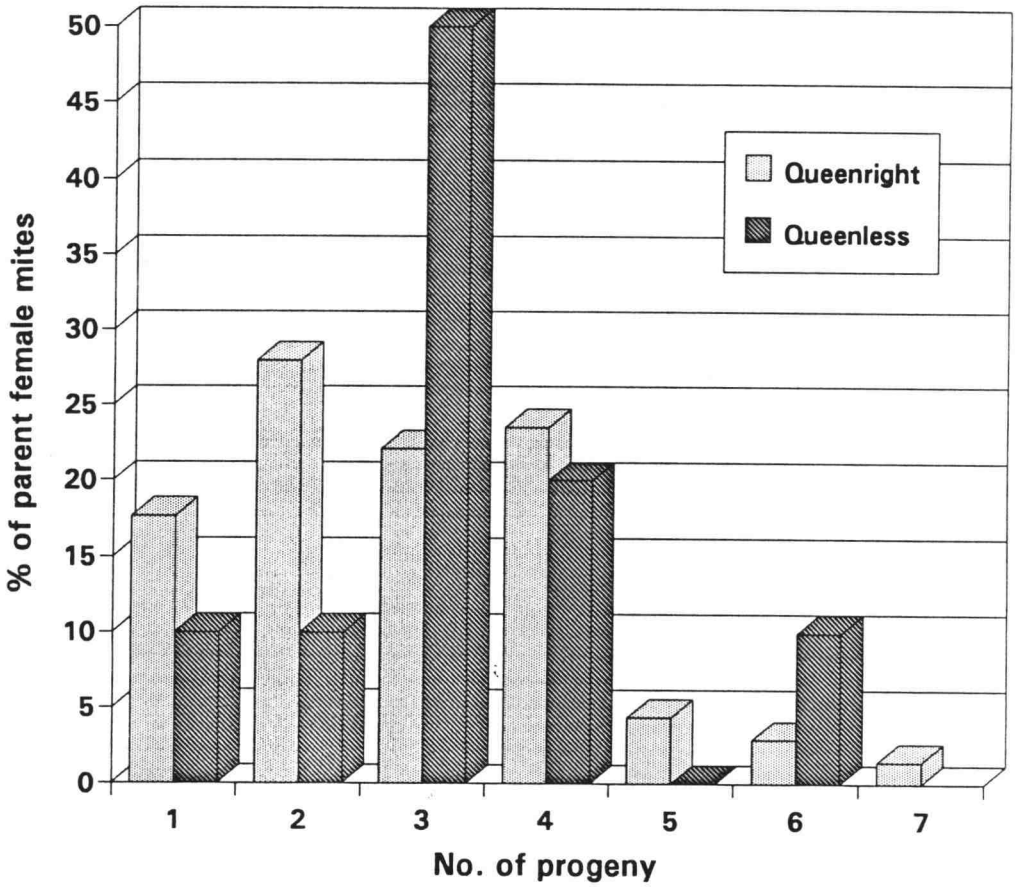

Figure VI. 4. Frequency distribution of progeny of a single reproducing female of *Euvarroa sinhai* in queenright and queenless *Apis florea* colonies

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SUMMARY

Euvarroa sinhai Delfinado and Baker parasitizes and reproduces only in the drone brood of the dwarf honey bee, *Apis florea* F. It remains unknown why *Euvarroa* mites prefer drone rather than worker brood. There are many possible factors such as period of development of sealed brood cells or nutrition or hormones from honey bee brood which cause the attraction of *Euvarroa* mites to the drone larvae. The infestation of this mite only on drone brood seems to be less harmful for the honey bee because the worker bees which take care of the important functions of colony maintenance are not damaged by the parasitic mite. Normally, prevalence infestation rate of *Euvarroa* in drone brood from queenright colonies was low. In this study, the level of mite infestation in queenless colonies was higher than in queenright colonies and queenless colonies also produced a high percentage of invasive female mites from infested brood cell. The reason might be a reduced ability of the honey bees in queenless colonies to defend against parasitic mites. The low number of drone brood in queenless colonies might be another factor that accounted for the high frequency of invasive female mites per infested cell.

The distribution patterns of *Euvarroa* mites in queenright and queenless *Apis florea* colonies were aggregated. A truncated negative binomial distribution of the mite on drone brood provided evidence that worker bees remove mite-infested drone pupae from the capped cells. Also, numbers of mites and dead drone pupae in the debris from *A. florea* colonies were positively correlated. This supports a general hypothesis that Asian honey bees have evolved specific mechanisms against their species-specific parasitic brood mites.

Population densities of *Euvarroa* mites were observed during various stages of *A. florea* colony development. The highest number of mites on adult bees occurred in colonies collected after swarming, as compared to swarms and other stages of colony development. It suggests that swarming affects mite population density within a colony

by leaving the parasitic mites on the mother colonies. Swarming behavior of honey bees seems to be one natural defense mechanism against brood mite parasites.

The survival and feeding ability of *Euvarroa* mites on adult worker bees was examined for several *Apis* species. Adult female mite survival was highest on *A. florea*, the adapted host, lower on *A. cerana*, and lowest on *A. mellifera*. Neither of the last two species are known to serve as *in vivo* hosts for *Euvarroa*. It is interesting that *Euvarroa* can survive as a phoretic so well on *A. cerana* but not on *A. mellifera*. Considering that *A. cerana* and *A. mellifera* are phylogenetically so close that one might have expected a similar *Euvarroa* survival rate on both species. As a caveat, the experimental conditions under which I examined phoresy might well have prevented the expression of *A. cerana* grooming behavior, which is known to be very effective at reducing phoretic adult *V. jacobsoni*.

The life cycles of *E. sinhai* and *V. jacobsoni* are very similar and both species are members of the same family (Varroidae), which gives rise to the question as to why *Euvarroa* mites have not been found to parasitize *A. cerana* colonies when *V. jacobsoni* is such a successful parasite of drone brood of that species. While *Euvarroa* mites are able to survive on the adult workers of *A. cerana* and reproduce on the drone and worker brood of *A. mellifera* under laboratory conditions, it is still unknown why they are not found *in vivo* as parasites of either *A. cerana* and *A. mellifera* in Southeast Asia.

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