CHAPTER 5

Comparative reproduction of *Varroa* spp. in *Apis cerana* and *Apis mellifera*

5.1 Introduction

Varroa is an acarine parasite of brood and adult bees (*Apis* spp.) and currently possesses a near worldwide distribution. The first identified species was *V. jacobsoni* Oudemans (1904) infesting the eastern honey bee, *Apis cerana,* in Java, Indonesia. Subsequent reports of *Varroa* in Southeast Asia include Singapore (Gunther, 1951) and Hong Kong (Delfinado, 1963), which reported *Varroa* using *A. mellifera* as the host bee species for the first time. Later, Akratanakul and Burgett (1975) found *Varroa* in Thailand. *Varroa* quickly spread throughout Asia since then due to the international importation of queen honey bees (*A. mellifera*) of. Recently, Anderson and Trueman (2000) redefined *V. jacobsoni* as two different species: *V. jacobsoni* and *V. destructor*.

Some of the earliest studies on *Varroa* reported that in its original host, *A. cerana*, it reproduces in worker brood only but not in worker brood (Koeniger et al., 1981, 1983; Koeniger, 1987; Rath and Drescher, 1990; Tewarson et al., 1992; Anderson and Sukarsih, 1996; Boot et al., 1997; Rath, 1999). However, De Jong (1988) demonstrated that *Varroa* can successfully reproduce in both worker and drone brood of *A. cerana* in South Korea, albeit with a higher prevalence in drone brood. In *A. mellifera*, the non-adapted host, *Varroa* also parasitizes both worker and drone brood with high prevalence in worker brood, but an increased fecundity in drone brood (Fuchs, 1990).

Varroa has been considered to be the most serious pest of honey bee brood and adult bees and has caused incalculable losses to commercial beekeeping worldwide. *Varroa* feeds on the haemolymph of the developing honey bee larva, pupa, and adult bees. Severely infested colonies usually express large numbers of de-capped (opened) brood cells. Additionally, *Varroa* is a vector of viral pathogens, e.g., deformed wing virus (Dietz and Hermann, 1988; Fries et al, 2003; Chen and Siede, 2007). Serious

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Varroa infestations result in the presence of moribund newly emerged bees with malformed wings, legs, and reduced abdomens. *Varroa* is considered one of the more serious contributors to CCD (Colony Collapse Disorder) (vanEngelsdorp et al., 2009).

Historically, *A. cerana* is the indigenous commercial honey bee throughout Thailand. Larger scale introductions of *A. mellifera* into Thailand began in the early 1980s (Wongsiri et al., 2000). Presently there are *ca*. 300,000 *A. mellifera* colonies in Thailand primarily located in northern provinces, particularly Chiang Mai and Lamphun Provinces. Both honey bee species serve as *Varroa* hosts.

A. cerana in Thailand is infested with three *V. jacobsoni* haplotypes, namely: Northern Thai, Malaysia, and Samui (Smith and Hagen, 1996); Warrit et al. 2006). Two additional haplotypes of *V. destructor* [Japanese or Japan/Thailand (J) and Korea or Russian (K or R)] area also found infesting *A. mellifera* in Thailand and are thought to possess different levels of virulence (de Guzman and Rinderer, 1999; Anderson, 2000; Anderson and Trueman, 2000, Navajas et al., 2010). Recently, variants of J (J1-6) and K (K1-4) as well as for other Varroa haplogroups have been identified (Navajas et al. 2010). The K1 haplotype is considered to be the most virulent; colonies of *A. mellifera* have been shown to survive infestations of the J1 haplotype (Vetharaniam and Barlow, 2006). Reproductive success is related to the genotypes of *Varroa*. *V. jacobsoni* is known not to reproduce in *A. cerana* worker brood. In South Korea, Anderson and Trueman (2000) also found the K haplotype of *V. destructor* in both *A. cerana* and *A. mellifera*.

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Since *Varroa* infestations ultimately bring about the death of host *A. mellifera* colonies, further studies of the host/parasite relationship are warranted considering that this honey bee species is a non-adapted but competent *Varroa* host. This study assessed the reproductive ability of *Varroa* mite spp. collected from *A. mellifera* and *A. cerana* colonies and cross-infested to their adapted and non-adapted honey bee host species.

5.2 Materials and methods

5.2.1 Host honey bee species (*A. mellifera* and *A. cerana*) sources and *Varroa* sources

Nine traditional *A. cerana* hives (indigenous to Northern Thailand) and 11 *A. mellifera* colonies were used as the sources for *Varroa* and placed in the same apiary site on the Chiang Mai University campus, Chiang Mai, Thailand. For the cross-infection trials, sufficient numbers of newly sealed larvae were required. The availability of *A. mellifera* and *A. cerana* L3 and L4 larvae was confirmed two days before the initiation of the experiments to ensure an adequate number of appropriately aged larval hosts. Also at this time, the *Varroa* infestation prevalence for both honey bee species hosts was assessed. P_1 adult *Varroa* necessary for inoculation were obtained at this time (two days pre-inoculation) by opening capped brood and extracting mites, which were then placed on adult worker bees of both *A. mellifera* and *A. cerana* held in cages. This step allowed the mites a two-day phoresy period prior to the cross-inoculation experiments. The P_1 foundress female mites used in these experiments were considered reproductive if their host brood cell from which they were extracted contained at least one progeny. During the 2-day phoresy period, the caged adult honey bees were fed sugar water (1:1 by weight). Adult honey bees were stocked at 10-12 bees per cage with one adult host carrying 1 or 2 mites.

To provide newly sealed brood, the location of L5 larvae was mapped on transparent sheets. After 6-8 h, the newly sealed brood area was marked. Because of the hygienic behavior of *A. cerana*, workers do not cap L5 larva as quickly as done by *A. mellifera* workers, therefore a layer of tissue paper was used to recap *A. cerana* L5 cells after inoculating the P_1 *Varroa*. *Varroa* from *A. cerana* host brood was inoculated into *A. mellifera* brood. Conversely, *Varroa* from *A. mellifera* was inoculated into *A. cerana* brood. For the control, *Varroa* originating from *A. cerana* brood was inoculated back to *A. cerana* brood, and the *Varroa* from *A. mellifera* to *A. mellifera* brood. During cross-inoculations, where cell caps were opened to introduce mites, an equal number of brood cells were opened and

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re-closed without mite inoculation, which served as a control for the inoculation technique. Brood combs were then incubated at 34.5˚C and 70% relative humidity. *A. mellifera* and *A. cerana* test brood cells were then de-capped 11 and 10 days, respectively after inoculating mites in order to assess the reproductive success of *Varroa*. Honey bee larval hosts developmental stages follow that of Human et al. (2013). Following the determination of P_1 reproductive success, the foundress mites and progeny were sampled for species verification using DNA analysis.

5.2.2 DNA analysis

Varroa species and haplotypes were identified based on the mtDNA sequence (328 bp *Cox-1* gene sequence) described in Warrit et al. (2006). PCR was performed using a *COI* 51-F primer (5'-GTAATTTGTATACAAAGAGGG-3') and a *COI* 1400-R primer (5'-CAATATCAATAGAAGAATTAGC-3') (Warrit et al., 2006). The PCR amplifications consisted of initial denaturation at 94˚C for 1 min, followed by 35 cycles of [94˚C, 40sec; 40˚C 1min 30sec; increase to 72˚C at rate of 0.3˚C/sec; and 72˚C for 2 min], then final extension 72˚C 5 min, finally hold at 4˚C. The PCR products were purified using the PureLink® Gel Extraction kit (Life Technologies, Carlsbad, CA). The species or haplotypes of *Varroa* collected from *A. mellifera* and *A. cerana* were confirmed as the Korea (K1) haplotype of *V. destructor* and Northern Thai (NThai) haplotype of *V. jacobsoni*, respectively.

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5.3 Results

V. destructor (K1) was able to reproduce on *A. cerana* worker brood however, at a lower level of prevalence compared to reproductive success when parasitizing *A. mellifera* worker brood (9.0% vs. 44.7%, respectively) (Table 5.1). This also seems to be the case for drone brood. However, the sample size for drone brood in this study is too low to confidently state that the K1 haplotype experiences a high prevalence rate on *A. mellifera* drone brood, while much lower prevalence on *A. cerana* drone brood (*A. mellifera* drone brood, 100% reproductive; *A. cerana* drone brood, 16.7%).

V. jacobsoni (NThai) supported reproduction success when infesting *A. cerana* worker brood however at a low prevalence (16.5%). NThai did not reproduce when infesting *A. mellifera* worker brood. This rate is lower than that for K1 in *A. mellifera* worker brood but higher than that for K1 when infesting *A. cerana* worker brood.

Table 5.1 Proportion of reproductive *Varroa* and fecundity of foundress of *V. destructor* and *V. jacobsoni* parasitizing *A. mellifera* and *A. cerana* worker brood.

Mite species	**Worker honey bee brood host**	
	A. mellifera	***A. cerana***
V. destructor (K1):		
reproductive/total inoculated cells	46/103	10/111
reproductive success	44.7%	9.0%
fecundity	3.6±1.5	4.1±1.2
V. jacobsoni (NThai):		
reproductive/total inoculated cells	0/75	13/79
reproductive success	0%	16.5%
fecundity	-	3.2±1.7

5.4 Discussion

A. cerana has an Asian distribution (Ruttner, 1988) and is the adapted host for both acarine brood parasites, *V. destructor* and *V. jacobsoni* (Smith and Hagen, 1996, 1999; Anderson and Trueman, 2000; de la Rua et al., 2000; Smith et al., 2000; Hepburn et al., 2001). Following the anthropogenic introduction of *A. mellifera* into Asia, and especially Southeast Asia, it was soon discovered that *A. mellifera* can serve as a competent, albeit non-adapted host for *V. destructor*.

Anderson and Trueman (2000) reported *Varroa* to be a species complex. They distinguished *Varroa* species *via* morphology and the mitochondrial Cytochrome Oxidase I (*COI*) gene sequencing. In 1999, de Guzman and Rinderer documented the presence of J and R (= K) in both *A. cerana* and *A. mellifera* colonies in Thailand. However, Anderson and Trueman (2000) found only the *V. destructor* J1 haplotype

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infesting *A. cerana* in central Thailand, while both the J and K haplotypes were shown to infest *A. mellifera*. Further studies also identified J1 infesting *A. mellifera* in Northern Thailand (Navajas et al. 2010). No evidence of J on A. cerana was reported by Warrit et al. (2006). These authors found three new haplotypes of *V. jacobsoni* (Malaysia, Northern Thai and Samui) infesting *A. cerana* in Thailand i.e.

Our results demonstrate, at least in an experimental arena, that the K1 haplotype is capable of reproductively successful when infesting *A. cerana* worker brood but at a lower rate than in *A. mellifera* worker brood. Based on a limited sample size, K1 haplotype may also reproduce in *A. cerana* drone brood at a lower rate as compared to that in *A. mellifera* drone brood.

My results support De Jong's (1988) observation indicating that *Varroa* is capable of reproducing in worker brood of *A. cerana*. The K haplotype is the only *Varroa* mite infesting *A. mellifera* and *A. cerana* in South Korea (Anderson and Trueman 2000). Thus, this study may explain de Jong’s results. This finding also demonstrates for the first time that NThai1 of *V jacobsoni* cannot reproduce in *A. mellifera* worker brood, an observation also reported by Anderson and Fuchs (1998) with the Java haplotype of *V. jacobsoni* infesting *A. mellifera* in Papua New Guinea.

Tropilaelaps ssp. were considered the most serious acarine pest for *A. mellifera* in SE Asia. To investigate comparative reproduction of *Tropilaelaps* on its adapted host (*A. dorsata*) vs. the non-adapted host (*A. mellifera*) is an ecologically interesting research question. *A. dorsata* are most prevalent during the late December to late May period in northern Thailand. *A. dorsata* most likely experiences low *Tropilaelaps* infestations because of their natural migratory absconding behavior. Thus, the seasonal abundance and incalculable prevalence of *Tropilaelaps* on *A. dorsata* colonies are some factors to include in the wider research program.

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