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## REARING CONDITIONS AND THE NUMBER OF SPERMS REACHING THE QUEENS' SPERMATHECA

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There is a great need to increase the number of sperms entering into the queen spermatheca. Only a small part of sperms injected to the queen oviducts enter into the spermatheca. The increase is especially important when the queen must be inseminated with a small amount of semen i.e. with semen of one drone only when she would be inseminated with the semen of diploid drones, which produce very small amounts of it.

The number of sperms reaching the spermatheca is influenced in natural matings not only by factors depending upon drones but also by factors depending upon queens, namely the size of spermatheca (Woyke 1966). It was suggested that this in turn may be influenced by the rearing conditions.

It was therefore investigated whether the rearing conditions of the larvae may affect the size of spermatheca and the results of natural and artificial insemination?

### MATERIAL AND METHODS

The investigated queens originated from a line derived from a cross of *A. mellifera* × *A. caucasica*. The virgins were reared from one queen and after she was lost, from her daughter. Altogether 98 inseminated queens were killed and investigated.

The virgins of every series were reared in one colony. Each series consisted of brood grafted as eggs (by the Örosi Pal method, or when this failed as just hatched larvae) and as 1, 2, 3 and 4 days old larvae. All virgins were weighed soon after emerging from the queen cell. Some queens reared from brood of every age

were put into the mating nuclei for natural mating. Other queens were mated artificially. Every day were inseminated queens of one series, reared from brood of every age. Queens of each repetition containing queens reared from larvae of every age were inseminated with semen of drones of the same age and origin.

Queens inseminated artificially were killed two days after last injection of semen. Those mated naturally, — after first eggs were laid. The ovarioles in ovaries were counted and the diameter of spermatheca deprived of the tracheal cover was measured. Then spermatozoa in the spermatheca were counted by the usual methods.

## RESULTS

Not always eggs transferred by the Örosi Pal methods to the queen cups were accepted by the bees. When this failed, just hatched larvae even not yet fed by the bees were grafted. Many larvae grafted in age of 4 days were not accepted.

The weight of the emerged virgins changed from hour to hour. But when several of them are weighed at the same time, it seems that the data are comparable.

Virgins reared from eggs and 1, 2, 3 and 4 days old larvae weighed in average 207, 189, 172, 144 and 119 mg. respectively. A decrease of weight of virgins reared from older larvae is visible.

The average numbers of ovarioles in queens reared from eggs and the 1, 2, 3 and 4 days old larvae were 319, 305, 291, 274, 233 respectively. The maximum numbers of ovariole were 340, 325, 324, 301, 128 respectively. Additionally, the variations in the numbers were greater in queens reared from older larvae. So the worse rearing conditions in the first days after hatching decreased the number of ovarioles.

The average volumina of the spermatheca (deprived of the tracheal cover) of the same five groups of queens were 1.23, 1.15, 1.00, 0.89 and 0.62 cu.mm. The maxima were 1.52, 1.34, 1.15, 1.09 and 0.79 cu.mm. respectively, and the minima were 1.09, 0.95, 0.79, 0.69 and 0.38 cu.mm. respectively. It can be noted very distinctly, that the spermatheca is much smaller when the queens were reared from older larvae.

Most of the queens reared from larvae 4 days old and left for natural mating were lost. Only one started to lay eggs. It was also very difficult to inseminate artificially such queens. The semen came out of the vagina during the insemination process of some queens and many others died.

The average numbers of spermatozoa, found in spermatheca of the five types of naturally mated queens were: 5.7, 5.4, 4.5, 3.5 and 1.5 mill. So the naturally mated queens reared from older larvae possessed in the spermatheca much less sperms than did the queens reared from the youngest brood.

Similar numbers of spermatozoa: 5.4, 5.3, 5.2 and 4.9 mill. were found in spermatheca of queens inseminated artificially twice with 8 cu.mm. of semen (queens reared of 4 days old larvae were here not inseminated). Thus queens with smaller spermatheca had less spermatozoa, independently whether they were inseminated naturally or artificially. One could assume, that the spermathecae were filled completely, there was no place for any more sperms and therefore queens with smaller spermatheca had less spermatozoa.

To know better the physiology of the sperms entering the spermatheca additional inseminations with smaller amount of semen were made.

Queens of the four groups inseminated with 8 cu.mm. of semen, contained in the spermatheca in average 3.8, 3.5, 3.2 and 2.6 mill. sperms. Those inseminated with 4 cu.mm. — 2.7, 2.4, 2.3 and 2.0 mill. and those with 1 cu.mm. — 1.6, 1.5, 1.3 and 1.1 mill. sperms. Thus although there was plenty of sperms in the oviducts (in 1 cu.mm. 7—7.5 mill.) to the smaller spermatheca entered less sperms, despite the fact that there was plenty of additional place for more sperms.

The above results show that different number of sperms injected to the queen oviducts is not the only cause of the different number of sperms entering the spermatheca (since from the same amount of semen different number of sperms enter different spermathecae).

To explain the question why less sperms enter smaller spermatheca in spite of a surplus of sperms in the oviducts and plenty of place in the spermatheca, the relations between the volumen of spermatheca and the number of sperms in it was taken into account. The number of sperms per 1 cu.mm. of spermatheca was

calculated. Concentration in the spermatheca of naturally inseminated queens reared from eggs, 1 day and 2 days old larvae was 4.51, 4.83 and 4.50 mill. sperms per 1 cu.mm. what is very similar for the three groups. Queens reared from 3 days old larvae had lower concentration (3.7 mill. cu.mm.). Perhaps their sexual behaviour was different or their sexual attraction for drones was lower. Queens of the four groups inseminated twice with 8 cu.mm. of semen had an average number of sperms of 4.49, 4.37, 4.38 and 4.49 mill./cu.mm., what is almost identical for all the groups.

Queens of the four groups inseminated with 8 cu.mm. of semen had in the spermatheca 3.08, 3.14, 3.10 and 3.05 mill. sperms per cu.mm., inseminated with 4 cu.mm. had 2.23, 2.05, 2.53 and 2.05 mill. per cu.mm. and those inseminated with only 1 cu.mm. of semen had 1.38, 1.35, 1.42 and 1.43 mill. sperms per cu.mm. of spermatheca. It is clear that the concentration of sperms in the spermatheca of queens inseminated with less semen is lower. But the most interesting result is that queens reared from brood of different age (inseminated with the same amount of semen) had almost the same concentration of sperms in the spermatheca despite the fact that its volumen was very different and the absolute number of sperms in it differed very much.

One can therefore conclude that to spermathecae of different volumen enter from the given amount of semen injected, such number of sperms which cause there the same concentration. As a result, less semen enter to smaller spermatheca despite the fact that there is plenty of room in it, and a surplus of sperms in the oviducts.

For practical value important is that more sperms will enter (from the given amount of semen injected, also by small quantities used) a greater spermatheca. This can be obtained by rearing the queens from eggs or the younger larvae.