CHAPTER - 4

LITERATURE REVIEW

4.1. Taxonomy of *A. dorsata* Fabricius 1793

Order : Hymenoptera
Family : Apidae
Genus : *Apis*
Species : *Apis dorsata*

4.2. Distribution of *A. dorsata*

A. dorsata is distributed almost all of the Indo-Malayan region. In the west *A. dorsata* is extended to the Indus river and almost to the Xerotherm coasts of the Persinan Gulf. In the east, *A. dorsata* is found in all of the Philippine islands including Wallace line as far as the Kei islands east of Timor and Borneo, Cambodia, India, Laos, Nepal, Palawan, Thailand and Vietnam (Figure 2) (Maa, 1953; Sakagami et al., 1980). Colonies of *A. dorsata* usually occur at altitudes of up to 1500 m, but may occur seasonally at up to 2000 m in different regions (Husain, 1938; Muttoo, 1956; Reddy, 1980; Gautum, 1984). In Thailand, *A. dorsata* is found from 10-1600 m from the sea level (Wongsiri et al., 1996a).

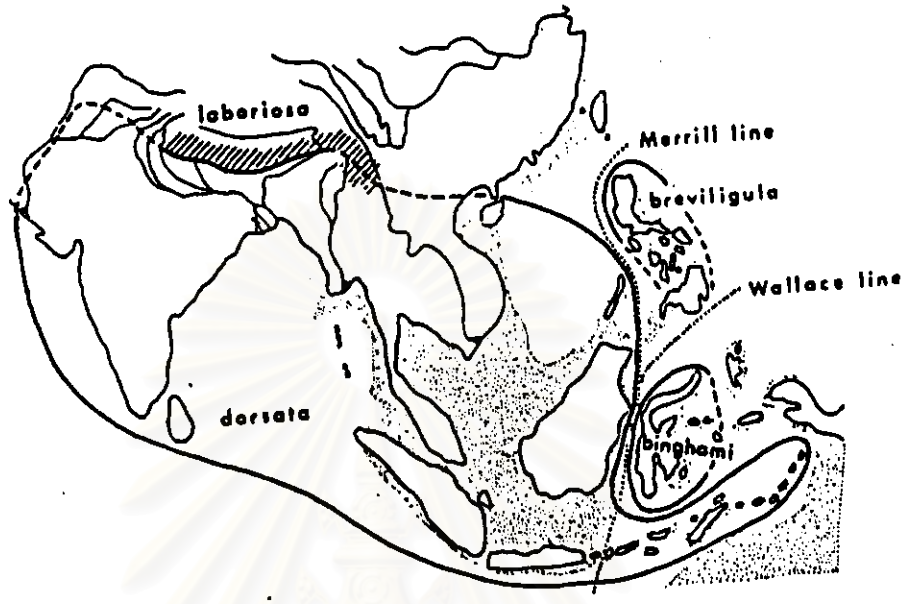

Figure 2. Distribution of *A. dorsata* in Asia.
(Adapted from Ruttner, 1980)

4.3. Caste

4.3.1. Queen

The queen is the colony's primary reproductive female. She does not have various glands possessed by workers such as wax producing glands, salivary glands for brood feeding and glands for the production of alarm pheromone. The queen is also lack of pollen baskets. She mates at least with 13-39 drones in flight before oviposition is initiated (Oldroyd et al., 1996).

A. dorsata constructs the queen cells like other *Apis* species on the lower edge of the combs 5-11 cells per colony in a row (Millen, 1942; Viswanathan, 1950; Thakar and Tonapi, 1961; Morse and Laigo, 1969). There is not much different in the physical size of

workers and queen of *A. dorsata* except in the thorax (Figure 3). The thorax of a queen is significantly larger than that of worker bees and this may be an adaptation for emergency flight (Koeniger and Koeniger, 1980).

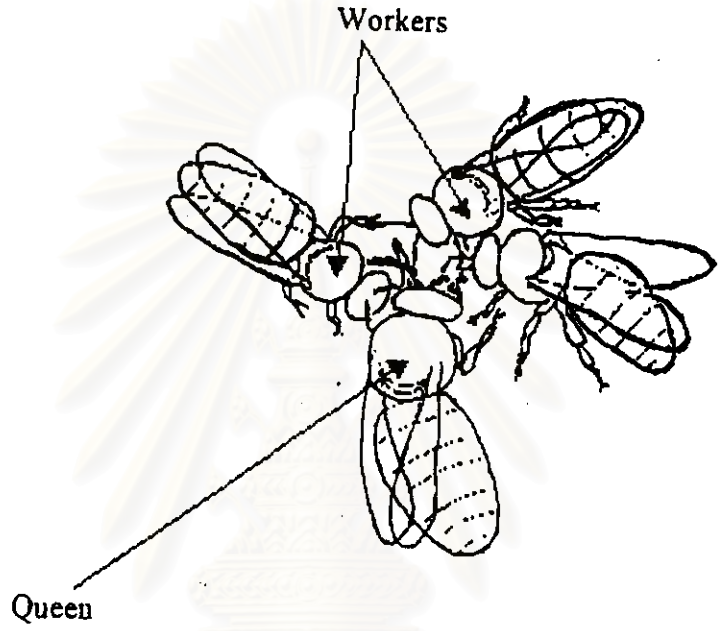

Figure 3. Comparison of the thorax of a queen and worker bees of *A. dorsata*. (Adapted from Koeniger and Koeniger, 1980).

4.3.2. Workers

Worker bees are sub-fertile or non-reproductive females. The head, thorax and abdomen are generally black and yellow-orange which are covered with dark brownish hairs. The main tasks of worker bees are brood care (feeding, cleaning), comb building, colony thermoregulation, defense against predators and foraging for pollen, nectar and water (Ruttner 1980).

4.3.3. Drones

Drones (*A. mellifera*) develop from unfertilized eggs (Dzierzon, 1848) has one set of chromosome and are therefore "haploid". The main task of drones is to fertilize virgin queens. A drone dies immediately after copulation. A single drone produces about 2.46 million spermatozoa (Koeniger et al., 1990). Drones usually mate in dusk under the canopy of a tall tree so-called "drone congregation area" (DCA) (Koeniger et al., 1994). Drone brood is irregularly scattered among worker brood. They are reared in the same cells as workers, but capping are slightly elevated (personal observation). However, it has been observed that during the main honey flow season, drone cells were constructed on the lower part of the combs as is *A. florea* and *A. andreniformis* (Wongsiri et al., 1996b). But in dearth season, (August) drones cells were randomly laid over the comb. Drones are seasonal (Seeley, 1985). Adult drones are found aggregated in the upper part of the brood comb, but usually concealed underneath the curtain formation bees (Morse and Laigo. 1969).

4.4. Life cycle

The development period of individual caste of *A. dorsata* is shorter than *A. mellifera* (Table 2) (Qayyum and Nabi, 1968). There has been no observation made on life span of individual bees of *A. dorsata* after emerging.

Table 2. Life cycle of *A. dorsata.*

Caste	Development periods (days)
Queen	13.00 - 13.5
Worker	16.00 - 20.00
Drone	20.00 - 23.5

Source: Qayyum and Nabi 1968

4.5. Foraging behavior

The maximum foraging range of *A. dorsata* is around 10 km (Seeley et al., 1982). *A. dorsata* is unique among *Apis* in that it can also forage and dance in the moonlight (Diwan and Salvi, 1965; Dyer, 1985). They were observed flying between 18:00-19:00 hours. The workers were collected in the light traps with pollen on their pollen baskets (Wongsiri, personal communication).

4.6. Colony defense

A. dorsata is the most ferocious stinging insect in the world. Usually 10-5000 bees and sometimes the whole colony attack nest intruders within a few seconds after being disturbed. For example, in Thailand March 1997, an old woman who wanted to harvest honey from a colony of *A. dorsata* was killed by the massive attacked by worker bees of *A. dorsata* in a village of Chuntaburi province (Bangkok post, 1997). During nest defending time, worker bees spray the alarm pheromone "iso-pentyl acetate" (Morse et al., 1967; Koeniger et al., 1979) and they can pursue intruders for long distances (Lindauer, 1961). However, defensive behavior of *A. dorsata* is strongly affected by high and low temperature, time of day (hours) and other environmental factors (Morse and Laigo, 1969).

4.7. Honey production

A typical nest of *A. dorsata* holds 1.8-4.0 kg of honey (Kallapur, 1950; Morse and Laigo, 1969) while the biggest nest holds 10-15 kg (Wongsiri et al., 1998). In China, a single colony of *A. dorsata* can produce 35.0-65.0 kg honey on average per hive per year and 15.0-29.0 kg per colony during the main honey flow season of *Brassica campestris* L. (Yaochun, 1984). However, in Thailand, *A. dorsata* colonies can produce not more than 2-17 kg honey during the main honey flow season of *Dimocarpus longan* L (Sapindaceae)

(Wongsiri *et al.*, 1998). In Nepal, the Gharti hill tribe people, harvest around 50.0 kg per colony (Stricland, 1982).

4.8. Colony relatedness

In diploid species each parent contributes exactly 50% of their genes to their offspring (F_1). So, the probability that any one parent will share a particular allele with an offspring is 0.5. This probability is denoted "r" (Wright, 1922).

In the case of honey bees, the queen is related to her offspring (sons and daughters) as in the diploid case (r = 0.5). However, because drones are haploid and can pass only one kind of gamete to their offspring, workers are related to their super-sisters by r = 0.75 (Table 3).

Table 3. Coefficient of relatedness for descendants and non descendants (Wright, 1922).

Coefficient of relatedness r	Descendant kin	Non descendant kin
0.50 (1/2)	offspring (F_1)	full sister
0.25 (1/4)	grandchildren (F_2)	half sibling,
0.125 (3/4)	great grandchildren (F_3)	cousins, nephews, nieces

4.9. Microsatellites

Microsatellites are sections deoxyribonucleic acid (DNA) that consists of tandem repeats (VNTRs) of very simple motifs such as (CT)n with 1-6 nucleotides per unit (Tautz, 1989; Queller et al., 1993; Choudary et al., 1993). They are generally less than 500 base pair (bp) in length. Most of these simple DNA motives are composed of one to

two nucleotides. Any sequence that is composed of either one to four or one to six bp repeated units is generally considered as a "microsatellite" (Glenn, 1994). Most microsatellites have twenty to two hundred bp per site. Ten or more repeated units are necessary for a hyper variable microsatellite (Weber, 1990). Microsatellites tend to be distributed through the euchromatin i.e. coding DNA, but the minisatellites (short sequence satellites) are distributed throughout the chromosomes and concentrated in the heterochromatin i.e. non-coding DNA (Stallings et al., 1991). They are mutated by a process called "slippage". During replication, repeated units become misaligned, leading either to increases or decreases in length (Schlottere and Tautz, 1992) (Figure 4).

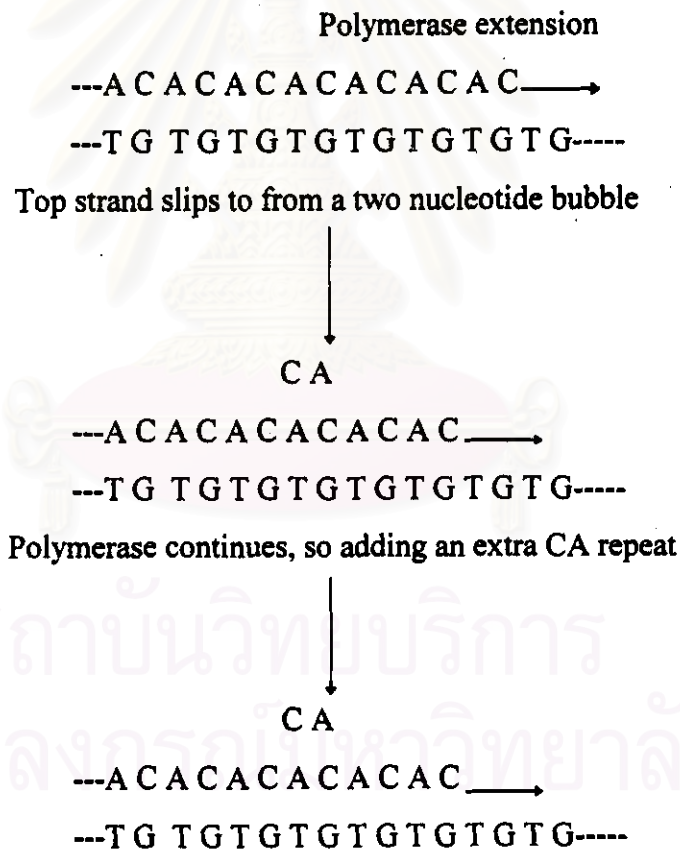

Figure 4. Microsatellite mutations by slippage.
(Adapted from Schlottere and Tautz, 1992)

4.10. Characteristic features of microsatellites

Microsatellite alleles are inherited in a Mendelian manner and have four unique characteristic features which allow them to be amplified by the Polymerase Chain Reaction (PCR) to produce easily scoreable alleles for pedigrees analysis:-

(i). The total size of a microsatellite regions is less than 500 bp (Glenn, 1994). However, the length of the actual repeated tend to be less than 300 bp. This facilitates amplification of the region for using see 4.13. PCR page 19.

(ii). The DNA flanking microsatellite regions tend to be a single copy of nuclear DNA which allows design of a specific PCR primer for annealing to the highly conserved regions of DNA.

(iii). Microsatellites mutate at a high rate 10^{-3}-10^{-4} per locus per generation for dinucleotides.

(iv). This mutation can occur by gain or loss of repeated units (Glenn, 1994). This creates different allele lengths at a given locus. For instance, one individual may have a sequence **ACACAC=$(AC)_3$** at a given locus, while another may have **ACACACACAC=$(AC)_5$**. This phenomenon allows us to distinguish the individual allele length sequence between the two PCR primers at a given locus.

4.11. Advantages of microsatellites

Microsatellites have several unique features which make them especially suited to genetic analysis:

i. Microsatellites PCR primers can often be transferred between closely related species (Schlottere et al., 1991; 1991; Deka et al., 1994),

ii. Microsatellites PCR primers can be used to exam the polyandry level in honey bees and bumblebess (Oldroyd et al., 1995),

iii. Microsatellites can be used as co-dominant markers,

iv. Microsatellites can use to maternity analysis and to investigate kinship patterns (Choudary et al., 1993; Peter et al., 1995),

v. Microsatellites can be used to determine relatedness between group members (Queller et al., 1993; Evans, 1996),

vi. Microsatellites can be used to determine how individuals disperse between social groups.

vii. Microsatellites are use to estimate the effective number of mating (number of drone copulated with a virgin queen (Estoup et al., 1994; Oldroyd et al., 1996, 1997)

4.12. Application of microsatellites

Microsatellites are being increasingly employed to answer the questions of relatedness. Microsatellite sequences are used as nuclear markers for identifying, population studies, linkage analysis and genetic mapping.

In 1993, Estoup and his colleagues developed a large number of PCRs microsatellite primers from *A. mellifera* L. which has allowed estimation of the number of drones mating with a queen of *A. mellifera* (Estoup et al., 1994) and *A. dorsata* (Moritz et al., 1995; Oldroyd et al., 1996) (Table 4). Oldroyd et al., have used different microsatellite loci to study levels of polyandry and intracolonial genetic relationships of all single open nest *Apis* species.

Table 4. Number of drones mate with a virgin queen of different single open nest *Apis* species determined by different microsatellite loci developed by Estoup et al., in 1994.

Investigators	*Apis* species	Number of drones mate with a virgin queen
Oldroyd et al., 1995	*A. florea*	5-14
Oldroyd et al., 1996	*A. dorsata*	13-39
Oldroyd et al., 1997	*A. andreniformis*	10-20

4.13. Polymerase chain reaction

Polymerase Chain Reaction (PCR) is a very useful method to amplify small quantities of relatively short target sequences of DNA so called "template DNA" that lies between two characteristic flanking sequences. PCR involves a series of repeated cycles

of DNA synthesis in buffer containing heat stable polymerase, two oligonucleotide primers and four deoxynucleaotide (dNTPs). During the reaction, the template DNA is first denatured to single strands by heating. When the DNA is cooled then primers anneal their complementary template so called "annealing stage'. Then DNA polymerase synthesis to form a new DNA extending 5'-3' direction (Figure 5).

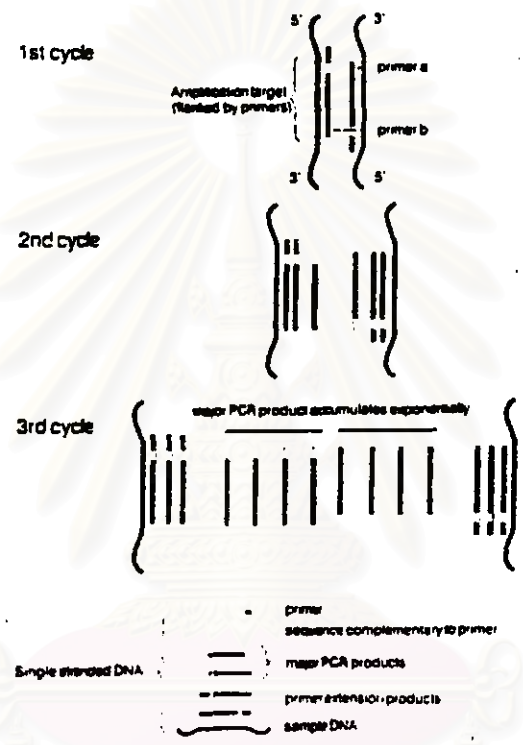

Figure 5. A simplified schematic view of the principle behind the polymerase chain reaction.

4.14. Roles of microsatellite in *Apis* species

Microsatellite technology has been broadly applied in social insects. Detailed accounts of the process of cloning and selecting microsatellite sequences are given by Queller et al., (1993) and Estoup et al., (1993). More than 100 microsatellite loci have been identified in *Apis* species, although only a few are needed to precisely identify the maternity of every individuals in a colony (Estoup et al., 1994).