CHAPTER 6

GENERAL DISCUSSION

This thesis has shown that worker sterility is strict in *A. dorsata*. Workers rarely or ever have developed ovaries. I detected no males that were sons of workers using genetic markers, indicating that policing behaviour is well express in this species. I found this a somewhat surprising finding, as the biology of *A. dorsata* suggests that the costs of an efficient policing system based on oophagy would be high in this species.

Costs of policing via oophagy

If workers erroneously remove queen-laid rather than worker-laid eggs, or erroneously rear a worker-laid egg, then these errors imposes a cost of worker policing on colony fitness. The relative importance of these errors will determine the optimal stimulus threshold at which a worker will decide to remove an egg. Figure 6.1. a) shows the situation where there is no ambiguity in the queen's signal. In this case workers will be selected to set a threshold for egg removal where no queen-laid eggs are removed and all worker-laid eggs are removed. The more interesting (and probably more realistic) situation occurs when not all worker-laid and queen-laid eggs can be distinguished (Figure 6.1.b)) i.e., their templates overlap. This situation is likely as there would be a selective pressure on workers to produce eggs that are more 'queenlike' (Oldroyd and Osborne, 1999; Oldroyd and Ratnieks, 2000; Barron et al., 2001). In this case (Figure 6.1.b)), the optimal threshold for the removal of an egg (i.e. the minimum level of queen-marking pheromone that stops egg removal) will be determined by the overall costs of two kinds of errors. These errors are: 1) the probability of retaining worker-laid eggs (E_W), and 2) the probability of removing queen-laid eggs (E_Q) and the associated costs can be defined as C_W and C_Q, respectively. The cost of policing errors (W) can therefore be defined as:

$$W = P_Q C_Q E_Q + (1-P_Q) C_W E_W \qquad \text{Eqn. 1}$$

Where : P_Q = the proportion of eggs that are queen-laid. E_Q and E_W are functions f1(T), f2(T) of the queen pheromone threshold at which an egg will be rejected (T) i.e. the template, which is presumably chemicals that signal queen-laid; but may be some queen-laid eggs are not properly marked or some worker-laid eggs are partially queen-like.

$$W = P_Q C_Q f1(T) + (1-P_Q) C_W f_2(T) \qquad \text{Eqn. 2}$$

Differentiating with respect to W :

$$\frac{dW}{dT} = P_Q C_Q f_1'(T) + (1 - P_Q) C_W f'_2(T) \qquad \text{Eqn. 3}$$

Selection will act to minimize W. This occurs when the differential of W is equal to zero. Thus:

$$\frac{P_Q C_Q}{(1 - P_Q) C_W} = \frac{-f_2'(T)}{f_1'(T)} \qquad \text{Eqn.4}$$

If P_Q, the proportion eggs that are queen-laid, is reduced then either $f_2'(T)$ (the slope of E_W) must reduce in absolute value or $f_1'(T)$ (the slope of E_Q) must increase. It is apparent from Figure 6.1.c) that in such a case, the acceptance threshold must increase. That is, when P_Q is lowered, less worker-laid eggs are retained.

One way in which this reduction in P_Q can be achieved is by the use of a special cell size to distinguish cells used for rearing male and female eggs. Diploid eggs, which are all queen-laid, are separated from the haploid male-producing eggs. Policing attention is directed towards eggs in drone cells. So the proportion of queen-laid eggs that are checked by policing workers (P_Q) is lower than if diploid and haploid eggs were laid haphazardly. The difference in drone cells occurs in many species of the honey bees; *A. mellifera*, *A. florea* and *A. cerana* with the exception in *A. dorsata*.

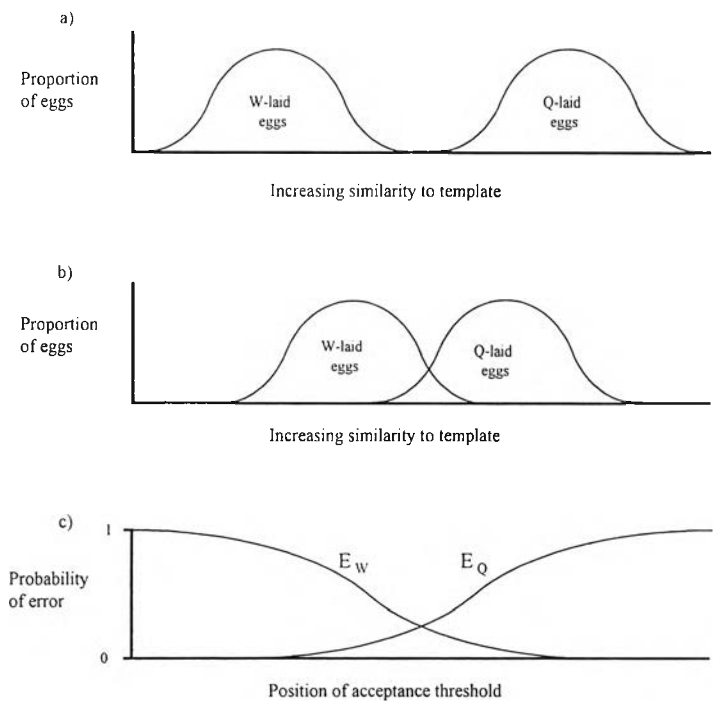

Figure 6.1 Model repesented the rate of egg misidentification. W = worker, Q = queen, EW = the probability of retaining worker-laid eggs, EQ = the probability of removing queen-laid eggs.

a) A situation where all worker-laid eggs are distinguishable from queen-laid eggs.
b) A situation where some worker and queen-laid eggs are indistinguishable.
c) Probability of misidentifying egg maternity assuming the situation in b). The "acceptance threshold" is the point where eggs are accepted as queen-laid eggs and are not removed.

In *A. dorsata*, males are reared in cells identical in shape and size to those used for rearing workers. This behavior is expected to increase the costs of policing and higher retention of worker-laid eggs is predicted. However, the finding in this thesis indicates that most, if not all of *A. dorsata* drones are sons of queens. The absence of workers with developed ovaries may mean that policing mechanism in this species is directed at workers with developed ovaries (Visscher and Dukas, 1995), reducing the need for policing via oophagy, and special cells for the rearing of males. However, if there are any of laying workers that can escape from this policing, other workers could only use pheromonal signal as discriminating cue to eliminate those worker eggs.

Because worker policing is demonstrated in three other species of honey bees, there is strong circumstantial evidence that worker sterility in *A. dorsata* is enforced by worker policing. Additional studies could focus on the mechanisms of policing in *A. dorsata*. Under some circumstances, *A. dorsata* colonies can be non-defensive and it is possible to manipulate them, so direct bio-assays of the survival of worker-laid eggs relative to queen-laid eggs might be possible. On the other hand, obtaining appropriate queen-laid and worker-laid eggs may be an insurmountable problem.

Polyandry

The level of polyandry that occurs in this species is the highest in this genus. The hypothesis that the queens mate with many males due to the greater availability males at the DCAs due to colony aggregations was rejected. Also, the hypothesis that inbreeding avoidance is insufficient to explain the extremely high level of polyandry as no significant difference was found between queen mating frequency of aggregation and singly nesting colonies (section 5.3).

The sex allele hypothesis for the evolution of polyandry (Page, 1981) that queens should mate many time in order to reduce the probability that a large proportion of offspring will be diploid drones, cannot explain the extremely high number of mating in *A. dorsata* queens, as the benefits of polyandry do not change beyond six matings.

Seasonal migration in this species may be one of the major causes of this extremely high degree of polyandry. During the migratory flight, honey bees must encounter many risks; environmental extremes, predators and parasites, in founding anew colony. This requires a broad range of worker genotypes that can cope with a broad range of environmental conditions Under these pressures, genotypic variation of workers is important for maintaining the new colony (Kolmes, Winston and Fergusson, 1989; Page and Mitchell, 1998; Robinson and Page, 1989 a). Therefore, these reasons may be a plausible explanation of the extremely high levels of polyandry seen in *A. dorsata*.

Extremely high degree of mating frequency in *A. dorsata* queens was also reported in this thesis. Polyandry arose after the divergence of the genus *Apis* and since worker policing is facilitated by polyandry, hence worker policing could evolve shortly after polyandry. Adding worker policing as one of the reproductive behaviour traits could redraw the cladogram of this genus (Figure 6.2).

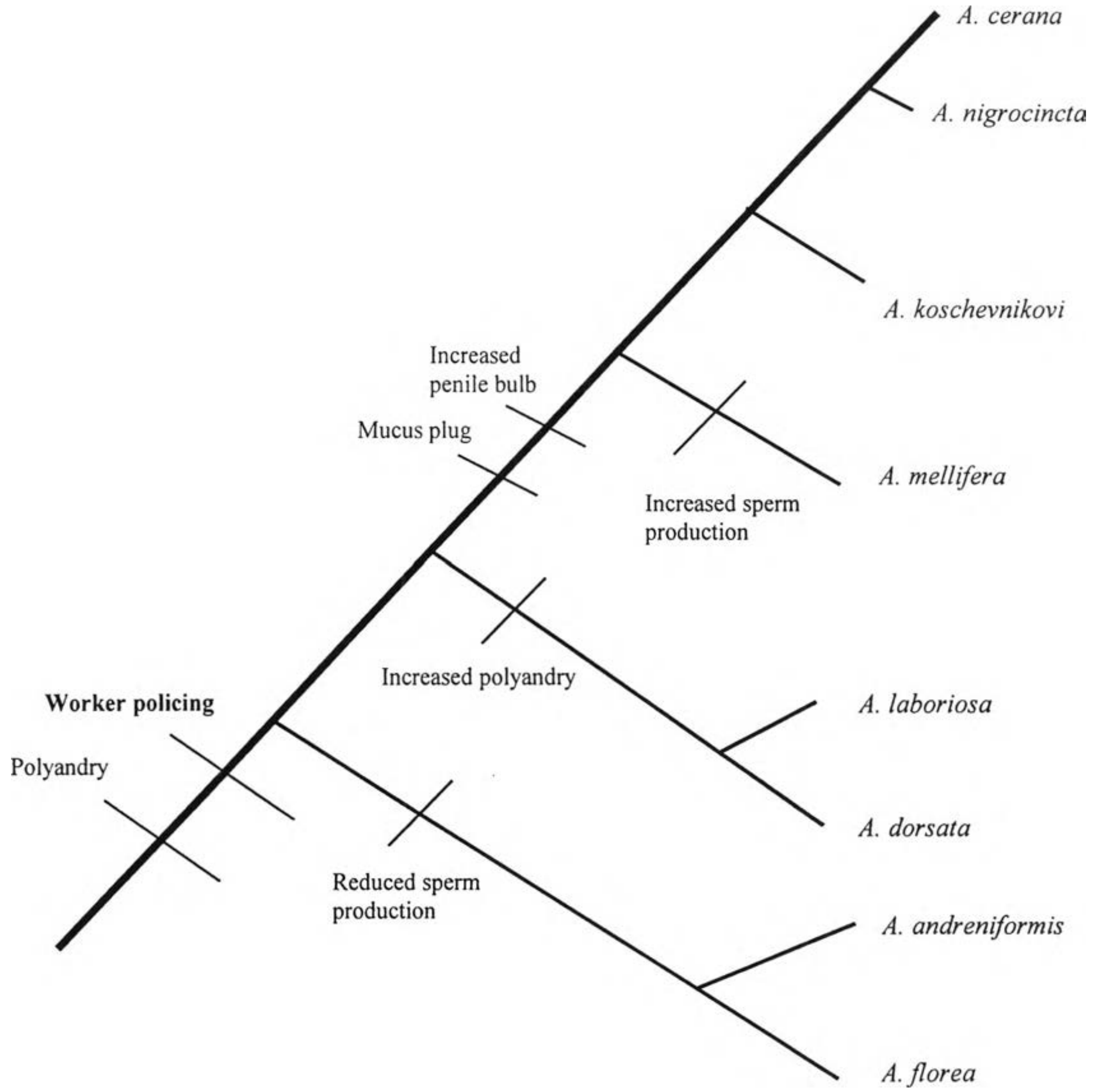

Figure 6.2 Reconstruction of cladogram of the genus *Apis* with the adding of worker policing behaviour.

Further research

In order to get better insights into the evolution of extreme mating frequencies in this species, further research should be carried out.

1). The characteristics of *A. dorsata* DCAs should be investigated in several aspects; location, distance from colony, number of drones and number of drone's subfamilies participate at the DCAs.

2). Comparative analysis on mating frequency across the genus *Apis* could enable an additional explanation in extreme mating frequency (Palmer and Oldroyd, 2000).

3). Worker policing in A. *dorsata* obviously occurs via preventing ovary-developed workers. But the mechanism of preventing ovaries from developing is not known.