

CHAPTER 2

LITERATURE REVIEW

2.1 Species Study

2.1.1. The genus *Apis*

Honey bees (genus *Apis*) belong to the family Apidae which comprise of 3 subfamilies; Bombinae (bumble bees), Meliponinae (stingless bees), and Apinae (honey bees) (Figure 2.1). Apidae are characterised by a complex organs used for collecting pollen called the pollen comb and pollen basket (corbiculae) (Ruttner, 1988; Hickman, Roberts and Larson, 1993) (Figure 2.2). Members of two subfamilies, Meliponinae and Apinae are highly social (eusocial).

The genus *Apis*, true honey bees, is a single genus in the subfamily Apinae. They live in a nest made from wax in a form of double-sided, vertical combs of hexagonal cells. The nests are built in various sites according to species. The nest architectures of different species are variable and are sometimes used for species identification (Rinderer et al., 1996).

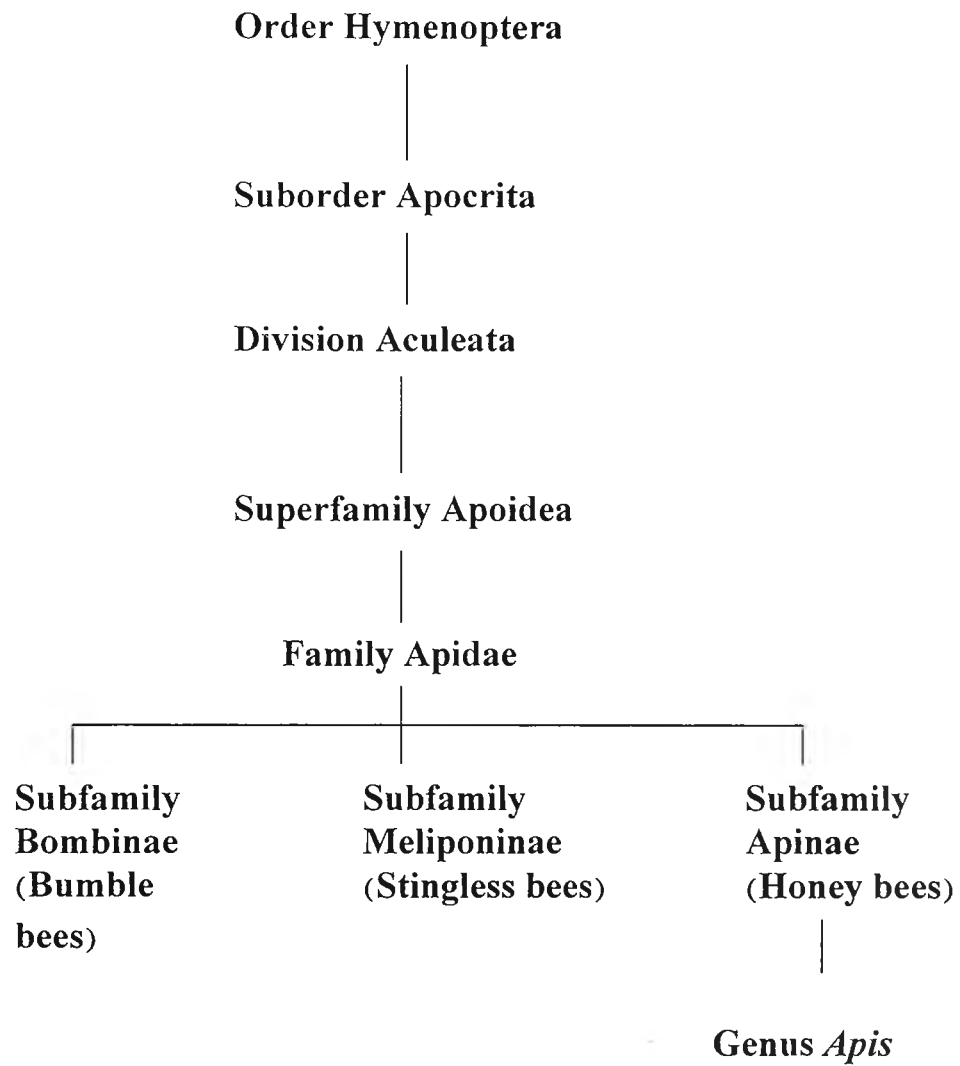


Figure 2.1 Classification of the family Apidae. (Modified from O'Toole and Raw, 1999).

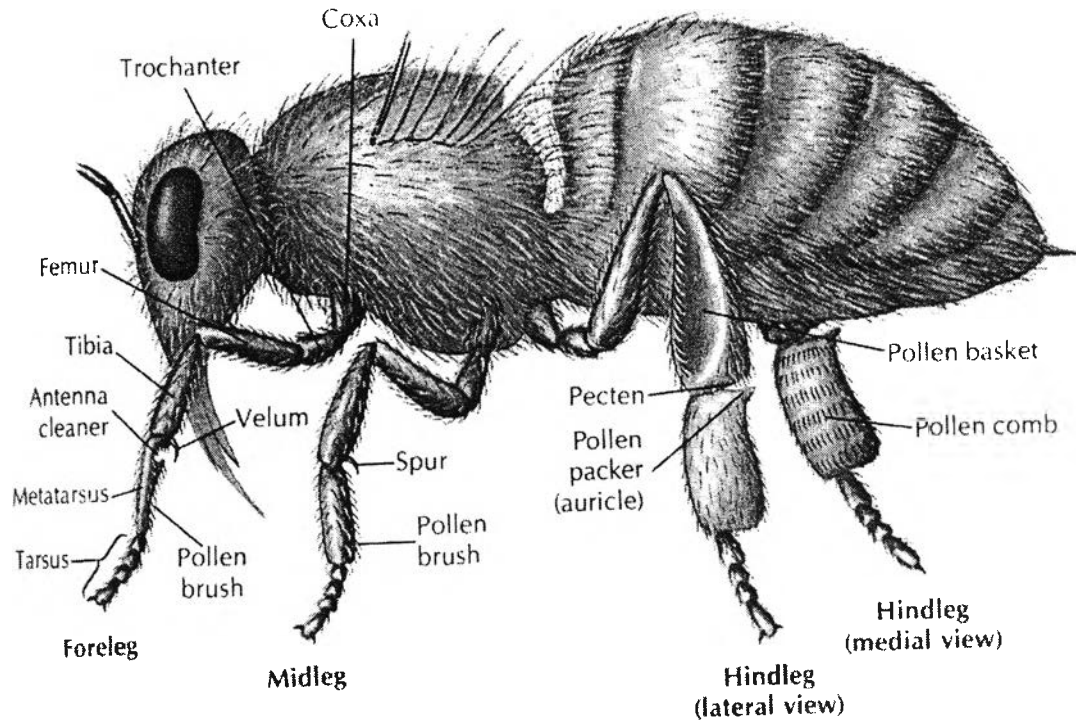


Figure 2.2 A worker honey bees (*Apis* sp.) showing the adaptive organs of the legs for pollen gathering and wax manipulation. The toothed indentation in the foreleg covered with velum is used to comb the antennae. The spur on the mid leg is used to remove wax from wax glands on the abdomen. Pollen picked up on body hairs are combed off by pollen brushes on the forelegs and mid legs. The pollen is deposited on pollen brushes of the hind legs. The elongated hairs of pecten on the hind leg are used to remove pollen from the brush of the opposite leg. The auricle (pollen packer) is used to press the pollen into a pollen basket when the leg joint is flexed back. (After Hickman et al., 1993).

This genus consists of 9 species which are highly diverse in morphology, behaviour and distribution (Figure 2.3) (Ruttner, 1988; O'Toole and Raw, 1999). They can be divided into three groups; the dwarf honey bees, the giant honey bees and the cavity-nesting honey bees.

1. Dwarf Honey Bees

1.1 Yellow dwarf honey bee : *A. florea* Fabricius 1787

1.2 Black dwarf honey bee : *A. andreniformis* (Smith, 1858)

2. Giant Honey Bees

2.1 Giant honey bee : *A. dorsata* Fabricius 1793

2.2 Rock honey bee : *A. laboriosa* F. Smith 1871

3. Cavity-nesting Honey Bees

3.1 European or western honey bees : *A. mellifera* Linneaus 1758

3.2 Eastern honey bees : *A. cerana* Fabricius 1798

3.3 *A. nigrocincta* Smith (1861)

3.4 *A. koshevnikovi* Buttel-Reepen 1906

3.5 *A. nuluensis*

Cavity nesting species construct multiple-comb nests inside cavities. They are commonly found inside caves or concealed spaces such as under roofs, tree hollows or inside buildings. They are distributed across a vast geographic range in tropical, subtropical and temperate areas. *A. mellifera* is commercial species and has been introduced world- wide. *A. mellifera* and *A. cerana* are close relatives and they are very similar species for which no pre-mating barrier exists (Ruttner, 1988).

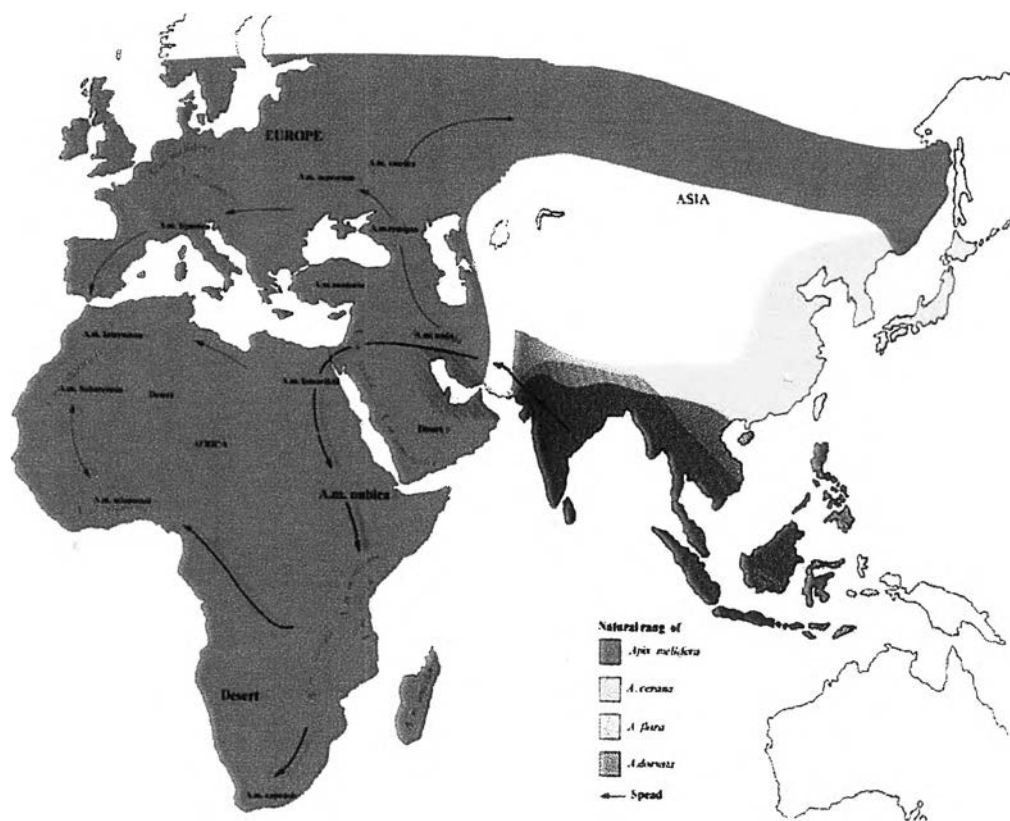


Figure 2.3 Distribution of honey bees in the genus *Apis* (Adapted from Gould and Gould, 1988).

Both dwarf honey bees and giant honey bees are open-air nesting species which produce single comb. They are restricted to the tropics and subtropics due to their nesting habit. *A. florea* and *A. andreniformis* are smaller in their body size and colony size than the other species. They are commonly found nesting on tree branches. Possibly because of heavy deforestation in Thailand, *A. florea* are now occasionally found nesting in human dwellings. *A. andreniformis* is more strict in its nest site requirements, they are only found in dense bush and in limited areas (pers. obs.). The architecture of the combs of these two species is different and can be used to distinguish them (Rinderer et al., 1996).

A. dorsata and *A. laboriosa* are the largest honey bees. *A. laboriosa* restricted to mountainous areas of the Himalayas. They construct large single combs on trees, cliffs or tall buildings. They are very aggressive and perform mass attack when colonies are disturbed from intruders. Aggregation of colonies and seasonal migration of *A. dorsata* are common.

2.1.2. Phylogeny of *Apis*

The phylogeny of the genus *Apis* (Figure 2.4) is simple. *A. florea* and *A. andreniformis* are believed to be the least derived forming a closely related basal group. *A. dorsata* and *A. laboriosa* form a sister clade to the dwarf honey bees. In cavity nesting honey bees, *A. mellifera* is a sister clade to *A. cerana* which is probably paired with *A. koschevnikovi*, *A. nuluensis* and *A. nigrocincta* (Alexander, 1991).

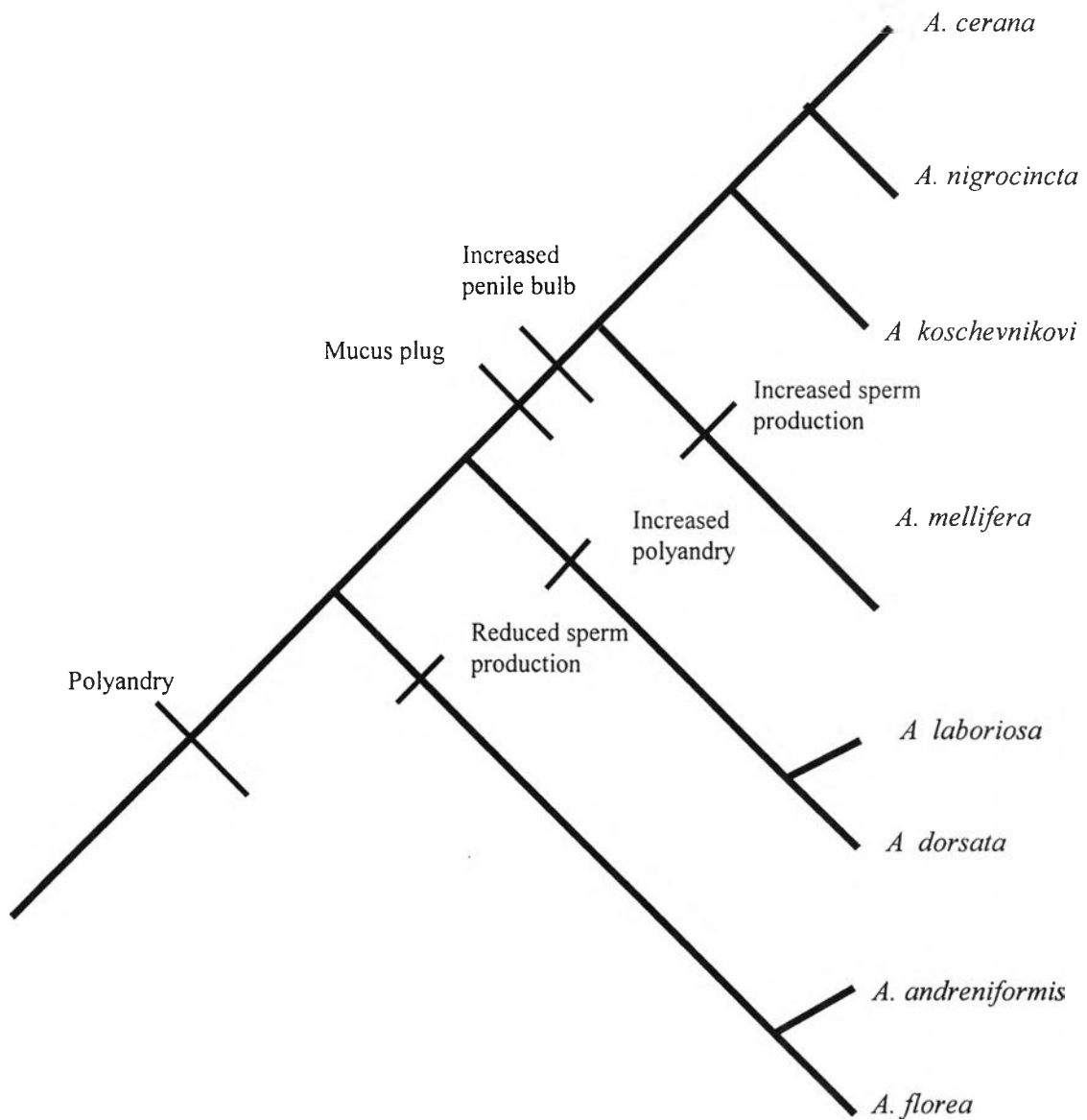


Figure 2.4 Cladogram of the genus *Apis* with reconstructions of reproductive behavioural traits (Adapted from Palmer and Oldroyd, 2000).

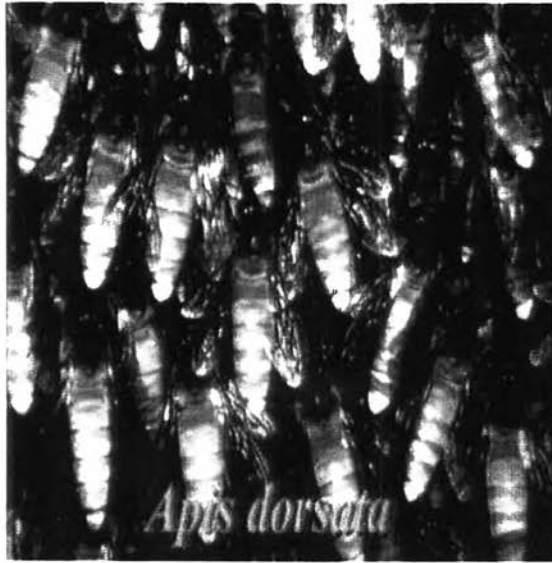
2.1.3. Giant Honey Bees: *A. dorsata*

The giant honey bee is distributed from the Indian subcontinent to south-east Asia. It is typically found in subtropical regions and high latitude areas such as north of India, Vietnam, south of China (Ruttner, 1988). In Thailand, this species is found throughout the country. It is well known for its large in body size and nest size. Unlike *A. mellifera*, differences in body size among the castes are small. Wing length in drones is greater than in workers but head width is smaller. The sealed drone and worker cells have flat capping which are different from the other species. To maintain the resistance in lower temperature and high latitude areas, where this species is commonly found such as in the North of India, the body size must be enlarged and the body hair is comparatively long (Ruttner, 1988).

This species construct their enormous nests in inaccessible places and attack intruders *en masse*. These group attacks may include thousands of bees (Gould and Gould, 1988).

Aggregated colonies of this species are commonly found (Seeley, Seeley and Akwatanakul, 1982; Dyer and Seeley, 1994). Colony tends to aggregate as groups and nest on a single tree (Koeniger and Koeniger, 1980). In Alor Setar, north of peninsular Malaysia, Oldroyd, Osborne and Mardan (2000) observed 120 *A.dorsata* colonies aggregated on a single tree. Wongsiri et al. (1996) also found 69 colonies nested on a bee tree (*Kompassia alaccensis*) in Mae Hong Son province in northern Thailand and I have personally seen 14 colonies on a single tree in Nan province, north of Thailand.

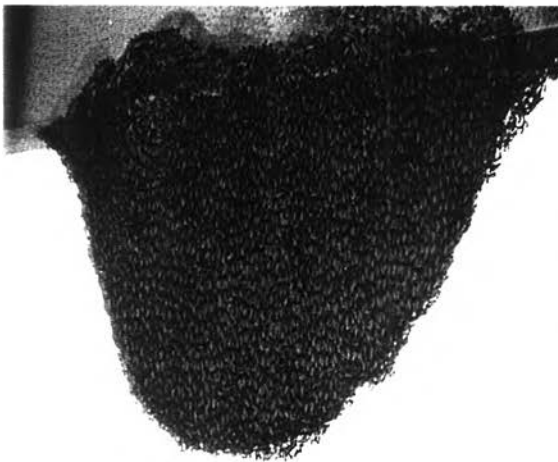
Seasonal migration is a familiar behaviour of *A. dorsata*. The distance of migration may be up to 200 km (Koeniger and Koeniger, 1980; Dyer and Seeley, 1994). In Thailand, colonies of *A. dorsata* arrive at nesting sites (such as trees, human buildings, cliffs), mostly adjacent to agricultural areas, in November-December. This is the peak blooming period in these areas, and therefore the forage is abundant. Newly-arrived swarms found colonies, forage and reproduce until the beginning of rainy season (May-June). In the rainy season almost of all colonies migrate, almost certainly to forest areas and come back again in subsequent dry season (Wongsiri-pers.cont., Piyapichart-pers.cont., and pers.obs). The selective forces for this seasonal migration are still ambiguous. However, predation by parasites and diseases is probably one of the selection pressures on all tropical honey bees including the giant honey bee (Ruttner, 1988). Infestation by parasitic mites, *Tropilaelaps* sp. is usually found when collecting *A. dorsata* colonies especially when colonies are fully-grown (pers.obs.). In addition, food resources available may play another important role in the selective force for its migration (Figure 2.5).



a).



b).



c).



d).

Figure 2.5 Giant honey bees; *A. dorsata*.

a). *A. dorsata*.

b). Colony of *A. dorsata* nests on the tree.

c). Colony of *A. dorsata* nests on the building.

d). Aggregated colonies of *A. dorsata* nest on the water tower.

2.1.4. Mating Behaviour of *A. dorsata*.

In *A. mellifera*, drones take their first flight, an orientation flight, about five to eight days after emergence from cells (Ruttner, 1966). The first queen mating flight in *A. mellifera* begins when they are ten days old. This flight lasts up to 30 minutes (Witherell, 1971). The destination of the mating flight in honey bees is a specific place called a *Drone Congregation Area* (DCA) which appears to be species-specific. DCAs are variable according to species and differences in geography. In *A. mellifera*, physiography has an important influence on flying drones and queens (Pechhacker, 1994). In two other species of cavity-dwelling honey bees, *A. cerana* and *A. koschevnikovi*, the DCAs seem to be variable within species, but mostly they occur in the open air nearby trees and under the thick cover of trees, respectively. The DCAs of *A. dorsata* observed in Borneo are found under the canopy of tall emergent trees at the height 10-35 m above the ground depending on the size of the tree. *A. dorsata* DCAs seem to be found at short distances from colonies (Koeniger et al., 1994).

The time of drone flight of sympatric honey bee species were discussed by Koeniger and Koeniger (2000). Mating time seems to provide a major behavioural barrier enhancing reproductive isolation. Observations of *A. dorsata* in Sri Lanka showed that the mating flight takes place shortly after dusk and lasts less than 1 hour (Koeniger and Wijayagunasekera, 1976). In Thailand, Rinderer et al (1993) observed drone flight of *A. dorsata* and found that drones take mating flight after sunset between about 18.45 h and 19.00 h. Nearly all drones take the mating flight simultaneously. The number of drones flying from colonies is highest at the middle of mating flight period. A similar pattern was observed in Borneo (Koeniger et al., 1994).

The mating flight of *A. dorsata* queens is shorter than that of the males. Queens return from mating flights after about 10 minutes (Rinderer et al., 1993; Koeniger et al., 1994). This brief mating period is similar to those observed in other species: *A. florea*, *A. andreniformis*, *A. koschevnikovi* and *A. mellifera* (reviewed in Koeniger and Koeniger, 2000).

Pheromones released by virgin queens attract flying drones. These sexual signals are produced from queen's mandibular glands and the main component is as (*E*)-9-oxo-2-decenoic acid (9-ODA) (Callow and Johnston (1960) reviewed in Koeniger and Koeniger, 2000). This sex attractant is also found in *A. mellifera*, *A. cerana*, *A. florea*, and *A. dorsata*. The quantity of 9-ODA in *A. mellifera*, *A. dorsata* and *A. cerana* are similar at 150-300 µg (reviewed in Koeniger and Koeniger, 2000).

2.2 Relevant Theories

2.2.1 Kin Selection

Considering kin selection theory, fitness can be defined as the relative ability of any particular genotype to survive and reproduce. Inclusive fitness can be defined as;

$$\text{Inclusive fitness} = \text{personal fitness (direct)} + \text{indirect fitness}$$

(Hamilton, 1964)

Direct fitness is the number of offspring of an individual relative to the population average, while indirect fitness is a function of the number of offspring of relatives corrected for relatedness. Thus, an individual may indirectly increase its inclusive fitness by helping relatives to increase their

reproductive success, even if this reduces personal reproduction (Hamilton, 1964). Thus, selection can act to increase the frequency of alleles which reduce personal reproduction, provided that there is a compensating increase in the reproductive success of relatives. This phenomenon has been termed “*kin selection*”, to distinguish it from direct selection on individuals (Maynard Smith, 1964).

The mechanism of sex determination in Hymenopterans (diploid females and haploid males) is thought to have particularly favoured kin selection as a powerful evolutionary force. Females are more related to their full-sisters ($r = 0.75$) than their own offspring ($r = 0.25$). In monandrous (queen is mated once) colonies, alleles favouring worker sterility and eusociality can increase in frequency due to the effects of kin selection. However, in polyandrous (queen mates more than twice) colonies, workers are more related to their own offspring than that of the queen. Asymmetrical relationships caused by multiple mating of the queen can lead to conflicts of interest between individuals over which of them should reproduce. This reduces the kin-selective benefits of social life. The maintenance of eusocial behaviour after the evolution of polyandry may be explained by the benefits gained by individuals if they still live socially (Oldroyd et al., 1996).

2.2.2 Sex Determination

In the Hymenopteran, sex determination combines male-haploidy with arrhenotoky in which males are derived from unfertilised eggs ($n=16$) and females from fertilised eggs ($2n=32$) (Figure 1.1, Chapter 1). Males are haploid, their germ line nuclei containing half the number of chromosomes than are present in the corresponding diploid nuclei of the female.

However, in most insects, for example honey bees, some of the somatic tissues exhibit high levels of endopolyploidy so that haploid males have about the same amount of DNA as females in some of their somatic tissues. This is because the nuclei of the male undergoes compensatory endomitosis so that the equal amounts of DNA are present (Hoy, 1994). A single multi-allelic locus controls sex determination. Individuals heterozygous at this locus are female while homozygotes or hemizygotes, (with a single allele at the sex locus) develop into males (Adams et al., 1977). However, in honey bees, homozygous diploid males are eaten by workers at an early stage so not much energy has been invested in the dying larvae (Pamilo, 1991; Crozier and Pamilo, 1996).

Sex determination via male-haploidy in Hymenoptera enables reproductive females to flexibility to control the sex of their offspring. Reproductive females (queens) can produce both reproductive haploid males (drones) and non-reproductive diploid females (workers) by controlling fertilisation of eggs. The spermathecal valve of the queen can be opened or closed in order to release or retain spermatozoa (Ratnieks and Keller, 1998).

An exception to this reproductive system has been observed in the cape honey bee (*A. mellifera capensis*). Workers of this subspecies are able to produce female offspring via automictic fertilisation of the eggs (thelytokous parthenogenesis). These eggs are reared to adulthood and being a part of colony members (Greeff, 1996; Moritz, Kryger and Allsopp, 1999).

2.2.3 Polyandry

Polyandry is widespread in eusocial insects and has the effect of lowering intra-colonial genetic relatedness. Because monandry and haplodiploidy have been considered as key factors facilitating the evolution of eusociality, the occurrence of polyandry has been a central of interest. Several hypotheses have been purposed to explain the occurrence of polyandry in eusocial hymenopteran insects. Genetic variance hypotheses (GV) have been regarded as the most plausible explanations of the extreme levels of polyandry in the genus *Apis* (Keller and Reeve, 1994, reviewed by Palmer and Oldroyd, 2000). These hypotheses propose that multiple mating by queen increases intra-colonial genetic variance and that this increases colony fitness. The GV hypotheses are of two broad types. The first group suggests the mechanism of sex determination system (haplodiploidy) places a genetic load on colonies that can be mitigated by multiple mating (Page 1980; Page and Metcalf 1982) and conflict between queens and workers over sex ratios (Moritz, 1985). The second group suggests that variation in worker genotypes increases colony fitness by increasing opportunities for task specialisation, and by conferring disease resistance (reviewed in Palmer and Oldroyd, 2000). A plausible sequence of the evolution to extremely high levels of polyandry is shown in Figure 2.6.

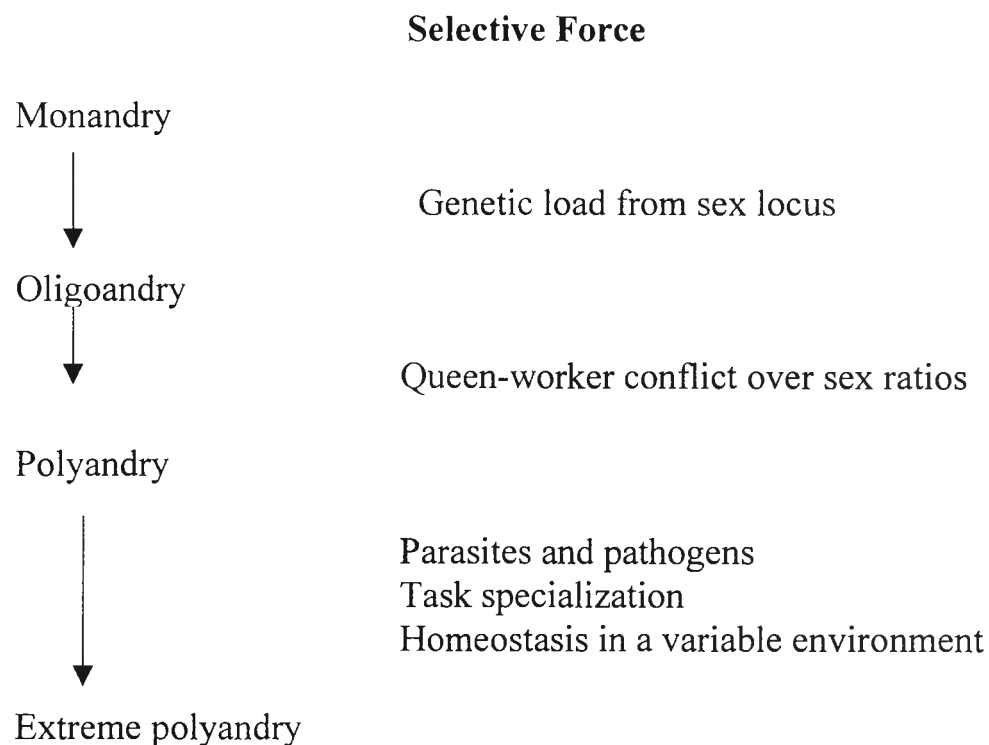


Figure 2.6 Plausible evolution of extreme polyandry in the genus *Apis* (After Palmer and Oldroyd, 2000).

2.2.4 Relatedness

The haplodiploid system of sex determination in Hymenoptera insects causes high relatedness among workers. However polyandry (multiple mating) or polygyny (multiple queens) is normal in many eusocial hymenoptera. and causes a reduction of the relatedness among offspring. Therefore, the relatedness of offspring is diverse and varies according to the number of males that the queen mates.

The relatedness is defined by Wright's Coefficient of relatedness (r). It is the proportion that two individuals share identical alleles. This relatedness in polyandry hymenopteran, i.e. honey bees, can be defined by:

$$r = (\phi + \phi')/2$$

Where : ϕ = probability that in two individuals the paternal allele is identical.

ϕ' = probability that in two individuals the maternal allele is identical.

If there is no paternal relationship, $\phi = 0$

If there is no maternal relationship, $\phi' = 0$

The pedigree and relationship between individuals in a polyandrous hymenopteran colony showed in Figure 2.6 (Hamilton, 1964 ; Crozier and Pamilo, 1996).

In honey bees, relatedness among workers varies according to their paternity (Figure 2.7). Offspring of the same father are related to each other as super-sisters ($r = 0.75$), while offspring of different fathers are related as half-sisters ($r = 0.25$) Workers relatedness to their brothers (queen's son) is 0.25. However, if workers produce their own male progeny (which are haploid), they are related to them by $r = 0.5$, which is greater than their relatedness to sons of a super-sister ($r = 0.375$), sons of the queen ($r = 0.25$), or sons of a half-sister ($r = 0.125$). Average relatedness between pairs of workers, if chosen by random, approaches $r = 0.25$ as the number of subfamilies increases within a colony (Page and Erickson, 1988; Ratnieks, 1988; Ratnieks and Visscher, 1989; Crozier and Pamilo, 1996).

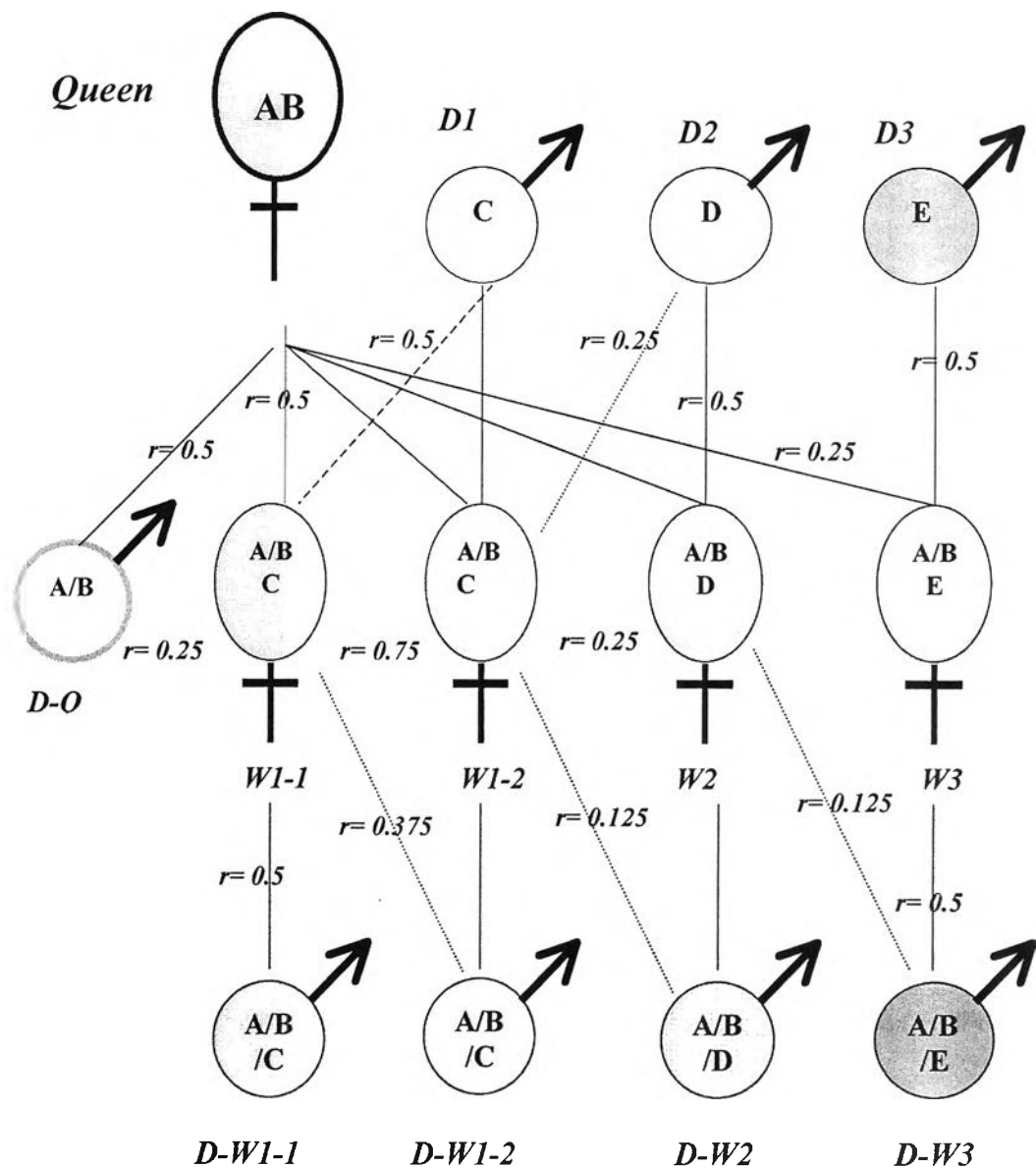


Figure 2.7 Parentage and relatedness of workers in a colony of honey bees. A, B, C, D, and E represented genotype carried by each bees. The relatedness among individual bees represented by r which varies according to paternity. Worker $W1-1$ and $W1-2$ are super-sister as they are daughters of the same father ($D1$). $W1-1$ and $W1-2$ are related to $W2$ and $W3$ as half-sisters. $D-W1-1$, $D-W1-2$, $D-W2$ and $D-W3$ are worker produced males.

2.2.5 Queen-worker Conflict

Multiple mating in eusocial insects lowers relatedness between workers and results in asymmetrical relationships among offspring. In order to maximise personal Darwinian fitness, workers should preferably reproduce their own male offspring ($r = 0.5$) instead of caring the male offspring of other workers ($r = 0.375$ or 0.125). However, if a large proportion of workers lay eggs, the consequence of the colony may be serious (Montague and Oldroyd, 1998, Barron, Oldroyd and Ratnieks, in press). Therefore, the compromising in evolution of eusociality is that workers rear close relatives, their brothers (the queen's sons) for which $r = 0.25$ (Ratnieks, 1988).

2.2.6 Worker policing

Worker policing is suggested as the mechanism by which polyandrous insects deal with lower relatedness among offspring. Queens in the genus *Apis* are polyandrous, so most of progeny are half-sisters. Therefore, workers are more closely related to their mother (queen) than they are to their sisters. Because workers are only distantly related to the sons of other workers ($r = 0.125$) workers should prefer to care for their closer relatives, i.e. brothers with $r = 0.25$. Workers prevent each other from reproducing via oophagy of worker-laid eggs aggressive behaviour towards laying workers (or developing ovaries workers) (Vissher and Dukas, 1995).

2.3 Genetic Markers

Molecular genetic techniques provide powerful tools for the study of insect biology, ecology and population genetics. Molecular techniques used include analyses of isozymes, molecular cytogenetics, DNA-DNA hybridization, restriction enzyme analyses of DNA and analysis of DNA sequences either directly or through PCR-based markers such as microsatellites. Each technique has virtues and limitations in the amount and type of information obtained, its degree of technical difficulty, and its cost. Analysis of DNA sequences, in both nuclear and mitochondrial DNA, can be used to study genealogies within species, for maternity or paternity testing, analysis of geographic variation, relatedness and for reconstruction of species phylogenies (Hoy, 1994). For example, Oldroyd et al. (1995 a) determined the genetic relationship among aggregations of *A. mellifera* using isozyme analysis, microsatellite analysis and mitochondrial DNA polymorphism.

2.3.1 Microsatellites

One type of repetitive DNA that does not encode products used by the cell and which is usually present at high frequency throughout the genome are so-called *Microsatellites*. Microsatellites or STRs (Short Tandem Repeat polymorphisms) are sequences composed of runs of repeat units 2 to 5 base pairs long. The repeats of the dinucleotide CA are commonly found. They are mostly found in regions of euchromatin in the genomes of many eukaryotes. They are sometime called “simple sequences” because of their simple structure. The differences in allele size (repeated sequence) within species are higher than those observed between species. Among individuals the differences in allele size are obvious even if

the cause of the allele size change is unclear. Therefore, microsatellite variation is a non-specific indicator of interspecific relationships (Hoy, 1994; Page and Holmes, 1998). The mutation rate of microsatellite sequences is high (10^{-4} and 10^{-5} per gamete, per generation) and this results in the differences in number of copies of the repeated sequence between individuals. Microsatellites have proven extremely valuable in distinguishing the difference and investigating the relatedness among individuals (Snustad and Simmons, 2000). Thus, high levels of genetic diversity combined with natural evolution, co-dominance and simple Mendelian inheritance mean that microsatellites have become a very popular set of molecular markers (Page and Holmes, 1998; Queller, Strassmann and Hughes, 1993).

In haplodiploid social insects such as honey bees, microsatellites show many alleles and high heterozygosity. Therefore, this technique is reliable and precise for determining the genetic structure of honey bee colonies (Estoup, Solignac and Cornuet, 1994).