Workers of *Apis cerana* reproduce during short periods of queenlessness

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Abstract: In six colonies of *Apis cerana* the queen was removed and occurrence of egg-laying by workers was studied. Three colonies had brood of all stages and were thus allowed to build emergency queen cells and to rear queens. In three other colonies no young brood to build emergency queen cells was left (hopelessly queenless), but older brood was present. Workers started laying within two to three days after removal of the queen, both in the colonies rearing queens and in the hopelessly queenless colonies. Four to five days after removal of the queen mass egg-laying by workers occurred in five of the six colonies. These eggs partly developed into capped drone brood. Thus, *A. cerana* workers may produce a substantial number of drones during short periods of queenlessness, even when new queens are reared simultaneously.

Keywords: *Apis cerana*, worker reproduction, drone production, egg removal.

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Introduction

In honey bees, genus *Apis*, both the queen and the workers can reproduce. The queen lays haploid eggs, which develop into males, and diploid eggs, which develop into females. Since workers do not mate, they can as a rule only lay haploid eggs. Only in exceptional cases, worker honeybees can produce diploid eggs by thelytoky (Verma & Ruttner, 1983). Thus, workers have two ways to increase their fitness. Firstly, they can help to increase the reproduction of the queen (mother) by performing tasks in the colony. Since they are related to the queen, this will increase their own fitness as well. Secondly, they can reproduce themselves and increase their fitness directly by contributing to the population of drones.

In queenright colonies, reproduction is apparently restricted to the queen, despite the fact that relatedness (G) of workers to self-produced drones (G=0.5) is higher than to the male offspring of the queen (G=0.25). Relatedness of workers to the offspring of other workers is still lower than 0.25, however, because the queen generally mates with a high number of drones: 10 to 20 in *Apis mellifera* Linnaeus and probably a similar number in *A. cerana* Fabricius (Ruttner et al., 1973; Page, 1986). Workers may therefore prefer drone production by the queen and prevent each other from reproduction, since relatedness to the offspring of a randomly chosen worker is lower than relatedness to the male offspring of the queen (Ratnieks, 1988). When the colony becomes queenless and when no brood to rear a new queen is available (hereafter called hopelessly queenless), workers start to reproduce since in this case they can increase fitness only by producing drones themselves before the colony dies (Winston, 1987; Moritz & Southwick, 1992).

In European races of *Apis mellifera* (*A. mellifera carnica* Pollmann, *A. mellifera ligustica* Spinola, *A. mellifera mellifera* Linnaeus, and *A. mellifera adami* Ruttner), workers start to reproduce 16 to 30 days after the colony has become hopelessly queenless (Ruttner & Hesse, 1981). In other honeybee races and species, however, laying workers may occur much earlier. In African races of *A. mellifera* (*A. mellifera intermissa* von Buttel-Reepen, *A. mellifera scutellata* Lepeletier, and *A. mellifera capensis* Escholz), laying workers

are found 5 to 10 days after becoming hopelessly queenless (Ruttner & Hesse, 1981). They may even appear while new queens are reared in the colony, which was found for *A. mellifera adansonii* Latreille (Fletcher & Ross, 1985), for *A. mellifera yemenitica* Ruttner (Woyke, 1993), for 'Africanized' bees in Costa Rica, which can be considered as a mixture of *A. mellifera scutellata* and *A. mellifera ligustica* (Van der Blom & Arce, 1991; Van der Blom et al., 1994), and for the dwarf honey bee, *A. florea* Fabricius (Woyke & Wongsiri, 1992). For the eastern honey bee, *A. cerana*, it is well known among beekeepers that laying workers may appear quickly, but this fact is poorly documented. Therefore, we studied whether and when workers started laying eggs, (1) in queenless colonies of *A. cerana* in which new queens were reared and (2) in hopelessly queenless colonies of *A. cerana*.

Materials and methods

The experiments were carried out in the South of Vietnam at the university of Agriculture and Forestry of Thu Duc, near Ho Chi Minh City, with the local *Apis cerana* race. *Apis cerana* from the South of Vietnam is considered to belong to *A. cerana indica* Fabricius (Ruttner, 1988).

Six colonies each occupying 3 to 4 combs were used. They contained brood in all stages and a laying queen. At the beginning of the experiment the queen was removed from the colonies. In three of the six colonies the brood nest was left undisturbed. Since young larvae and eggs were present, the bees started to build emergency queen cells and to rear queens. Nine days later a new queen had emerged in all three colonies, after which we removed the queen cells left. From the other three colonies all young brood was removed to make them hopelessly queenless. However, two combs with capped brood were left in each of the three colonies to prevent the bees from absconding.

After removal of the queen, the colonies were inspected daily for a period of fourteen days. During inspections we recorded rearing of new queens, emergence of queens, the occurrence of worker-laid eggs and the development of these eggs.

Results

Colonies rearing queens

During the first two days after removal of the queen, 1 to 4 eggs were found in emergency queen cells, but later these eggs had been removed by the bees. Whether these eggs had been laid by the queen or by workers could not be said with certainty, since worker-laid eggs could not be distinguished from queen-laid eggs and because development time of eggs is almost three days (Tan et al., 1993). However, the first worker laid eggs seemed to appear within three days after removal of the queen in queen cells, worker cells and drone cells. From the fourth to fifth day large numbers of eggs were found in colonies 1 and 2 (fig. 1). These eggs must have been laid by workers. At first many cells with one egg were found. Later, cells often contained several eggs, up to more than 10 eggs per cell (fig. 3). In colony 1, egg-laying by workers decreased after emergence of the new queen, which happened at the ninth day after queen removal. Five days after her emergence only a few eggs were still found. In colony 2, egg-laying by workers continued, probably because the freshly emerged queen was lost. In colony 3, egg-laying by workers started at the same time as in colonies 1 and 2, but more than a few eggs were never found.

Part of the eggs developed into larvae. The bees seemed to nurse this drone brood badly, however, since only a small proportion of the larvae developed into pupae. In colony 2, the first worker laid eggs were probably removed before development into larvae, because the first larvae appeared five days instead of three days after the first eggs. In colony 3, the few worker laid eggs never developed into larvae. The eggs were evidently removed by the bees because every day a few eggs were found in different cells.

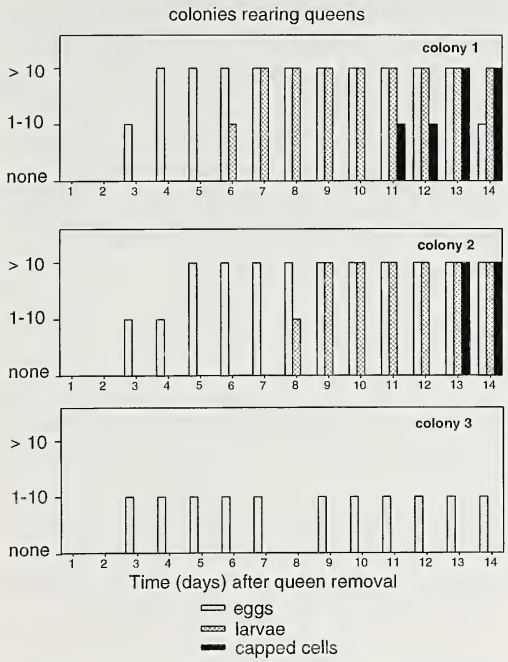

Figure 1. Number of cells with brood of worker origin in the three colonies that were rearing queens.

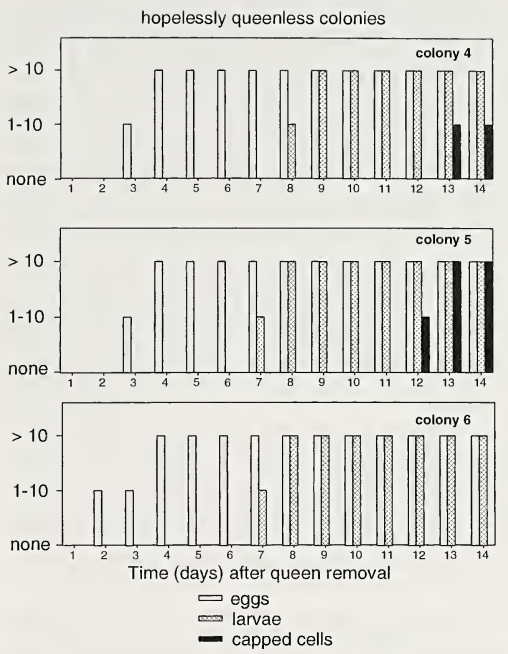

Figure 2. Number of cells with brood of worker origin in three colonies that had been made hopelessly queenless.

Hopelessly queenless colonies

In the three colonies made hopelessly queenless, all the eggs found were laid by workers, since young brood had been removed before. Similar to the colonies rearing queens, eggs were found in all types of cell of the hopelessly queenless colonies within two to three days after queen removal (fig. 2). From the fourth day many worker-laid eggs were found in all three colonies. Larvae appeared four to five days after appearance of the first eggs. However, only a small proportion of the cells with larvae were eventually capped.

Discussion

In queenless colonies of *Apis cerana*, workers started to oviposit within two to three days after removal of the queen, both in colonies rearing queens and in hopelessly queenless colonies. Thus, in the colonies rearing queens the presence of queen larvae or pupae did not inhibit egg-laying by workers. This is in agreement with studies on ovary development of *A. mellifera* workers (Jay, 1968; Jay, 1970), since ovaries of *A. mellifera* workers have been found to develop despite the presence of queen larvae or pupae. However, in *A. mellifera* a strong inhibiting effect of the presence of worker larvae and pupae on the development of workers' ovaries has been reported (Jay, 1970; Kropácova & Haslbachová, 1970, 1971). Egg-laying by *A. cerana* workers, which implies development of the ovaries, was apparently not inhibited by the presence of worker brood. After a young queen had emerged, however, egg-laying by workers seemed to stop within a few days. The same was found in *A. florea* by Woyke & Wongsiri (1992) and by Van der Blom et al. (1994) in 'Africanized' bees.

It is unlikely that the eggs found in queen cells at the first and second day after queen removal in the colonies that were rearing queens, had been laid before by the queen. Emergency queen cells built on worker or drone cells containing an egg has not been described, which suggests that it is not a normal event in honeybee colonies. In addition, remo-

val of eggs by the bees, as happened here, is not expected when the eggs are laid by the queen, since the bees seem to remove the larvae and not the eggs when the size of the broodnest has to be adjusted during periods of dearth (Woyke, 1976). In contrast, eggs laid by workers are likely to be removed because workers may prevent each other from reproduction (Ratnieks, 1988; Ratnieks & Visscher, 1989), or because worker laid eggs may not be viable. Ratnieks (1993) showed that even in queenright *A. mellifera* colonies egg-laying workers may occur. However, only a few eggs are laid by workers and most eggs laid by workers are removed. In our experiment, removal of worker-laid eggs was evident in colony 3. Per day, only a few eggs were found and the following day these eggs were always removed.

Thus, the following picture emerges. When the queen is lost, the removal response of bees to worker-laid eggs may partly be inhibited. Subsequently, worker-laid eggs have a chance to develop into larvae and eventually into drones, either because a few laying workers are already producing eggs or because egg-laying by workers quickly develops after the loss of the queen. The results show that mass egg-laying by workers of *A. cerana* may appear quickly even if the colony is rearing queens at the same time. Though much of the worker originated brood was nursed badly in our colonies, this still means that *A. cerana* workers may produce a substantial number of drones during short periods of temporal queenlessness and not only when a colony becomes hopelessly queenless. Therefore, the contribution of laying workers to the drone population may be much higher for *A. cerana* than for European *A. mellifera* races.

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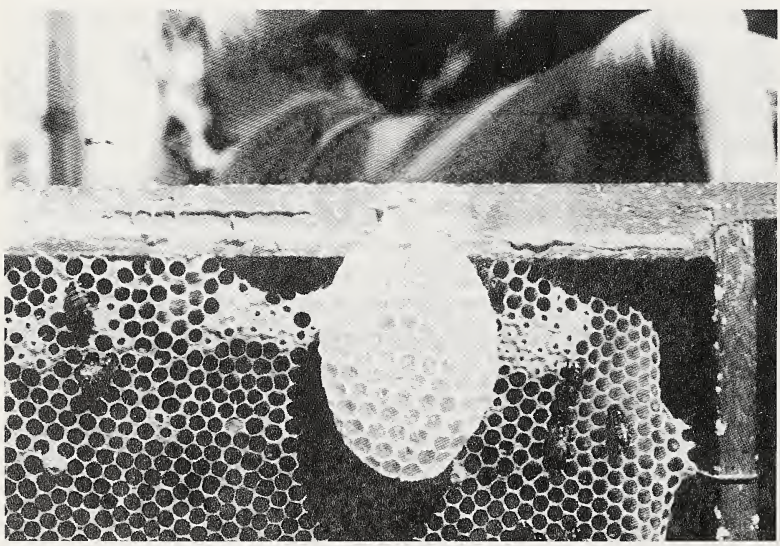

Figure 3. Massive egg-laying by *A. cerana* workers. Note the high number of eggs per cell.

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