

Stimulating natural supersedure of honeybee queens, *Apis mellifera*

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When a honeybee queen starts to fail, she is often superseded by a young queen that takes over reproduction inside the colony. Natural supersedure in winter leads to an unfertilised young queen and colony loss. To reduce these losses we tried to stimulate supersedure of queens earlier in the season. In 50 colonies we introduced queen cells with one-day-old larvae and capped queen cells. Although many larvae were fed initially, few of them were reared to mature queens and none of the cases resulted in supersedure. This suggests that supersedure cannot be evoked by artificially bypassing the initial phases of the process.

Keywords: Supersedure, *Apis mellifera*, queen cells, larvae

When the quality of honeybee queens decreases she has to be superseded. In the process of natural supersedure in honeybee colonies (*Apis mellifera*) the bees rear a few new queens, one of which replaces the resident queen and takes over the reproduction in the colony (Hooper & Morse 1985). In order to stimulate supersedure of old or failing queens beekeepers use several methods. They may introduce a ripe queen cell (induced supersedure) or a virgin queen in the colony ('Virgin drop'). In this way the old queen remains in the colony while a new queen is introduced. This is in contrast to conventional requeening, in which the resident queen is removed and replaced with a mated queen (Johansson & Johansson 1971). Stimulating supersedure compared to conventional requeening has the benefit that it is cheap, since production of mated queens is not needed. In addition, the bees themselves decide if they accept the young queen or not, which warrants unnecessary replacement of well-functioning queens.

Reported replacement rates of induced supersedures using closed queen cells are in the range of 13-17% (Boch & Avitabile 1979, Jay 1981, Szabo 1982). With variable success, replacement rates can be improved by repetitive introductions of queen cells, or by introducing queen cells in tubes that prevent destruction by the bees. Queen cells can also be protected by introduction of the cells in a section isolated from the resident queen using division boards or queen excluders (Skirkevicius 1963, 1965, Forster 1972, Reid 1977, Boch & Avitabile 1979).

Honeybee queens normally suppress their replacement by pheromones that inhibit worker bees to construct queen cells (Winston *et al.* 1989). If natural supersedure occurs, this pheromonal inhibition apparently does not function any more. The old queen lays eggs in a few queen cups, after which the worker bees rear 1 to 3 supersedure queens in 16 days. One new queen remains alive, and after mating she starts laying eggs. The old queen may remain in the colony for some time, but eventually she disappears, either as a result of natural death, or as result of antagonistic behaviour of the new queen or workers (Deyel 1980, Hooper & Morse 1985, Droege 1993). When queen cells are introduced in the brood nest, they are often destroyed by the worker bees (Caron & Greve 1979). This destruction is thought to be induced by pheromones of the resident queen (Reid 1977, Jay 1981).

Queens in honeybee colonies used in apiaries maintained for pollination may suffer from transport and unnatural conditions in glasshouses and tunnels (personal observation). Up to 20% of these queens is superseded in the overwintering colonies (personal observation). Since winter conditions limit possibilities for mating flights and drones are hardly available, these supersedures often lead to unfertilised young queens and thus to colony losses. To reduce these losses we tried to stimulate supersedure of queens earlier in the season. A new form of induced supersedure is tested by introducing queen cells with young larvae, instead of introducing capped queen cells. Introduction of queen cells with young larvae bypasses the initial steps of the natural supersedure process. Therefore, bees may be stimulated to supersede queens immediately that would otherwise be superseded during winter.

The present study was designed to examine the fate of queen cells with young larvae introduced into queenright honeybee colonies in order to stimulate supersedure. The research question of the study is: are queen cells with larvae readily accepted and is the old queen superseded later? A possible age effect of resident queens is considered by using queenright colonies with young and with old queens.

MATERIAL AND METHODS

To follow the fate of introduced queen cells with larvae in honeybee colonies, 50 queenright colonies were selected from our apiary in Wageningen, The Netherlands. The colonies were housed in single storey hives and occupied 3-5 combs. Prior to the experiments, the colonies were treated against *Varroa destructor* by spraying ± 1.5 ml oxalic acid (3%) per comb side bees. Half of the colonies had queens from previous years and had recently returned from pollination services. The other half had queens ageing 2-4 months. All queens were marked with a quick drying paint. The colonies were given fresh combs and were continuously fed with sugar candy. The test colonies were transferred to a new site where the experiments took place.

One day before the experiments queen cells were produced by the standard method of grafting: zero to 1-day-old larvae were grafted into 120 queen cells, placed on frames and put into a dequeened colony. The following day about 90 cells were found accepted as visible by a surplus of royal jelly and extended building of the cell walls.

Experiment 1: Queen cells containing 1-2 day-old larvae

On the first day of the experiment, 50 queen cells were taken from the dequeened starter colony and were introduced between top bars in the brood area of the test colonies. It was carefully checked whether queen cells were still intact after 1, 6 and 9 days. After 13 days it was checked if young queens had successfully hatched by inspecting the empty queen cells. Three weeks after hatch, it was checked if the old queen was superseded by a new queen.

Experiment 2: Capped queen cells

Of the remaining queen cells in the starter colony, 33 capped cells were introduced into the test colonies that had rejected the queen cells on day 1 or day 6 in Exp. 1. This second introduction was done on day 9, and the cells were also placed between top bars in the brood area. The cells were of same origin and developed parallel to the cells used in Exp. 1. Their fate was checked together with Exp. 1.

Observation

Parallel to the experiments a closed queen cell (day 9) was introduced into an observation hive housing a queenright colony in order to get additional information concerning acceptance or rejection of introduced queen cells.

RESULTS

Experiment 1

Introduction of queen cells with larvae did not result in successful supersedures in the test colonies. Initially 12/24 (50%) of the colonies with young queens and 13/23 (57%) of the colonies with old queens accepted the introduced cells with larvae (Fig. 1A). After nine days, however, all of the cells in the colonies with young queens had been aborted. In the colonies with old queens 3 cells were left on the 9th day. These cells (13%) did also hatch according to the inspection on day 13 (Fig. 1A). Three weeks later no young queen was found and all resident queens were still present in the colonies.

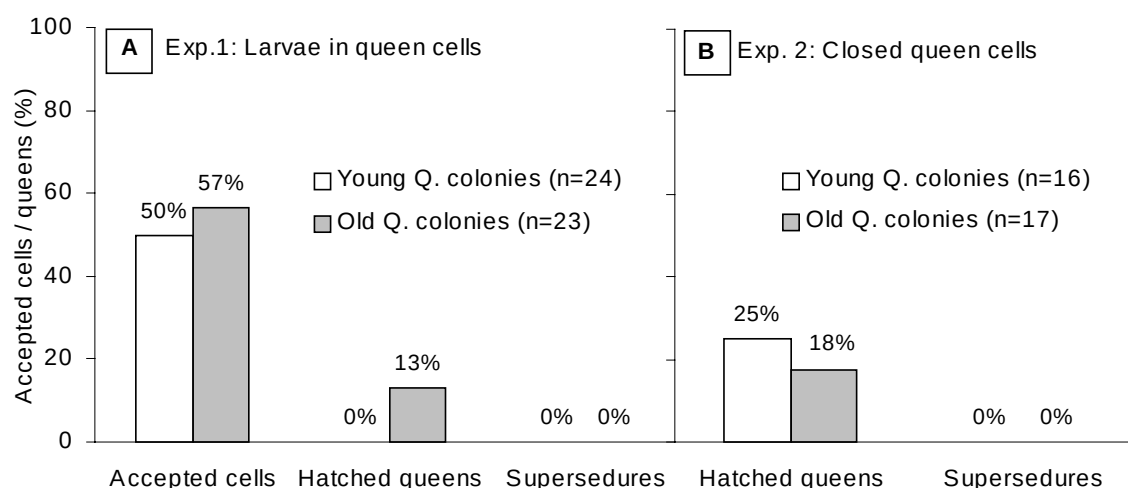


Figure 1. Fate of queen cells introduced into queenright colonies with young and old queens. 1A: Introduction of queen cells with larvae. We defined cells 'accepted' when they were still intact after one day. 1B: Introduction with ripe queen cells in colonies that had earlier rejected queen cells with larvae.

Experiment 2

After introduction with capped queen cells in 33 colonies that had rejected the queen cells with larvae of the first introduction, most colonies also rejected the cells for the second time. Of the introduced cells only 4/16 (25%) did hatch in colonies with old queens and 3/17 (18%) did hatch in colonies with young queens (Fig. 1B). All original queens were present three weeks later and no new queen was found.

Observation

After introduction, the ripe queen cell in the observation hive remained intact. When the young queen hatched, the worker bees balled her and she died (Pettis *et al.* 1989). In the following week another young queen was directly introduced into the hive, in order to observe this queen elimination more closely. The queen was grabbed immediately by worker bees and was balled by approximately 40 bees. The bees acted very aggressively, formed a ball around the young queen and were stinging each other. Besides killing the queen, approximately 30 bees died.

DISCUSSION

The introduction of queen cells with young larvae and ripe queen cells did not result in successful supersedures. If inspection next spring shows that supersedures in winter remain absent, the replacement of possible inferior queens was indeed redundant. If supersedures do occur, Exp. 1 and 2 indicate that the queen cell introductions are inadequate to prevent colony losses at the apiary due to supersedures in winter.

Since supersedures did not occur in Exp. 1 and 2, a possible effect on supersedure success considering queen age could not be determined. On the acceptance of queen cells with larvae (Exp. 1) a small age effect is observed. The 24 colonies with young queens aborted all introduced cells with young larvae, however from the 23 colonies with old queens 3 accepted the cells and reared new queens (13%). This higher acceptance in colonies with old queens is conform results of experiments testing capped queen cell introductions, in which the replacement rate of queens was found to be higher in colonies with relatively older resident queens (Reid 1977, Boch & Avitabile 1979, Jay 1981, Szabo 1982). After introduction of capped queen cells (Exp. 2) the numbers of hatching are low and do not show a trend considering the age of resident queens (Fig. 1B). Worker

bees most likely killed queens that did hatch (as observed in the observation hive). The 0% replacement rate in Exp. 2 is perhaps not surprising if we consider that the same colonies already rejected queen cells with larvae in Exp. 1.

Just before we started Exp. 1, a natural supersedure process was going on in two colonies, and before we introduced a new queen cell the natural queen cells were destroyed. These colonies were among the three colonies that accepted the introduced cells with larvae and reared new queens (Exp. 1). Obviously these colonies did want to supersede their queen. This observation gives a strong indication that only colonies already trying to supersede their queen, are willing to accept introduced cells with larvae to rear a new queen. It is remarkable, however, that the successful hatching of the cells did not lead to successful supersedures. Three weeks after hatching, one of the three colonies was found to be queenless. In the other two colonies there was no successful replacement observed. Possibly, the bees killed the young queen (as seen in the observation hive) or the new queen did not return from her mating flight. It is also possible that the observation date (3 weeks after hatch of the queen cells) was early considering the time a new queen needs to become active in a colony, and she may have been overlooked.

One could think that the bees do not want to replace their queen by an unrelated specimen introduced by us. This is unlikely since rearing of unrelated queens is common practice in beekeeping, so apparently kin-recognition does not play a role (Breed *et al.* 1994). Instead, relatedness may play a role in a general reluctance of bees to supersede their queen. Bees are much more related to the sexual progeny of their mother ($r=0.5$ for drones, $r=0.75$ for a young queen that is a full sister, and $r=0.25$ for a young queen that is a half sister) than to that of a sister that would be the new queen ($r=0.375$ for a drone or a queen from a full sister, and $r=0.125$ for a drone or a queen from a half sister) (Pamilo & Crozier 1982). Therefore, supersedure is only expected when the loss of fitness due to decreased reproduction of the old queen outweighs the loss of fitness due to the decreased relatedness with the progeny of the new queen. In practise we observed that bees kill immature or mature young queens as long as the old queen is considered good enough. Apparently, bypassing the initial phases of natural supersedure by introducing queen cells with larvae does not evoke earlier supersedure of the queen.

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