# CHAPTER 4

# POPULATION AND SEX RATIO OF *Tropilaelaps clareae*IN DEBRIS AND DESERTED COMBS OF *Apis dorsata* IN THAILAND

#### Abstract

Debris from five *Apis dorsata* colonies was collected between May 2000 and July 2002 in Thailand. A total of 9,163 and 1,806 *Tropilaelaps clareae* were recovered from the colonies 1 and 2, respectively, located in Chiang Mai during a period of 250 and 176 days, respectively. The percentage of injured mite collected in colonies 1 and 2 was 94.7% (8,674 mites) and 90% (1,625 mites), respectively. The mite infestation rate on adult bees of colony 1 was 0.2%. No mites were found on 965 adult bees of colony 2. The three colonies located in Samut Songkhram province were either mite-free or had low levels (1-10 mites) of *T. clareae* infestation. The rate of *T. clareae* infestation on *A. dorsata* brood from deserted combs of colonies 1, 2 and 3 was 11.3, 21.8 and 0.9%, respectively. The bees of colonies 1, 2 and 3 had left 63, 53 and 3 mites in their own deserted combs after they migrated. These suggest that *A. dorsata* has efficient grooming behavior to remove mites from their bodies and also kill them, and *T. clareae* was not a main cause of migrations of these *A. dorsata* colonies. The male to female sex ratio of *T. clareae* from the debris ranged from 1:8 to 1:56. A lower ratio of 1:8.2 was observed on *A. dorsata* brood of deserted combs.

Key words: Tropilaelaps clareae / Apis doorsata / debris / sex ratio / deserted comb / Thailand

#### Introduction

Tropilaelaps clareae Delfinado & Baker (Acari: Laelapidae) is a natural brood parasite of *Apis dorsata* Fabr., a native honey bee species of South-East Asia (Burgett and Krantz, 1984; Wongsiri et al., 1989). *A. dorsata* lives in the open, in single comb nests hanging on cliffs, tree branches or eaves of buildings. This bee species has effective defense behavior to its foes, migrates seasonally over long distances and stops brood rearing while preparing for migrations (Ruttner, 1988).

The geographical distribution of *T. clareae* seems to be limited to tropical Asia and coincides with the indigenous areas of *A. dorsata* (Burgett et al., 1983; Burgett and Akratanakul, 1985; Delfinado-Baker and Aggarwal, 1987; Delfinado-Baker et al., 1989). However, infestation of *T. clareae* in *A. mellifera* brood was observed in Afghanistan and South Korea, which are outside the range of *A. dorsata* (Woyke, 1984; Woo and Lee, 2001; Otis and Kralj, 2001). *T. clareae* reproduces in sealed brood of honey bees. To date, *T. clareae* has been found associated with five species of honey bees: *A. dorsata*, *A. cerana*, *A. florea*, *A. laboriosa* and *A. mellifera* (Delfinado-Baker et al., 1992). Like *Varroa destructor*, *T. clareae* is a very serious pest of *A. mellifera* but not considered to be a dangerous pest of *A. dorsata* (Wongsiri et al., 1989).

When mite populations increase beyond the grooming capacity, *A. dorsata* colonies may start migrating in order to decrease the mite populations by leaving large numbers of mites behind (Wongsiri et al., 1989; Koeniger et al., 1993). Thapa (1998) contradicted these theories since only 7 mites were found from 22 cells examined in a deserted comb of *A. dorsata*. However, the grooming behavior of *A. dorsata* as measured by natural mortality of *T. clareae* through time and the clear impact of this mite on migrations of *A. dorsata* colonies have not been studied.

The sex ratio of *T. clareae* biased in favor of females and varied considerably. The male to female sex ratio under laboratory conditions was about 1:5 (Rath et al., 1991). The sex ratio (male: female) on *A. dorsata* brood in Thailand was 1:7.6 (Burgett

and Kitprasert, 1989). In debris of *A. mellifera* colonies, the ratio was 1:4 in Afghanistan (Woyke, 1990) and 1:2.6 to 1:3.1 in Thailand (Rath et al., 1991). An even lower sex ratio of this mite (1:1.8 to 1:6.7) was observed in debris of *A. dorsata* collected for about 2 months (Rath and Delfinado-Baker, 1990). However, variation of sex ratios of *T. clareae* within a bee colony through time has yet to be studied. Therefore, the objectives of this study were to investigate *T. clareae* populations and sex ratios in the debris across many months and in deserted combs of debris collected colonies.

# Materials and Methods

Debris of A. dorsata was collected from 5 colonies. Colonies 1 and 2, located at Maejo University in Chiang Mai (Northern Thailand), were monitored from October 2000 to July 2002. The ages of colonies 1 and 2 were approximately 10 and 3-months-old, respectively. These colonies built their nests under the eaves of a building (the same building). Three other colonies were located in Samut Songkhram province (Central Thailand) and were monitored from May 2000 to March 2001. These colonies built their nests underneath tree limbs. The ages of colonies 3 and 4 were uncertain; colony 5 was approximately 3-weeks-old. Debris from each colony was collected onto a white plastic sheet (1.4 m x 2 m; sprayed with vegetable oil). The traps were placed about 1 m below each nest using bamboo poles (Rath, 1991). The bamboo poles were smeared with engine oil to prevent scavengers from getting into the traps. Debris from all colonies was collected weekly except in November 2000 when debris was collected daily from colony 1. Collection of debris from colonies 1, 2 and 3 stopped when the colonies absconded, and deserted combs of these colonies were collected. All capped brood of each comb was examined for mites. A sample of adult bees from the nests of colonies 1 and 2 was also collected and examined for mites. Debris collections of colonies 4 and 5 ended when the colonies were harvested by honey hunters. Dead mites on the traps were retrieved using a fine paint brush. Mites from the debris and deserted combs were examined and classified by sex (using a stereomicroscope at 40X magnification). Populations, infestation rates and sex ratios of mites from deserted combs were estimated. Injured types of mites from debris were also determined. Injuries to the mites were classified as: (1) injured legs (missing legs or parts of legs), (2) injured body only, or (3) injured legs+injured body. Due to the ferocity of *A. dorsata* and the poor accessibility of the colonies, bee populations in each colony were not estimated. However, changes in exposed comb area were noted at each sampling time. Bee population was considered to have changed when exposed comb area increased. The entire comb of a populous colony is usually well covered by adult bees and thus cannot be seen without disturbing the bees.

#### Results

## T. clareae populations in debris and deserted combs of A. dorsata

The number of *T. clareae* adults collected in the debris and deserted combs greatly differed among the *A. dorsata* colonies (Table 4.1, 4.2). In Chiang Mai for colony 1, a total of 9,163 mites were recovered from the debris during the 250-day collection period. Of the mites collected, 94.7% (8,674 mites) were injured. Most mites (51.7%) suffered injuries on both legs and body, 48.1% had injured legs only and 0.2% showed body injury only. The majority of mites (4,790 mites) from the debris were collected in November 2000. Mite numbers decreased (625 mites) sharply in December 2000 and decreased (59 mites) dramatically again in March 2001. The number of mites increased (137 mites) gradually in April 2001. After that, mite numbers decreased (57 mites) sharply in May 2001 and decreased (7 mites) sharply again in June 2001. Only 7 mites were detected in the debris for 23-day collection period before the bees left the nest. The mite infestation rate on adult bees collected from the nest (in October 2000) was very low (0.2%). For deserted comb of colony 1 (comb1), the infestation percentage of *T. clareae* on *A. dorsata* brood was 11.3%, and 63 mites were left in the comb after the bees absconded (Table 4.2).

For colony 2, out of a total of 1,806 mites collected in the debris during the 176-day collection period, 90% (1,625 mites) of these being injured. Of the damaged mites, 68.5% had injured legs only, 31.1% were badly damaged on both legs and body, and

0.4% injured body only. The number of mites increased (179 to 195 mites) slightly from February to March 2002. Mite numbers decreased (72 mites) dramatically in April 2002. The number of mites increased (692 mites) sharply in May then decreased (604 mites) slightly in June and decreased (64 mites) sharply again in July 2002. Only 64 mites were detected in the debris for 26-day collection period before the bees absconded. *T. clareae* was not found on 965 adult bees collected from the nest (in May 2002). In deserted comb of colony 2 (comb 2), the mite infestation rate on *A. dorsata* brood was 21.8%, and only 53 mites had been left after the bees migrated (Table 4.2).

Very few mites were found in the debris of the three *A. dorsata* colonies located in Samut Songkhram province (Table 4.1). For 38 days, 1 injured mite (in the debris) and 9 uninjured mites (on dead adult bees on the trap) were recovered from colony 3. For deserted comb of colony 3 (comb 3), the infestation rate of *T. clareae* in capped brood was very low (0.9%), and only 3 mites were left in the nest after the colony migration (Table 4.2). Only 1 injured female mite was found in the debris of colony 4 during the 28-day collection period. No mites were collected from colony 5 for the entire 94-day collection period.

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Table 4.1 Numbers, sex ratios and injuries of *T. clareae* in debris of *A. dorsata* colonies in Thailand (colonies 1, 2 = Chiang Mai, colonies 3, 4, 5 = Samut Songkhram).

Colony number	Months	Collection days	Total Mites	Injured mites (%)				Male	Female I	Unknown	Sex ratio
				Total	Leg	Body	Leg+body			sex	(M / F)
	0 . 003	4.5	4.044	4.070 (00.4)	1.042	4	825	59	1,834	48	1/31.1
	Oct.00°	15	1,941	1,872 (96.4)	1,043			1	4,422		1/17.7
	Nov.	30	4,790	4,583 (95.7)	1,941	8	2,634	250		118	
	Dec.	31	625	589 (94.2)	270	3	316	21	596	8	1/28.4
1	Jan.01	31	761	733 (96.3)	317	1	415	50	705	6	1/14.1
	Feb.	28	786	690 (87.8)	428	1	261	85	698	3	1/8.2
	Mar.	31	59	49 (83.1)	32	1	16	5	54	0	1 / 10.8
	Apr.	30	137	109 (79.6)	97	0	12	5	132	0	1/26.4
	May	31	57	45 (78.9)	43	0	2	1	56	0	1 / 56
	Jun.	23	7	4 (57.1)	4	0	0	0	7	0	-
	Total	250	9,163	8,674 (94.7)	4,175 (48.1)	18 (0.2)	4,481 (51.7)	476	8,504	183	1 / 17.9
	F=1 00	20	179	149 (83.2)	116	1	32	0	179	0	
	Feb.02	28		100000000000000000000000000000000000000	117		55	4	187	4	1 / 46.8
	Mar.	31	195	174 (89.2)		2			69		1740.0
0	Apr.	30	72	64 (88.9)	33	1	30	0		3	1/22
2	May	31	692	643 (92.9)	421	2	220	20	660	12	1/33
	Jun.	30	604	559 (92.5)	393	1	165	14	588	2	1/42
	Jul.	26	64	36 (56.3)	33	0	3	0	64	0	-
	Total	176	1,806	1,625 (90.0)	1,113 (68.5)	7 (0.4)	505 (31.1)	38	1,747	21	1 / 45.9
	May.00	11	1	1 (100)	0	0	1	0	1	0	+
3	Jun.	27	9°	0	0	0	0	1°	8°	0	1/8
	Total	38	10	1 (10.0)	0	0	1 (100)	1	9	0	1/9
	N	00	14	4 (400)	2	0	0	0	1	0	
	Nov.00	23	1	1 (100)	0	0	0	0	1	0	
4	Dec. <sup>d</sup> Total	5 <b>28</b>	0	1 (100)	1 (100)	0 <b>0</b>	0	0	0	0	-
								V			
5°	Dec.00	26	0	0	0	0	0	0	0	0	-
	Jan.01	31	0	0	0	0	0	0	0	0	-
	Feb.	28	0	0	0	0	0	0	0	0	-
	Mar.d	9	0	0	0	0	0	0	0	0	
	Total	94	0	0	0	0	0	0	0	0	-
Overall			10,980	10,301 (93.8)	5,289 (51.3)	25 (0.03)	4,987 (48.4)	515	10,261	204	1 / 19.9

<sup>&</sup>lt;sup>a</sup>Two *T. clareae* females were found from 1,270 adult bees collected from the nest.

<sup>&</sup>lt;sup>b</sup>No mites were found on 965 adult bees collected from the nest.

<sup>&</sup>lt;sup>c</sup>Mites on dead adult bees (131 workers and 3,457 drones) collected from the trap.

<sup>&</sup>lt;sup>d</sup>Colonies were harvested by bee hunters.

<sup>&</sup>lt;sup>e</sup>Colony arrived at the site about 3 weeks before collecting the debris.

#### Sex ratios of T. clareae in debris and deserted combs of A. dorsata

T. clareae adults in the debris and brood of A. dorsata greatly favored females. Sex ratios (male to female) of T. clareae found in the debris of A. dorsata ranged from 1:8 to 1:56 (Table 4.1). A lower sex ratio of 1:7.8 to 1:8 was found in A. dorsata brood from deserted combs of their own colonies (Table 4.2).

Table 4.2 Numbers, infestation rates and sex ratios of *T. clareae* adults in *A. dorsata* capped brood from deserted combs of debris collected colonies (combs 1, 2= Chiang Mai, comb 3=Samut Songkhram).

Comb	No. of capped	No. of Infested		No.	Sex ratio		
number	brood examined	Infested	brood	Total	Male	Female	(M:F)
		brood	rates (%)				
1	213	24	11.3	63	7	56	1: 8
2	78	17	21.8	53	6	47	1: 7.8
3	215	2	0.9	3	0	3	-
4*	No deserted comb		1186	-	-	-	-
5*	No deserted comb	-	-		-	-	-
Total	506	43	8.5	119	13	106	1: 8.2

Colonies were harvested by bee hunters.

#### Discussion

The colonies of *A. dorsata* (colonies 1 and 2) in Chiang Mai, where most of Thailand's *A. mellifera* colonies were located, exhibited efficient grooming behavior as indicated by the numbers of injured *T. clareae* and the low levels of infested adult bees in their colonies (0.2% in colony 1 and no mites on 965 adult bees in colony 2) (Table 4.1). The number of injured mites (90-94.7%) from the debris was higher than the numbers previously reported by Rath and Delfinado-Baker (1990) in Thailand (73–76%) and Koeniger et al. (2002) in Malaysia (84%). Increased grooming behavior, which

researchers (Koeniger and Koeniger, 1980; Wongsiri et al., 1989; Rath, 1991). Self-cleaning (auto-grooming) behavior by *A. dorsata* in response to *T. clareae* infestation has also been reported (Buchler et al., 1992). Koeniger and Muzaffar (1988) and Rinderer et al. (1994) reported that *T. clareae* cannot survive more than 3 days on adult bees of *A. dorsata*. Thus, this grooming behavior and inability of *T. clareae* for survival long on adult bees may be responsible to the low levels of *T. clareae* infestation on *A. dorsata* adult bees in this study (0.2% in colony 1 and no mites on 965 adult bees in colony 2).

For colony 1, the highest number of T. clareae in the debris was collected in November when the colony was high bee population. The decrease in T. clareae numbers coincided with a slight decline in bee population in December. The colony was presumed to have started issuing swarms at this time because low bee population was observed although no queen cells were noticeable. In March, multiple queen cells were observed. Several swarms were thought to have left the colony due to the sharp decrease in bee population. One half of the comb surface was exposed, having about 40% sealed brood. The increase in mite numbers coincided with a slight increase in bee population from brood emerging in April. Approximately, 30% of the comb surface were exposed, having 20% sealed brood. In May and June, the sharp decrease in Tropilaelaps numbers were found to coincide with a sharp decline in bee population. Several swarms had left the colony at this time because of appearing of 8 queen cells. About 70% of the comb surface were exposed, having about 10% sealed brood in May. A small number of sealed brood was found in the nest in the late of June before the bees absconded. The reductions in the number of mites coincided with the reductions in adult bee populations. However, proportionately the mites appeared to be reduced at greater proportions.

For colony 2, the bee population and nest size continuously increased from February to May. The increase of *T. clareae* numbers in the debris coincided with the increase in bee populations from February to May except in April the mite numbers decreased while the bee population increased. The highest *T. clareae* number was

found in May when the colony was most populous. In June, the slight decrease in mite numbers coincided with a slight decline in bee population. Swarms had left the colony at this time since 4 queen cells were observed. The sharp decrease in *T. clareae* numbers coincided with a sharp decrease in bee population in July. Several swarms had left the colony in this month because 6 queen cells were detected. About 70% of the comb surface were exposed, having a small number of sealed brood in the late of July before the colony migration.

In Samut Songkhram, colony 3 was monitored at the time the bees were preparing for migration. The low number of mites recovered from this colony might be due to either the colony being only slightly infested (0.9% infested brood in deserted comb) initially or most mites having been already killed in preparation for migration before collection started. It is interesting to note that no mites were found in debris of colony 5 during the 3-month collection period. This colony arrived at the research site 3 weeks before the study started. This observation suggests that new colonies may start with uninfested or slightly infested bees. This speculation can be supported by the significant decrease in the number of mites collected from the debris of colonies 1 and 2 during their swarming period. In addition, 3,457 dead adult drones were recovered from the debris of colony 3 ten days before the bees left the nest. This behavior indicate that worker bees may kill drones prior to migration, thus regulating mite infestation in the new colony; *T. clareae* are known to prefer drones over workers (Ritter and Schneider-Ritter, 1988). Sample collections from colonies 4 and 5 were halted after honey hunters destroyed the nests for honey harvest.

This finding on the highest number of mites in the debris is inconsistent with the previous report by Rath and Delfinado-Baker (1990). According to their report, the highest number of mites in the debris from 2 *A. dorsata* colonies during 32-and-8-day collecting periods was on 5 days before the bees migrated. For long collecting periods in this study, the highest numbers of *T. clareae* in the debris were found when the colonies were populous while the very low numbers of mites were detected when the colonies nearly absconded. From the data obtained, the mite populations in the debris

seem to have relationship with bee population and brood size in the colonies. *A. dorsata* had stopped brood rearing in preparing for migration (Ruttner, 1988) and several swarms had left the colonies. A number of mites are thought to have leaving the nests by going with swarms. Most *Tropilaelaps* mites are in brood cells because the mites re-entered brood cells within 2-3 days after the mite emerging (Woyke, 1987). The colonies had both low levels in bee population and sealed brood number when the colonies nearly migrated. Thus, the low number of mites in the debris was found at this time although almost mites emerged with the last emerging brood and they were killed by bees or died because of no food (bee brood).

In Thailand, there were 4 carriers of *Tropilaelaps* in every location: *A. dorsata*, *A. florea*, *A. cerana* and *A. mellifera*. Thus, *A. dorsata* colonies in all regions could not avoid infestation by *T. clareae*. Most *A. mellifera* colonies in Thailand are concentrated in the North, espectially in Chiang Mai. *T. clareae* is a more serious problem to *A. mellifera* colonies than *A. dorsata* due to their lack of defense mechanisms necessary to regulate mite populations. *A. mellifera* beekeeping may be a main cause of more higher numbers of *T. clareae* in the debris of the colonies located in Chiang Mai than in Samut Songkhram.

The present study found, similar to the observation of Thapa (1998), low numbers (3-63 mites) and low infestation rates (0.9-21.8%) of *T. clareae* in *A. dorsata* deserted combs of debris collected colonies. This observation suggests that the inability of *T. clareae* populations to grow in *A. dorsata* colonies to a dangerous level is due to the bees' grooming behavior, and *T. clareae* was not a main impact on migrations of these *A. dorsata* colonies. The bees migrated after several swarms left the colony and insufficient bees remained to manage the colony (similar to Woyke et al. 2000). It is possible that the departure of swarms may be a significant factor on migrations of the 3 *A. dorsata* colonies because there was other *A. dorsata* colonies, some nectar and pollen available in the areas when the bees left the nests. However, Koeniger et al. (1993) reported that an *A. dorsata* colony migrated because of a high rate of brood infestation (63%) and a large number of mites (1,060 mites) in a deserted comb in Malaysia.

In this study, the female biased sex ratio of T. clareae (male to female) in A. dorsata brood from deserted combs was 1:8.2. This is similar to the observation of Burgett and Kitprasert (1989) on A. dorsata brood from established colonies (1:7.6). The observation on uneven sex ratios (male to female) of T. clareae in the debris (1:56) were significantly higher than any estimate so far reported. Rath et al. (1991) estimated a ratio of about 1:7. Rath and Delfinado-Baker (1990) reported that the unequal sex ratio of T. clareae adults in the debris seems to be influenced by the much shorter life span of the males (not more than 5 days) as compared to that of females (about 28 days). However, the unbalanced sex ratio of T. clareae adults observed in the present study also may be attributed to differences in the proportion of initial male to female eggs and differences in mortality between gender of immatures as observed in spider mites (Wrensch and Young, 1983). Location, environment, time period and colony strength also have likely to influence on the observed sex ratios. Rath et al. (1991) reported that multiple mating of T. clareae was observed in males whereas such a circumstance was not found in females, and the mating can be occurred both inside and outside of the brood cells. Since a male of this mite can easily serve a number of females, the biased sex ratio in favor of females should have a benefit to the increase of the total number of offspring produced.

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