

CHAPTER 5

NON-REPRODUCTION OF THE BEE MITE, *Tropilaelaps clareae*, IN *Apis dorsata* AND TWO STOCKS OF *Apis mellifera* IN THAILAND

Abstract

Eleven *Apis dorsata*, 10 ARS Primorsky honey bee (*A. mellifera*) and 10 domestic (Thai) colonies (*A. mellifera*), Italian honey bee hybrids, were used to investigate of *Tropilaelaps clareae* non-reproduction and the number of progeny produced by reproductive mites (or fecundity). *A. dorsata* brood combs were collected from different locations in Thailand between April 2000 and September 2002. Primorsky and domestic colonies were established and placed in an apiary in Chiang Mai, Thailand. Capped brood cells of the Primorsky and domestic colonies were examined and determined for non-reproductive mites and fecundity every month during March 2002 to February 2003. Dark brown eyes with light pigmented thorax pupae and older of the *A. dorsata*, Primorsky and domestic colonies were examined, and only cells that had been invaded by a single female mite were evaluated for non-reproductive mites and fecundity. Criteria for non-reproduction were mites that did not lay eggs and mites with progeny that were too young to mature before the host bee emerged from its cell. The percent mean of non-reproductive mites on the worker pupae of *A. dorsata* colonies ($65.2 \pm 5.1\%$) was significantly higher ($P=0.029$) than that of domestic ($50 \pm 3.8\%$) and Primorsky ($48 \pm 5.2\%$) colonies (percent mean \pm standard error). The percent mean of non-reproductive mites in the Primorsky and domestic colonies was not significantly different. *T. clareae* fecundity of reproductive mites in *A. dorsata*, domestic and Primorsky colonies was not significantly different ($P=0.100$) with the means of 1.7 ± 0.4 , 1.8 ± 0.3 and 2.0 ± 0.3 progeny (mean \pm standard deviation), respectively. Overall, a reproductive *T. clareae* produced 1.8 ± 0.4 progeny. About 42% of reproductive mites had produced 1, 37% had produced 2, 16.2% had produced 3, 3.2% had produced 4 and 0.3-0.8% had produced 5-8 progeny.

Key words: *Tropilaelaps clareae* / *Apis dorsata* / *Apis mellifera* / Primorsky / non-reproduction / reproductive mites / fecundity / Thailand

Introduction

Tropilaelaps clareae Delfinado & Baker (Acari: Laelapidae) feeds on haemolymph of bees (Kitprasert, 1984; Delfinado-Baker et al., 1992) and is a much more serious pest of *Apis mellifera* L. but not considered to be a severe pest of its natural host, *A. dorsata* Fabr. (Wongsiri et al., 1989). *A. dorsata*, a native honey bee species of South-East Asia, lives in the open, in single comb nests hanging high on cliffs, limbs or branches of trees, eaves and ledges of human buildings (Ruttner, 1988; Wongsiri et al., 1989, 1996). *T. clareae* populations can not grow in *A. dorsata* colonies to a dangerous level because of the effective defense mechanisms of this bee species such as colony migrations, broodless periods, grooming behavior and hygienic behavior (Wongsiri et al., 1989; Burgett and Rossignol, 1990; Rath and Delfinado-Baker, 1990; Koeniger and Koeniger, 1993; Koeniger et al., 2002). Non-reproduction of mites is one of many characteristics of honey bees that has been associated with resistance to mites (Buchler and Drescher, 1990; Spivak and Reuter, 1998). However, non-reproduction of *T. clareae* in *A. dorsata* colonies has never been studied.

The regional distribution of *T. clareae* appears to be limited to tropical Asia and coincides with the indigenous areas of *A. dorsata* (Burgett et al., 1983; Burgett and Akkratanakul, 1985; Delfinado-Baker and Aggarwal, 1987; Delfinado-Baker et al., 1989). However, the infestation of *T. clareae* in *A. mellifera* colonies outside the range of *A. dorsata* was reported in Afghanistan and South Korea (Woyke, 1984; Woo and Lee, 2001). When *A. mellifera* was introduced to Asia, *T. clareae* has infested this honey bee species and become a serious problem (like *Varroa destructor*) to *A. mellifera* colonies due to their lack of defensive mechanisms necessary to regulate the mite populations (Wongsiri et al., 1989). The use of chemical, physical and combination of chemical and biotechnical methods to control *T. clareae* populations in *A. mellifera* colonies provide some relief but nothing offer complete control (Wongsiri and Tangkanasing, 1987). In

addition, these methods tend to be labor-intensive, time-consuming, reduce bee populations or contaminate hive products. Thus, the use of *A. mellifera* stocks resistant to *T. clareae* has been thought to be a better solution to the *Tropilaelaps* problem in Asia. However, studies on non-reproduction of *T. clareae* in *A. mellifera* as a mechanism of resistance are very limited. Non-reproduction of *T. clareae* in *A. mellifera* colonies was 18.3% in Vietnam, 7.3% in Afghanistan (Woyke, 1990) and 27% in Thailand (Ritter and Schneider-Ritter, 1988). ARS Primorsky honey bees (*A. mellifera*) are known to have natural defensive mechanisms to *V. jacobsoni* and *V. destructor* (Danka et al., 1995; Rinderer et al., 1997, 1999). Possibly, the ARS Primorsky bees may have some natural defensive mechanisms to *T. clareae*. Therefore, this study was conducted to compare non-reproduction of *T. clareae* and the number of progeny produced by reproductive mites in *A. dorsata*, ARS Primorsky and domestic honey bee colonies.

Materials and Methods

Eleven *A. dorsata*, 10 ARS Primorsky (*A. mellifera*) and 10 domestic (Thai) colonies (*A. mellifera*), Italian honey bee hybrids, were used to estimate for non-reproduction of *T. clareae*. *A. dorsata* brood combs were collected from different locations in Thailand between April 2000 and September 2002. Four combs were collected in Northern (Kamphaeng Phet, Lampang and Chiang Mai), five combs in Central (Samut Songkhram and Ratchaburi) and two combs in Southern (Surat Thani and Songkhla). The whole combs of *A. dorsata* were cut and immediately frozen.

For *A. mellifera*, ARS Primorsky queens received from the USDA, Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, Louisiana, USA, and commercial domestic queens received from Supa's apiary in Chiang Mai, Thailand. These honey bees were established in Langstroth hives in November 2001 and placed in an apiary in Chiang Mai, Thailand. Approximately, 100 *Tropilaelaps* mites were inoculated into each colony by using an infested sealed brood section technique in February 2002. For each colony, about 200 sealed worker brood cells were cut from two different combs (about 50 cells on one side) and immediately frozen. Sealed drone brood cells

(depending on the availability) containing the same developmental stages as worker pupae were also examined and determined for non-reproductive and reproductive mites. Worker and drone brood cells in each colony were examined and determined for non-reproductive and reproductive mites every month until the colonies died or to the end of the experiment (12 months from March 2002 to February 2003).

The frozen capped brood of *A. dorsata*, Primorsky and domestic bees was later thawed, and the cells that containing dark brown eyes with light pigmented thorax pupae (Pdl stage, 17-18 days old from egg laying) or older were opened to determine for non-reproductive and reproductive mites. The capped brood cells were uncapped and the brood pulled out. The stage of brood development was determined. Age of pupae was estimated according to characteristics given by Rembold et al. (1980). Mites were collected on the brood or inside the cell from which the brood had been pulled out. The collected adult mites and progeny were examined and classified for sex and nymphal stages under a stereomicroscope at 40X magnification. Morphological characterization of *T. clareae* nymphal and adult stages was classified according to Kitprasert (1984) and Delfinado-Baker (1985). Duration of development stages of *T. clareae* was estimated according to Woyke (1987).

Percentage of non-reproductive mites

A reproductive mite was one that could produce at least 1 mature daughter before the host bee emerged from its cell. Bee pupae in Pdl stage or older was examined to determine the reproductive success by mites in cells that had been infested with a single female mite. At this stage of host development, it is too late for mite eggs to mature to adult mites. Mites were considered non-reproduction when they had no progeny beyond duetonymph by the time bee pupae had matured to the Pdl stage. Harbo and Harris (1999)'criteria for non-reproduction was divided into 2 categories : (1) mites that did not lay eggs, and (2) mites with progeny that were too young to mature before the host bee emerged from its cell. Measurements of non-reproductive mites per colony made only 1 time for *A. dorsata* colonies and 3-12 times for Primorsky and

domestic colonies (every month until the colonies died or the end of the experiment). Because the number of non-reproductive mites in each colony of Primorsky and domestic bees for each month was low, thus the data in each month for each colony were pooled together. The percentage of non-reproductive mites in each colony of *A. dorsata*, Primorsky and domestic bees was calculated. The percent means of non-reproductive mites in *A. dorsata*, Primorsky and domestic colonies were analyzed using one-way ANOVA (analysis of variance) for a completely randomized design (CRD) (SPSS statistic program). The honey bee colonies were used as replications. The means were compared using Duncan's New Multiple Range test.

Fecundity of reproductive mites

The number of progeny produced by reproductive mites (or fecundity) from infested cells by one female mite was recorded during each evaluation period. Only mites from worker pupae in Pdl stage or mites on pupae in older developmental stages were counted. This analysis did not include non-reproductive mites, which includes mites that produced progeny too late to mature, and progeny from reproductive mites on drone pupae. The data on fecundity of reproductive mites in each colony of Primorsky and domestic bees for each month were pooled together. The average number of progeny produced by a reproductive mite in each colony was calculated. The data on fecundity of reproductive mites in *A. dorsata*, Primorsky and domestic colonies was analyzed by one-way ANOVA in CRD (SPSS statistic program). The colonies of honey bees were used as replications. Means were compared using Duncan's new multiple range test. Pearson correlation analysis was used to test the relationships between the number of progeny produced by reproductive mites and the number of non-reproductive mites.

Results

Percentage of non-reproductive mites

In drone brood cells containing dark brown eyes with light pigmented thorax pupae (Pdl stage) and older of domestic colonies, the number of non-reproductive *T. clareae* detected was 6, and only 5 reproductive mites were found (did not show data). Unfortunately, the data on non-reproductive and reproduction mites on drone brood of *A. dorsata* and Primorsky honey bees was uncertain in this study.

In worker brood cells infested with one *T. clareae* female, the percentage of non-reproductive mites on Pdl pupae and older of *A. dorsata* colonies was significantly higher than that of domestic and Primorsky colonies ($P=0.029$, $df=28$), while non-reproductive mite percentage of the Primorsky and domestic colonies was not significantly different (Table 5.1). The percent mean of non-reproductive mites in *A. dorsata*, domestic and Primorsky colonies was 65.2 ± 5.1 , 50.0 ± 3.8 and $48.0\pm 5.2\%$ (percent mean \pm standard error), respectively. The percentage of non-reproductive mites that did not lay eggs and mites with progeny that were too young to mature before the host bee emerged from its cell was 71.7 and 28.3%, respectively (Table 5.1).

Mite fecundity

The number of progeny per reproductive *T. clareae* in *A. dorsata*, domestic and Primorsky colonies was not significantly different ($P=0.100$, $df=28$) with the means of 1.7 ± 0.4 , 1.8 ± 0.3 and 2.0 ± 0.3 progeny (mean \pm standard deviation), respectively (Table 5.2). Overall, a reproductive mite on Pdl and older worker pupae of *A. dorsata*, domestic and Primorsky honey bees produced 1.8 ± 0.4 progeny. Correlation between fecundity of reproductive mites and the number of non-reproductive mites was not found in *A. dorsata* ($r=-0.145$, $P=0.671$), domestic ($r=0.070$, $P=0.848$) and Primorsky ($r=0.229$, $P=0.524$) colonies. The maximum number of progeny produced by a single reproductive *T. clareae* was found to be 8 (Table 5.3). About 42% of reproductive mites had produced 1,

37% had produced 2, 16.2% had produced 3, 3.2% had produced 4 and 0.3-0.8% had produced 5-8 progeny.

Table 5.1 Numbers and percentages of non-reproductive *T. clareae* on worker pupae in dark brown eyes with light pigmented thorax and older developmental stages of *A. dorsata*, ARS Primorsky and domestic honey bees in Thailand.

Bee types	Colony number	No. of cells Infested by 1 female mite	Non-reproductive mites			
			Numbers	Percentage (%)	Mites that did not lay eggs	Mites with too young progeny
<i>A. dorsata</i>	1	5	4	80	4	-
	2	5	1	20	1	-
	3	6	5	83.3	5	-
	4	23	16	69.6	15	1
	5	14	10	71.4	10	-
	6	4	3	75	3	-
	7	3	2	66.7	1	1
	8	35	21	60	20	1
	9	9	5	55.6	5	-
	10	12	8	66.7	8	-
	11	13	9	69.2	9	-
	Total	129	84	*Mean=65.2±5.1^a	81 (96.4%)	3 (3.6%)
Domestic	1	14	11	78.6	10	1
	2	13	7	53.9	6	1
	3	21	7	33.3	4	3
	4	18	8	44.4	3	5
	5	86	42	48.8	28	14
	6	17	9	52.9	8	1
	7	30	12	40.0	9	3
	8	40	18	45.0	4	14
	9	48	25	52.1	20	5
	10	35	18	51.4	16	2
		Total	332	157	*Mean=50.0±3.8^b	108(68.8%)
Primorsky	1	32	15	46.9	7	8
	2	24	20	83.3	14	6
	3	47	23	48.9	10	13
	4	20	11	55.0	8	3
	5	15	5	33.3	2	3
	6	26	15	57.7	9	6
	7	26	6	23.1	4	2
	8	63	25	39.7	16	9
	9	30	16	53.3	11	5
	10	13	5	38.5	4	1
		Total	296	141	*Mean=48.0±5.2^b	85 (60.3%)
	Overall	757	382	*Mean=54.8±3.0	274(71.7%)	108 (28.3%)

*Percent mean ± standard error

Similar letters in the same column are not significantly different at the 0.05 level

Table 5.2 Progeny numbers of reproductive *T. clareae* on worker pupae in dark brown eyes with light pigmented thorax and older developmental stages of *A. dorsata*, ARS Primorsky and domestic honey bees.

Bee types	Colony number	No. of cells infested by 1 female mite	No. of reproductive mites	No. of progeny	No. of progeny / reproductive mite
<i>A. dorsata</i>	1	5	1	2	2
	2	5	4	8	2
	3	6	1	2	2
	4	23	7	16	2.3
	5	14	4	7	1.7
	6	4	1	2	2
	7	3	1	2	2
	8	35	14	23	1.6
	9	9	4	5	1.3
	10	12	4	4	1
	11	13	4	4	1
	Total	129	45	75	*Mean=1.7±0.4^a
Domestic	1	14	3	6	2
	2	13	6	11	1.8
	3	21	14	20	1.4
	4	18	10	22	2.2
	5	86	44	78	1.8
	6	17	8	11	1.4
	7	30	18	30	1.7
	8	40	22	35	1.6
	9	48	23	42	1.8
	10	35	17	35	2.1
	Total	332	165	290	*Mean=1.8±0.3^a
Primorsky	1	32	17	35	2.1
	2	24	4	10	2.5
	3	47	24	43	1.8
	4	20	9	18	2
	5	15	10	18	1.8
	6	26	11	23	2.1
	7	26	20	41	2.1
	8	63	38	70	1.8
	9	30	14	35	2.5
	10	13	8	14	1.8
	Total	296	155	307	*Mean=2.0±0.3^a
	Overall	757	365	672	*Mean=1.8±0.4

*Mean ± standard deviation

Similar letters in the same column are not significantly different at the 0.05 level

Table 5.3 Frequency of reproductive *T. clareae* that produced different numbers of progeny.

Bee types	No. of reproductive mites	No. of progeny produced by a reproductive mite							
		1	2	3	4	5	6	7	8
<i>A. dorsata</i>	45	23	16	4	2	-	-	-	-
Domestic	170*	72	69	25	3	1	-	-	-
Primorsky	155	60	52	31	7	-	3	1	1
Total	370	155	137	60	12	1	3	1	1
(%)		(41.9)	(37)	(16.2)	(3.2)	(0.3)	(0.8)	(0.3)	(0.3)

*included 5 reproductive mites from drone brood

Discussion

Non-reproductive mites

Mite non-reproduction is a genetic trait of bees (Harris and Harbo, 2001) and one of many resistant mechanisms of honey bees to mites (Buchler and Drescher, 1990; Boecking et al., 1993). Mite growth was low in colonies with the high percentage of non-reproductive mites (Harris and Harbo, 2001). In this study, the percent mean of non-reproductive mites on Pdl and older worker pupae from *A. dorsata* colonies ($65.2 \pm 5.1\%$) was significantly higher than that of *A. mellifera* colonies ($48 \pm 5.2\%$ for Primorsky colonies and $50 \pm 3.8\%$ for domestic colonies) (Table 5.1). This suggests that *A. dorsata* had more resistant genetic trait to *T. clareae* than *A. mellifera*. *T. clareae* is a natural brood parasite but not considered to be a serious pest of *A. dorsata* (Wongsiri et al., 1989). *A. dorsata* and *T. clareae* have coexisted for long time and have co-evolved. This bee species can limit the population growth of *T. clareae* in its colony by developing many defensive mechanisms such as colony migrations, grooming behavior, hygienic behavior and broodless periods (Wongsiri et al., 1989; Burgett and Rossignol, 1990; Rath and Delfinado-Baker, 1990; Koeniger and Koeniger, 1993; Koeniger et al., 2002). The result

here showed that *A. dorsata* has developed a high level of genetic trait to be resistant to its natural parasite by limiting the reproduction of *T. clareae*.

In *A. mellifera* colonies, the percent mean of non-reproductive mites in the Primorsky (48 ± 5.2) and domestic ($50 \pm 3.8\%$) colonies was not significantly different (Table 5.1). This result showed that the Primorsky and domestic bees had the same level of the resistant genetic trait to suppress reproduction of *T. clareae*. The percentage of non-reproductive mites in the domestic colonies (50%) in this study was higher than the previous estimation (27%) by Ritter and Schneider-Ritter (1988). It is possible that the domestic bees have increased a level of resistant genetics to the mite. In addition, the non-reproductive percentage of *T. clareae* in the Primorsky and domestic colonies (48-50%) in the present study was higher than that of *A. mellifera* in Vietnam (18.3%) and Afghanistan (7.3%) (Woyke, 1990).

When the western honey bee *A. mellifera* was introduced to Tropical Asia, *T. clareae* successfully switched hosts from *A. dorsata* to be *A. mellifera* and has become a severe pest of *A. mellifera* (Rath and Delfinado-Baker, 1990). Wongsiri et al. (1989) reported that *A. mellifera* lack of essential defensive mechanisms to limiting the population growth of *T. clareae* in its colony. In Thailand, *A. mellifera* was introduced in the early 1940s for the first time and in 1953 for the second time but did not succeed to maintain the bee until the early 1970s (Wongsiri and Chen, 1995). *T. clareae* and *V. jacobsoni* (or *V. destructor*) are considered to be the most important limiting factors to the development and expansion of *A. mellifera* beekeeping in Thailand and Tropical Asia (De Jong et al., 1982; Nyein and Zmarlicki, 1982). Thus, *A. mellifera* in Thailand (domestic honey bees) and *T. clareae* have coexisted and evolved for about 30 years. This time period might be too short for *A. mellifera* to develop a high level of resistant genetics to *T. clareae*. For example, the Primorsky bees have coexisted with *V. jacobsoni* more than 150 years to development a high level of resistant genetics to this mite (Danka et al., 1995). The Primorsky honey bees are known to have resistant genetics to *V. jacobsoni* and *V. destructor* (Danka et al., 1995; Rinderer et al., 1997,

1999). However, this is the first time for introducing the bees to Thailand to encounter with *T. clareae*. Possibly, the Primorsky bees might not know to their new pest.

Non-reproduction of mites is a mite-bee interaction in colonies in which mites show impaired reproductive ability (Anderson, 1994). The cause of this non-reproduction is exactly unknown. However, evidence suggests that certain factors can be possible causes. Harbo and Harris(1999, 2001) reported that a genetic characteristic of bees caused mites to become non-reproductive and is a heritable characteristic of honey bees. Chemicals from larva and prepupa's haemolymph stimulate *Varroa* mites to produce and lay eggs (Harris and Harbo, 2001). Several researchers have reported on the role of juvenile hormone as a trigger for *Varroa* mite reproduction. Exogenous application of juvenile hormone to bee larvae increased reproduction of *Varroa* mites (Hanel, 1983, 1986; Hanel and Koeniger, 1986). However, endogenous juvenile hormone titers are similar in bee larvae from races and species of bees that differ dramatically in their abilities to support mite reproduction (Rosenkranz et al., 1989, 1993). Woyke (1989) reported that the thickness of *T. clareae* females in brood cells after cell sealing 48 to 96 h increased to be double of mites moving freely on the combs because they feed on haemolymph of larva and pupa bees. Hence, it is possible that some substances in bee's haemolymph may affect reproduction of *T. clareae*. However, the affect of juvenile hormone in bee's blood to reproduction of *T. clareae* has not been studied.

From the data obtained, 71.7% of non-reproductive mites did not lay eggs (Table 5.2). This suggests that non-reproduction could be related to non-mating of the mites. *T. clareae* mates by podospermy and multiple mating was observed in males whereas such a circumstance was not found in females (Rath et al., 1991). Harris and Harbo (2001) reported that many non-laying egg *Varroa* mites had no stored sperm, and *Varroa* females used the stored sperm to produce eggs after entering brood cells for reproductive cycles. Possibly, non-laying egg *Tropilaelaps* females may not mate or fail to store sperm, like in non-laying egg *Varroa* females. Various environmental factors affect the percentage of non-reproductive *Varroa* mites in colonies of bees. High temperatures and relative humidity increased the percentage of non-reproductive *Varroa*

mites (Le Cont et al., 1990; Kraus and Velthuis, 1997). A higher percentage of non-reproductive *Varroa* mites occurred in colonies from tropical climates than in those from moderate climates (Harris and Harbo, 2001). However, the affect of environmental factors to non-reproductive *Tropilaelaps* mites in bee colonies has yet been studied.

In this study, 28.3% of non-reproductive mites produced too young progeny to mature before emerging of the host bee (Table 5.2). This finding was inconsistent with the previous report by Ritter and Schneider-Ritter (1988) that all *T. clareae* offspring could develop to be adults before hatching of *A. mellifera* (in Thailand) host bees. *Tropilaelaps* mites that were sandwiched between the cocoon that is spun by the host larva and the cell wall (entrapped by the cocoon) were not found in this study. Few entrapped *Varroa* mites (1-2%) were found in unselected *A. mellifera* colonies, but more than 50% of mites in colonies of bees bred for suppression of mite reproduction were entrapped (Harris and Harbo, 2001).

Mite fecundity

The actual reproductive rate for *Tropilaelaps* was calculated from reproductive mites. The number of progeny per reproductive *T. clareae* in *A. dorsata*, domestic and Primorsky colonies was not significantly different (Table 5.2). Overall, a reproductive *T. clareae* produced 1.8 progeny. The maximum number of offspring produced by a single *T. clareae* female in this study was found to be 8 while Woyke (1987) reported that the maximum number of progeny was 4. Most reproductive mites (42%) produced 1 progeny (Table 5.3). 37% of reproductive mites produced 2, 16.2% produced 3, 3.2% produced 4 and 0.3-0.8% produced 5-8 progeny. These percentages agree well with the previous report by Ritter and Schneider-Ritter (1988) that 64% of the *Tropilaelaps* females from *A. mellifera* brood in Thailand had produced 1, 33% had produced 2 and 3% had produced 3 offspring. The result here showed that the number of progeny produced of reproductive *Tropilaelaps* mites (or fecundity) was not correlated with the number of non-reproductive mites in the colonies of the three honey bees. This incidence was consistent with previous reports from several researchers that the number of progeny

produced by reproductive *Varroa* mites was independent of the frequency of non-reproductive mites in a colony (Rosenkranz and Engels, 1994; Martin, 1995).

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