CHAPTER 3

Apis dorsata F. comb structure and *Tropilaelaps* infestation

3.1 Introduction

Apis dorsata F. is one of two species of giant honey bees with a range throughout the Indian subcontinent, Southeast Asia and numerous southwestern Pacific Archipelagos (Ruttner, 1988). The large single comb nest of the giant honey bee can accommodate adult bee populations in excess of 70,000 individuals (Morse and Laigo, 1969). Giant honey bee colonies are known to exhibit a migratory life style with several colony movements during an annual cycle (Koeniger and Koeniger, 1980; Oldroyd et al., 2000; Woyke et al., 2000). The single comb nests can attain a large size, with reported dimensions of 1.5 m x 1 m (Oldroyd and Wongsiri, 2006). The construction of new nest comb represents a major investment of energy and wax material. The wax comb is comprised of hexagonal cells with the overall comb structure commonly assuming a semicircular shape under most circumstances. The nest combs usually hang from the underside of larger diameter tree branches, but often colonies will be established on the eves of building. Both giant honeybee species utilize the same diameter cell for rearing worker and drone brood, which is unique from other *Apis* species which construct larger cells for rearing drone brood. The food storage area (honey and pollen) is found in the upper top most section of the nest while the brood area makes up the majority of the total comb area.

A. dorsata is the indigenous host of *Tropilaelaps* (Burgett and Kitprasert, 1990; Burgett *et al*., 1990). *Tropilaelaps mercedesae* is also capable of infesting *A. mellifera* brood as a non-adapted or alternate host. For Southeast Asian *A. mellifera* beekeeping, *T. mercedesae* has become the paramount acarine parasite (Oldroyd and Wongsiri, 2006; Anderson and Morgan, 2007). When infesting its indigenous host (*A. dorsata*), *Tropilaelaps* can reproduce using both worker and drone brood but does not exhibit a preference for brood gender, unlike *Varroa* which is limited to drone brood when

infesting its natural host *A. cerana* (Koeniger et al., 1981; 1983). However, it is unknown whether *Tropilaelaps* prefers worker or drone brood when parasitizing the non-adapted host *A. mellifera*.

This study examined *A. dorsata* combs to derive several metrics that would allow for an accurate summarization of comb architecture as it relates to total comb volume, partitioning of comb between brood and food storage, gravimetric holding capacity, and potential honey storage. Additionally, this study demonstrated whether or not *Tropilaelaps* exhibits a preference for brood host gender.

3.2 Materials and methods

3.2.1 Source of samples

1) *A. dorsata* comb samples

Recently abandoned combs (bee- and brood-free) were obtained during January and February 2011 from the Chiang Mai University campus, and a private residence dormitory in metropolitan Chiang Mai, Thailand (18° 58’ N, 98° 59’ E). The colonies had been established in late 2010 and early 2011.

2) *Tropilaelaps* mite samples

Three mature *A. dorsata* brood combs were obtained in mid-January and early March, 2012. These colonies had an abundance of worker and drone brood for this study. The colonies were located in the Chiang Mai metropolitan area. To examine for the prevalence of *Tropilaelaps*, 1,000 sealed drone brood cells and 1,000 sealed worker brood cells were de-capped. *Tropilaelaps* samples were also collected kept at -20˚C for identification using DNA analysis.

3.2.2 Genotypic identification of *Tropilaelaps*

The genomic DNA was extracted from each of four *Tropilaelaps* mites. The entire ITS1-5.8S-ITS2 gene region was amplified using an ITS4 primer (5′-TCCT CCGCTTATTGATATGC-3′) and an ITS5 primer (5′-GGAAGTAAAAGTCGTA

ACAAGG-3′) in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) (White et al., 1990). The PCR component reactions was modified from the methods of Anderson and Morgan (2007): the mixture was initially denatured at 94ºC for 4 min and then processed with 30 amplification cycles, each consisting of 1 min at 94ºC, 1 min at 56ºC and 1 min at 72ºC. The reactions were completed by a final extension step spend time for 10 min at 72ºC. The 5 µl PCR products were separated using electrophoresis on a 1.5% agarose gel. The gel was stained by ethidium bromide a nd then visualized under UV illumination. The PCR band was purified by using NucleoSpin® Exact II (Germany).

3.2.3 *A. dorsata* combs measurement

The following comb parameters were measured: cell diameter, cell depth, total comb area (sub-divided into honey comb area and brood comb area), total number of brood cells, total honey storage cells, brood comb volume, and honey comb volume. To determine cell diameters, a series of 10 linear cells were measured. Additionally, individual cells were measured with an ocular micrometer. To determine the volume of cells, the internal cell wall diameter was used for calculating. For establishing the number of cells/cm^2, cell diameter was measured obliquely from the interior to the exterior of the opposing cell wall (septum). To determine cell depth, the distance from cell top to bottom from linear cell series cut along a sagittal plane through the comb was measured. The depth of the honey storage cells was variable within a comb and between combs. Thus for establishing an average depth of honey storage cells, a series of measurements were made both latitudinally and longitudinally through the honey storage area. Due to inter- and intra-colony variation for honey cells, an average honey cell depth was calculated for the honey storage areas of each comb. The total comb area was determined by tracing the comb outline onto paper. The tracings were then scanned to make a digital .jpg format. These images were imported to the public domain software program “ImageJ” (U.S. National Institutes of Health), which calculated and converted area to cm^2. Additionally, the honey and brood

comb areas were separately determined for each of the six combs. The mean cell diameters of brood and honey cells were compared by a one-tail ANOVA.

To determine a potential for weight and volume of honey storage, an average weight was determined for honey (1 liter). This, combined with the known honey comb area, allowed us to calculate honey storage potential. For the total weight of the brood, the average weight of brood comb was calculated by area (g/cm^2 of comb surface area). Combining honey weight and brood comb weight, I was able to determine a reliable estimate of total colony weight. To determine honey and brood sample weights, samples were taken from three active *A. dorsata* colonies in January 2012.

3.3 Results

3.3.1 DNA sequencing

When compared in the Gen Bank database using the BioEdit program (Hall, 1999), the DNA sequences (465-573 bp) derived from our samples (Accession number KP774522, KP774527-774529) showed a 100% similarity to sequences from *T. mercedesae* collected from China [EF025472.1]. Consequently *Tropilaelaps* used in this study were identified as *T. mercedesae* (Anderson and Morgan, 2007).

3.3.2 *A. dorsata* comb structure

All six combs examined conformed to the stereotypic semicircular form. Comb width was measured across the tops of the combs and ranged from 33.5 cm to 91 cm. The depth of combs, by measuring from the top to the comb bottom, ranged from 30.5 cm to 73 cm. The largest comb (C4) had an area, both comb sides, of 8,314 cm^2, while the smallest (C5) an area of 1,744 cm^2. Table 3.1 and Figure 3.1 show the diameter and depth of both brood cells and honey cells (the average cell sizes). They are in close agreement with previous studies (Ruttner, 1988; Oldroyd and Wongsiri, 2006; Tan, 2007). The average diameter and depth of honey cells were larger than that of brood cells. This range of brood cell diameters (5.1 – 6.1 mm) closely matches those reported by Tan (2007) (5.2 – 6.1

mm). I was not able to discriminate any cell size difference between worker brood cells and drone cells as reported by Tan (2007), albeit the combs in this study possessed no brood, therefore I was unable to determine if a brood cell had been used to produce a drone or worker. The difference between the mean cell diameters of brood and honey cells was statistically significant ($P < 0.001$, ANOVA test).

Table 3.1 Mean cell dimensions as determined from six *Apis dorsata* combs.

Cell Type	Diameter± SD (mm)	Area ± SD (mm^2)[1]	Cells/cm^2± SD	Depth±SD (mm)	Volume±SD (mm^3)[2]
Brood	5.54 ±0.16	26.58 ±0.60	3.76 ± 0.09	16.79 ± 0.65	406.89 ± 14.98
Honey	6.25 ±0.39	33.83 ± 1.15	2.96 ± 0.10	variable	variable

[1]The area of a hexagon = the diameter^2 x $\sqrt{3}/2$

[2]For cell volume determination, the cell interior diameter was used (*i.e.*, 5.29 mm for brood cells, 6.00 mm for honey cells)

Table 3.2 is the metrics summary of the six combs. Determining the total number of cells per comb was done by multiplying the known comb area (cm^2) by the number of cells per cm^2 (3.76 and 2.96 cells per cm^2 for brood and honey cells respectively). The largest comb (C4) possessed nearly 5 times as many cells as the smallest comb examined (C5). As the combs used in this study were from colonies that had absconded prior to reproductive maturity and therefore represent colonies of varying ages, it is not unexpected to see a broad range of variation in comb area and the total number of cells between the six combs. One measure that possessed only a small difference between colonies was that of the proportion of comb area devoted to brood production *vs*. food storage. On average, 82.7% of the total comb area was used for brood production with a range of 80.2% to 88.0%, with the remainder of the comb used for food storage, primarily nectar and honey. The average volume of a brood cell was 406.9 mm^3. A universal average volume for a honey storage cell could not be computed due to the great variability in honey cell depth. However, by calculating an average honey cell depth for each individual comb's honey storage area, I was able to derive a mean honey cell volume on a per comb basis. Following the determination of average brood and

honey cell volumes, it is a straight forward matter to calculate the total volumes for both the brood comb and the honey storage comb for each examined colony.

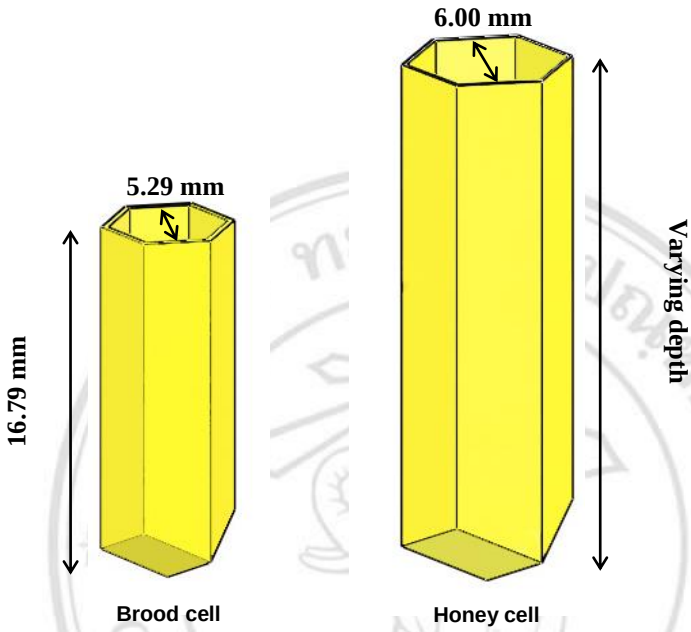

Figure 3.1 *A. dorsata* brood and honey cells average dimensions model *via* Sketchup 8. The bases of the hexagonal cells are trihedral, with only one of the three planes shown. Schematic by Mr. Prapat Thongjun, Post-Harvest Technology Research Institute, Chiang Mai University.

Table 3.2 Summarized metrics for *Apis dorsata* combs 1–6.

	C1	C2	C3	C4	C5	C6
∑comb area (cm^2)	2,790	6,366	6,956	8,314	1,744	3,120
Honey comb area (cm^2)	552	764	1,252	1,568	322	526
Brood comb area (cm^2)	2,238	5,602	5,704	6,746	1,422	2,594
% brood comb	80.2	88.0	82.0	81.1	81.5	83.1
∑ cells	10,043	23,317	25,140	29,991	6,296	11,310
Honey cells	1,628	2,254	3,693	4,626	950	1,557
Brood cells	8,415	21,063	21,447	25,365	5,346	9,753
∑comb vol. (l)	4.97	11.07	13.57	20.29	3.08	6.73
Honey comb vol. (l)	1.21	1.67	3.99	8.96	0.69	2.76
Brood comb vol. (l)	3.76	9.40	9.58	11.33	2.39	3.97

A hypothetical gravimetric capacity for a comb can be obtained by summing the weights of the honey and brood. *A. dorsata* honey weight varies slightly according to the moisture content, but from a series of honey samples taken from separately harvested honey combs, I obtained a weight of 1.35 kg/l. Brood weight estimates are based on capped brood samples which averaged 0.9 g/cm^2 of comb surface area. Table 3.3 provides estimates of the gravimetric holding capacity for the six combs examined. An additional weight, which I have not taken into consideration, is that of the adult bees. From samples of curtain bees taken from living *A. dorsata* colonies, I derived an average worker bee weight of 160.5 ± 21.8 mg which is *ca*. 6,230 worker bees/kg. Because the combs used in this study had been abandoned, I have no way of knowing the populations of adult bees prior to absconding. Worker bee density per unit area of comb has only been determined

for the European honey bee, *A. mellifera* (Burgett and Burikam, 1985) and as no published data exist regarding *A. dorsata* worker bee density per cm^2 of comb, I was unable to estimate adult bee populations from the comb area for the combs used in this study.

Table 3.3 The potential gravimetric capacity of *A. dorsata* combs 1-6, is based on the assumption that the honey storage area is completely utilized and the brood comb is at 80% capacity.

***A. dorsata* Comb**	**Brood (kg)[1]**	**Honey (kg)[2]**	**∑ brood & honey (kg)**
1	1.61	1.63	3.24
2	4.03	2.25	6.28
3	4.10	5.39	9.49
4	4.86	12.10	16.96
5	1.04	0.93	1.97
6	1.86	3.73	5.59

[1]brood wt.=0.9 g/cm^2 capped brood

[2]honey = 1.35 kg/l

3.3.3 *Tropilaelaps* mite infestation in *A. dorsata*

These results show that *T. mercedesae* does not preferentially infest *A. dorsata* drone brood over worker brood (Table 3.4). Moreover, the number of offspring per *T. mercedesae* foundress infesting drone brood is less than the progeny from worker infested brood.

Table 3.4 *T. mercedesae* infestation rate of *A. dorsata* brood and the number of offspring per foundress by host brood gender. (n = 1000 worker pupae and 1000 drone pupae per colony, ∑ = combine sample = 6,000 *A. dorsata* pupae)

A. dorsata sample	Infestation Rate		Offspring/P_1 Female	
	Worker brood	Drone brood	Worker brood	Drone brood
Colony 1	4.1%	1.4%	2.1	1.7
Colony 2	0.5%	0	3.8	n/a
Colony 3	9.8%	4.7%	3.5	3.1
∑	4.8%	2.0%	3.2	2.8

P_1 = First parent; n/a = not available

3.4 Discussion

This study concerning brood and honey cell dimensions agrees with previously reports. Tan (2007) however found a statistically significant cell width difference between brood cells used to rear drones and workers. I do not dispute his finding *per se*, but note that his range of widths for 'worker' brood cells is 5.2-6.1 mm, and the range for 'drone' brood cells is 5.5-6.1mm. From my results, the brood cell range was 5.1-6.1 mm. My interpretation is that within the combined ranges Tan reported (5.2-6.1 mm), drones are produced in the mid- to upper range cell widths, while worker brood are successfully reared in any cell size within the entire range of brood cell diameters.

Table 3.5 summarizes previous reports of comb cell metrics between five *Apis* species. For both dwarf honeybee species (*A. andreniformis* and *A. florea*), and the cavity nesting *A. cerana* and *A. mellifera*, there are two distinct cell types involved in brood rearing; the larger brood cells for rearing drones and the smaller cells for rearing worker bees. Additionally for the two dwarf honeybee species, the honey storage cells are much deeper (elongated) than cells used for rearing brood (Burgett pers. obs.) and therefore an average depth is not attainable except on a per colony basis.

Table 3.5 Comparisons of cell width and cell depth among five honey bee species.

Cell Type	*A. andreniformis*	*A. florea*	*A. cerana*	*A. mellifera*	*A. dorsata*
Worker					
Width (mm)	2.78±0.23[1]	2.98±0.15[1]	3.6-4.9[2,3,4,5]	5.2[6]	4.5-5.9 [7,8,9]
Depth (mm)	7.60±0.20[1]	9.30±0.70[1]	10.1[2]	11.0[6]	16.0-19.0[7,8,9]
Drone					
Width (mm)	4.18±0.24[1]	4.88±0.21[1]	4.7-5.3[3]	6.2[6]	5.81±0.14[9]
Depth (mm)	14.50±7.10[1]	13.30±0.70[1]	ND	12.5[6]	19.0±0.4[9]
Honey					
Width (mm)	ND	ND	ND	ND	6.39±0.3[9]
Depth (mm)	ND	ND	ND	ND	variable[9]

[1]Rinderer et al. (1996); [2]Inoue et al. (1990); [3]Crane (1993); [4]Tingek et al. (1996);
[5]Ruttner (1988); [6]Seeley and Morse (1976); [7]Doedikar et al. (1977); [8]Thakar and Tonapi (1961);
[9]Tan (2007); *ND: No data available

My study of giant honeybee comb architecture reiterates that cells for rearing both drone and worker brood are of a uniform size, albeit with *ca*. an 18% variability in width. Honey storage cells are greater in both width and depth than brood cells which are more uniform, suggesting that there is natural adaptation to have larger honey cells that, while economizing surface area, also possess greater volume, resulting in an efficient use of wax.

Concerning honey storage, Tan (2007) reported that colonies can possess *ca*. 4 kg of honey 3-4 weeks following nest initiation. From a large sample size of 152 colonies, he reported a maximum of 15.7 kg of honey. The six combs that I examined varied in potential honey storage (based on the volumes of the honey storage areas), but the largest comb could have theoretically held *ca*. 12 kg of honey.

Table 3.3 summarizes hypothetical colony weights for the six combs examined, which ranged from a low of 1.97 kg (C5) to a high of 16.96 kg (C6). This does not include the weight of the adult bees. Relying on the limited data of Morse and Laigo (1969), who reported colonies with as many as 70,000 workers, a colony large enough to have such a population would possess *ca*. 11 kilograms of adult bees in addition to

the weight of the comb, brood and food stores. In a study examining the mechanical properties of wax from four honeybee species, Buchwald et al. (2006) reported that the wax from *A. dorsata* was the strongest and stiffest wax of the four species examined. Due to the weight of the single comb nest, attached to the underside of a substrate, the findings of Buchwald et al. (2006) are not unanticipated, and, as they point out, conform to the nesting ecology of this giant honeybee species.

Based on size (comb area), the number of adult bees and weight, colonies of the giant honeybee *A. dorsata* should be considered as a species of the mega-faunal community in the tropical ecosystems where they exist; playing a vital role as pollinators contributing to the botanical biodiversity wherever they occur. This study would agree with Oldroyd and Wongsiri (2006) who comment that a colony of *A. dorsata* "…is a thing of wonder…".

Drone brood in *A. dorsata* colonies is not aggregated as it is in the cavity nesting species of the genus *Apis*, or the dwarf honey bee species *A. florea* and *A. andreniformis*, but rather drone brood is randomly spread throughout the *A. dorsata* brood nest (Burgett et al., 1990; Tan, 2007). If searching out preferred hosts based on gender, *T. mercedesae* would therefore not encounter well defined patches of male brood, hence a strategy of gender preference could potentially involves an elongated search period on the part of gravid female mites which may not be naturally selected as an adaptive trait. Additionally, while *A. dorsata* drones do have a 4-day longer developmental period than worker brood, *T. mercedesae* has a notably short developmental period (egg to adult) of 6-7 days (Rath et al., 1991). Considering that the research location for the Rath et al. (1991) report was northern Thailand, it is highly probably that they were observing *T. mercedesae* as opposed to their *T. clareae* nomen. Additionally their findings are based on *Tropilaelaps* utilizing *A. mellifera* as the host bee species. As pointed out by Oldroyd and Wongsiri (2006) and Warrit and Lekprayoon (2011), past studies on *Tropilaelaps* life history have rarely utilized their indigenous giant honey bee hosts, but have been largely based on observations of the parasites when infesting *A. mellifera*, the alternate, non-adapted host.

Varroa prefers drones over workers in *A. mellifera* (non-adapted) and *A. cerana* (adapted) (Koeniger et al., 1981; 1983; De Jong et al., 1982). *Tropilaelaps* prefers drones over workers in *A. mellifera* (non-adapted) (Burgett et al., 1983), but what about n its adapted host.

It is not common to find *A. dorsata* combs with a great deal of drone brood. Nevertheless, I found 3 *A. dorsata* combs that possessed an abundance of drone brood when compare to the usual amount of brood. These combs were used for the *T. mercedesae in vivo* infestation of *A. dorsata*. Because drone immature development time is longer than worker development one could anticipate a mite preference for drone brood over worker brood. From our results it appears that the mite is unable to distinguish the gender of a host brood. These findings support the alternative hypothesis that when *T. mercedesae* infests its adapted host, the mite parasite does not preferentially infest drone brood. *T. mercedesae* experiences reproductive success with both brood genders. Base on host gender, the data suggest that *T. mercedesae* experiences a higher parasitism rate, and "*r*" value on worker brood than drone brood.