CHAPTER 3

Tropilaelaps clareae POPULATIONS IN NEW, ESTABLISHED AND DESERTED NESTS OF *Apis dorsata* IN THAILAND

Abstract

Six new and 8 established colonies of *A. dorsata* were collected in Samut Songkhram, Thailand, between April 2000 and September 2002. The presence of *Tropilaelaps* was examined from adult bees, unsealed and sealed brood of each colony. *T. clareae* was not found in the 6 new colonies. The 8 established colonies had 2-119 mites in the sampled sealed brood, and 3 of 8 established colonies had 1-11 mites on the adult bees.

Sixteen established and 13 deserted colonies of *A. dorsata* were collected from different locations in Thailand from March 2000 to October 2002. Capped brood cells and adult bees of each colony were examined for the presence of *Tropilaelaps* adults. The sixteen established colonies had 0-146 *T. clareae* in the sampled capped brood, and 0-647 mites were found in sealed brood of the 13 deserted colonies. The average infestation rate was 0.3% on the adult bees, 1.8 and 13.5% in sealed brood cells of the established and deserted colonies respectively. The sex ratio (male to female) of *T. clareae* was 1:2.4 on the adult bees, 1:4.5 and 1:5.5 in capped brood cells of established and deserted colonies respectively.

Key words: *Tropilaelaps clareae* / *Apis dorsata* / honey bees / parasitic mites / deserted combs / 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 human buildings. *A. dorsata* colonies migrate regularly during the year and stop brood rearing in preparation for migration (Ruttner, 1988). This means that during migrations there is a period of broodlessness. *T. clareae* cannot survive more than 3 days on adult bees of *A. dorsata* (Rinderer et al., 1994) because their chelicerae (mouth parts) are not specialized for feeding on adult bees (Kitprasert, 1984; Delfinado-Baker et al., 1992). Thus the origin of infestations in colonies that have recently undergone migration is as yet unknown.

When *T. clareae* populations build up beyond the grooming capacity of *A. dorsata* workers, colonies may migrate which decreases the mite populations (Wongsiri et al., 1989). Koeniger et al. (1993) observed this phenomenon by finding a large number (1,060) of *T. clareae* left in a deserted comb of *A. dorsata* in Malaysia. However, Thapa (1998) contradicted this observation after finding only 7 mites in a deserted comb of *A. dorsata* in Thailand. At present, the impact of *T. clareae* on the migrations of *A. dorsata* colonies is unclear.

The male to female sex ratio of *T. clareae* varies considerably. Under laboratory conditions, the sex ratio (male: female) was nearly 1:5 (Rath et al., 1991). The sex ratio of *T. clareae* in debris of *A. dorsata* colonies ranged from 1:1.8 to 1:6.7 (Rath and Delfinado-Baker, 1990). The ratio of male to females in *A. dorsata* brood in Thailand was 1:7.6 (Burgett and Kitprasert, 1989). However, variation of sex ratios of *T. clareae* in brood cells and on adult bees of *A. dorsata* has yet to be studied. Therefore, the objectives of this study were to observe *Tropilaelaps* infestations in new, established and deserted nests of *A. dorsata* and to investigate sex ratios of *T. clareae* in brood cells and on adult bees of *A. dorsata*.

Materials and Methods

T. clareae populations in new and established colonies of *A. dorsata*

Six new (swarms or colonies with the first generation of brood) and eight established (with more than the first generation of brood) colonies of *A. dorsata* were examined for the presence of *Tropilaelaps* in Samut Songkhram, Thailand, between April 2000 and September 2002. For the new colonies, the whole nests (adult bees and brood combs containing unsealed and sealed brood) were collected and examined. The ages of the new colonies were inferred from the stage of the brood present. Colony 1 was a swarm (without comb), which had arrived at the site about 8 days before being sampled. Colonies 2 and 3 were 9-and-13-days-old respectively. Colonies 4 and 5 were both 14-days-old while colony 6 was 20-days-old. The established colonies were all more than two months old. The presence of *Tropilaelaps* in the established colonies 1-4 was determined by cutting a brood comb section (about 10 cm x 18 cm) from each of the colonies. Both unsealed and sealed brood within the cut sections was examined (Table 3.1). The entire nest of colonies 5-8 were collected and examined for the presence of mites.

A sample of adult bees (n = 52-13,070) was obtained from each colony. These were washed in 70% ethyl alcohol to remove any mites present. The bees were then removed with a sieve, and mites were counted in the alcohol.

Populations and sex ratios of *T. clareae* in established and deserted colonies of *A. dorsata*

Sixteen established colonies and thirteen deserted combs of *A. dorsata* were collected and examined for the presence of *Tropilaelaps* mites from different locations in Thailand during March 2000 to October 2002. For the established colonies, eight colonies were collected in Central (Samut Songkhram), three colonies in Southern (Songkhla and Surat Thani) and five colonies in Northern (Kamphaeng Phet, Lampang

and Chiang Mai) Thailand. The presence of *T. clareae* in eleven established colonies was determined by cutting a brood comb section (about 10 cm x 20 cm) from each of the colonies. Sealed brood cells within the cut sections were examined (Table 3.2). The whole nests of five established colonies were collected and all capped brood cells in each colony were examined for mites. Deserted combs of *A. dorsata* were collected from three different locations: 4 combs in Central (Aung Thong, Samut Songkhram and Bangkok), one comb in Eastern (Chanthaburi) and 8 combs in Northern (Nan and Chiang Mai). All capped brood cells, which contained dead or living pupae, of each comb were examined for the presence of *Tropilaelaps* mites. Both the mite and brood numbers in each colony were counted to determine for the mite infestation rates.

Adult bees (workers and drones) from established colonies were also sampled. The adult bees collected were pooled and washed in 70% ethyl alcohol. Both the mite and bee numbers in each colony were counted to determine the infestation rates on adult bees. The collected mites from sealed brood cells and on adult bees were examined and classified according to sex under a stereomicroscope at 40X magnification to determine sex ratios.

Results

T. clareae populations in new and established colonies of *A. dorsata*

T. clareae was not found in the 6 new colonies (Table 3.1). The eight established colonies had 2-119 mites in the sampled sealed brood, and 9, 1 and 11 mites were collected from adult bees of colonies 6, 7 and 8 (established colonies), respectively. No mites were found in the unsealed brood cells of the new and established colonies of *A. dorsata*.

Table 3.1 Numbers of *T. clareae* adults in new and established colonies of *A. dorsata* in Samut Songkhram, Thailand.

Colony type	Colony number	No. of unsealed brood examined	No. of sealed brood examined	No. of adult bees examined
New	1	No comb	No comb	9,782 (0)
	2	1,262 (0)	No sealed brood	5,055 (0)
	3	1,440 (0)	620* (0)	5,181 (0)
	4	1,281 (0)	425* (0)	3,767 (0)
	5	394 (0)	No sealed brood	3,930 (0)
	6	976 (0)	3925 (0)	10,885 (0)
		Total = 5,353 (0)	**4,970 (0)**	**38,600 (0)**
Established	1	200 (0)	254 (16)	57 (0)
	2	173 (0)	390 (27)	521 (0)
	3	136 (0)	883 (2)	52 (0)
	4	218 (0)	819 (2)	538 (0)
	5	341 (0)	3,366 (10)	1,232 (0)
	6	529 (0)	3,413 (5)	5,056 (9)
	7	402 (0)	2,721 (119)	2,751 (1)
	8	572 (0)	3,586 (26)	13,070 (11)
		Total = 2,571 (0)	**15,432 (207)**	**23,277(21)**

Numbers inside ( ) indicate numbers of detected mites.

*Sealed larvae

T. clareae populations in established and deserted nests of _A. dorsata_

The sixteen established colonies had 0-146 *T. clareae* adults in the sampled capped brood. Sealed brood cells of the thirteen deserted combs contained 0-647 mites (Table 3.2). The infestation rate of *T. clareae* in brood cells ranged from 0-9.8% in the established colonies and 0-74.3% in the deserted nests. The infested brood percentages of the established and deserted colonies were 1.8 and 13.5% respectively. This study

showed the infested brood rates of the established colonies at the levels of 1.0, 4.3 and 4.7% in the central, southern and northern areas of Thailand, respectively. In deserted colonies, the infested brood rates were 1.7, 8.6 and 18.1% in the central, eastern and northern areas of Thailand, respectively. The infested established and deserted nests occurred in all test locations.

The average infestation rate of *T. clareae* on *A. dorsata* adult bees was 0.3% (Table 3.2). The mite infestation levels on the adult bees in the central, southern and northern areas of Thailand were 0.1, 0.2 and 1.6%, respectively.

Sex ratios

T. clareae adults in brood cells and on adult bees of *A. dorsata* favored females. Sex ratios (male to female) of *T. clareae* found in brood cells ranged from 1:1.5 to 1:22 for the established colonies and 1:2.8 to 1:13 for the deserted combs (Table 3.2). Sex ratios of *T. clareae* on the adult bees of *A. dorsata* ranged from 1:1 to 1:8. The average ratio (male: female) of *T. clareae* was 1:2.4 on the adult bees, 1:4.5 and 1:5.5 in brood cells of established and deserted colonies respectively.

Table 3.2 Numbers, infestation rates and sex ratios of *T. clareae* adults in established and deserted nests of *A. dorsata* from different parts of Thailand (*T. c.*= *T. clareae*, M=male, F=female).

Colony type	Locations	Colony number	Sealed brood							Adult bees					
			No. of sealed brood examined	No. of Infested brood	Infested brood rates (%)	No. of *T. c.*	M	F	Sex ratio (M:F)	No. of adult bees examined	Infestation rates (%)	No. of *T. c.*	M	F	Sex ratio (M:F
Established	Central	1	254	15	5.9	16	1	15	1: 15	57	0	0	0	0	-
		2	390	18	4.6	27	7	20	1: 2.9	521	0	0	0	0	-
		3	883	1	0.1	2	0	2	-	52	0	0	0	0	-
		4	819	2	0.2	2	0	2	-	538	0	0	0	0	-
		5	3,366	10	0.3	10	4	6	1: 1.5	1,232	0	0	0	0	-
		6	3,413	3	0.1	5	1	4	1: 4	5,056	0.2	9	1	8	1: 8
		7	2,721	84	3.1	119	19	100	1: 5.3	2,751	0.04	1	0	1	-
		8	3,586	20	0.6	26	3	23	1: 7.7	13,070	0.1	11	5	6	1: 1.
		Total	**15,432**	**153**	**1.0**	**207**	**35**	**172**	**1: 4.9**	**23,277**	**0.1**	**21**	**6**	**15**	**1: 2**
	Southern	1	369	0	0	0	0	0	-	1,691	0.1	1	0	1	-
		2	560	29	5.2	50	15	35	1: 2.3	1,347	0.3	4	2	2	1:
		3	591	37	6.3	61	10	51	1: 5.1	26	3.9	1	0	1	-
		Total	**1,520**	**66**	**4.3**	**111**	**25**	**86**	**1: 3.4**	**3,064**	**0.2**	**6**	**2**	**4**	**1: 2**
	Northern	1*	1,138	10	0.9	19	2	17	1: 8.5	-	-	-	-	-	-
		2*	513	29	5.7	41	7	34	1: 4.9	-	-	-	-	-	-
		3*	236	8	3.4	17	1	16	1: 16	-	-	-	-	-	-
		4	701	69	9.8	146	32	114	1: 3.6	1,882	2.4	46	13	33	1: 2
		5	243	16	6.6	23	1	22	1: 22	955	-	0	-	-	-
		Total	**2,831**	**132**	**4.7**	**246**	**43**	**203**	**1: 4.7**	**2,837**	**1.6**	**46**	**13**	**33**	**1: 2**
		Overall	**19,783**	**351**	**1.8**	**564**	**103**	**461**	**1: 4.5**	**29,178**	**0.3**	**73**	**21**	**52**	**1: 2**
Deserted	Central	1	17	2	11.8	15	4	11	1: 2.8						
		2	215	2	0.9	3	0	3	-						
		3	3	0	0	0	0	0	-						
		4	7	0	0	0	0	0	-						
		Total	**242**	**4**	**1.7**	**18**	**4**	**14**	**1: 3.5**						
	Eastern	1	58	5	8.6	8	0	8	-						
		Total	**58**	**5**	**8.6**	**8**	**0**	**8**	-						
	Northern	1	49	6	12.2	36	7	29	1: 3.3						
		2	44	1	2.3	4	0	4	-						
		3	213	24	11.3	63	7	56	1: 8						
		4	78	17	21.8	53	6	47	1: 7.8						
		5	20	6	30.0	14	2	12	1: 6						
		6	49	8	16.3	15	5	10	1: 2						
		7	159	7	4.4	14	1	13	1: 13						
		8	74	55	74.3	647	102	545	1: 5.3						
		Total	**686**	**124**	**18.1**	**846**	**130**	**716**	**1: 5.5**						
		Overall	**986**	**133**	**13.5**	**872**	**134**	**738**	**1: 5.5**						

*Adult bees were not collected.

Discussion

T. clareae populations in new and established colonies of *A. dorsata*

The absence of *T. clareae* in the 6 new colonies (Table 3.1) suggests that *A. dorsata* colonies start with uninfested bees. This mite-free condition is probably due to broodlessness during the swarming events that interrupt the mite's life cycle. This period can be very lengthy. Koeniger and Koeniger (1980) reported that *A. dorsata* swarms fly in several stages and often rest between stages of the flight. They showed that swarms often rest one to three days without building any comb and can spend more than one month on their migration. In the present study, colony 1 did not commence comb building for eight days after its arrival at the nest site, providing a further period of broodlessness.

The extensive period of broodlessness suggests that any mites traveling phoretically on a swarm are likely to die during swarming events before having the chance to enter brood cells of the new nest. This suggestion is supported by the absence of *T. clareae* in the new colonies. In Borneo, Koeniger et al. (2002) observed infestations of *T clareae* in a newly settled swarm that had originated from an infested colony nearby. This shows that *T. clareae* can spread via reproductive swarms, but probably not, as this study has shown, in migratory swarms.

The presence of *Tropilaelaps* mites in the sampled sealed brood (2-119) and on the adult bees (1-11) of the established colonies (Table 3.1) confirmed that *T. clareae* populations gradually build up in new *A. dorsata* nests after re-infestation. Most often uninfested colonies must acquire *T. clareae* from other *Apis* species or nests of *A. dorsata* present in the area. In Southeast Asia, *T. clareae* has proved to be a more serious pest of *A. mellifera* than either *Varroa destructor* or *V. jacobsoni* . Since brood production in *Apis mellifera* colonies is year around in Thailand, these and non-migrating *A. dorsata* colonies may serve as constant reservoirs of *Tropilaelaps* for new colonies. *T. clareae* is also known to reproduce in *A. cerana* drone brood, and to be phoretic on adult

bees of *A. dorsata*, *A. laboriosa*, *A. mellifera*, *A. florea* and *A. cerana* (Aggarwal, 1988). In Samut Songkhram, all of these bee species are found except *A. laboriosa*. *A. cerana* and *A. dorsata* have been reported to forage simultaneously on the same inflorescence of king palm in Thailand (Oldroyd et al., 1992). In Malaysia, *A. cerana* adult bees have been observed to rob *A. dorsata* colonies (Koeniger et al., 2002). Thus, foraging of different bee species in the same flower or robbing must be considered as a potential source of *T. clareae* infestation for uninfested colonies.

T. clareae populations in established and deserted nests of _A. dorsata_

That *Tropilaelaps* populations gradually build up in *A. dorsata* colonies after re-infestation is supported by the observation of higher infestation rates in brood cells of the deserted nests (13.5%) than that of established colonies (1.8%) (Table 3.2). The absence of *T. clareae* in the 2 deserted combs and low numbers of mites (3-63) in the ten deserted combs suggests that *T. clareae* do not seem to be significant cause of the migrations of these colonies. This result is consistent with the finding of Thapa (1998) that found 7 mites from a deserted comb of *A. dorsata*. The presence of 4-8 emerged queen cells in each deserted comb suggests that these colonies migrated after one or more swarms issued from the colonies. Also, these honey bees are thought to migrate because nectar and pollen were no longer available in the area of the nest (Woyke, et al., 2000). However, a large number (647) and a high rate of brood infestation (74.3%) of *T. clareae* was found in the deserted comb of *A. dorsata*. Perhaps, this colony did migrate in response to the high infestation rate of *T. clareae*. This observation agrees with the finding of Koeniger et al. (1993) that the bees of an *A. dorsata* colony had left a large number of *T. clareae* in its deserted comb when they absconded.

The infested brood rate of the established colonies (1.8%) was about 6 times higher than that on the adult bees (0.3%) (Table 3.2). This result indicates that *T. clareae* feeds mainly on brood and can not survive long on adult bees (Woyke, 1985; Koeniger and Muzaffar, 1988; Rinderer et al., 1994). Woyke (1987) reported that *Tropilaelaps* adults re-entered brood cells within 2-3 days after emerging. Furthermore, *A. dorsata*

workers had an efficient grooming behavior which removed mites from their bodies (Koeniger and Koeniger, 1980; Rath and Delfinado-Baker, 1990; Koeniger et al., 2002). Thus, the short phoretic period, the restriction of reproductive mites in brood cells and efficiently grooming behavior of *A. dorsata* are all causes of the very low infestation rate of *T. clareae* on the adult bees of *A. dorsata*.

The infested adult bees and infested colonies (both established and deserted colonies) of *A. dorsata* were found in all test regions (Table 3.2). 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*. However, most *A. mellifera* colonies in Thailand are concentrated in the North. *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 the high infestation percentages on the adult bees (1.6%) and in brood cells of the established (4.7%) and deserted (18.1%) colonies in the north.

Sex ratios

The uneven sex ratio (male to female) of *T. clareae* adults in *A. dorsata* brood (1:4.5-1:5.5) in this study (Table 3.2) was similar to the sex ratio under laboratory conditions (1:5) (Rath et al., 1991). The male to female sex ratio in brood cells of *A. dorsata* in this study was lower than the previous sex ratios in *A. dorsata* brood in India (1:29) and in Thailand (1:7.6) (Aggarwal and Kapil, 1986; Burgett and Kitprasert, 1989). The unbalanced sex ratio of *T. clareae* adults seems to be influenced by the much shorter life span of the males as compared to that of females. *T. clareae* males do not survive more than 5 days while the life span of females is about 28 days (Rath and Delfinado-Baker, 1990). However, the unequal sex ratio of *T. clareae* adults observed in this study also may be attributed to differences in the proportion of initial male to female eggs and differences in mortality between sexes of immatures as observed in spider mites (Wrensch and Young, 1983). Environment, location and time period are also likely

to influence sex ratios. Rath et al. (1991) reported that *T. clareae* mates by podospermy and can be observed inside or outside of the brood cells, and multiple mating was observed in males (not found in females). Since one male of *T. clareae* can easily serve a number of females, it might be an advantage for this mite species. Because the sex ratio was biased in favor of females, this would increase the high total number of offspring produced (Pianka, 1983).

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