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AMOEBA DISEASE IN THE QUEEN HONEYBEE

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SUMMARY

Queen honeybees have hitherto been found resistant to *Malpighamoeba mellificae*. For example 516 queens were examined by the author in 1954–61, and none was infected. He has now found amoeba cysts in 3 out of 7 dead queens wintered in cages with attendant workers (instead of in a colony). Reasons for this difference between queens free in colonies and caged in incubators are discussed.

INTRODUCTION

One of the interesting problems about amoeba disease in honeybees (*Apis mellifera*) is that the queen is resistant, although it would seem that she could easily become infected from worker bees in her colony. She is long-lived, hence there would be ample time for the slowly developing amoeba, *Malpighamoeba mellificae* Prell, to multiply in her, and also to form the easily recognizable cysts. (It is well known that the disease is often difficult to demonstrate in workers, because most of them die before cysts can form.) The queen would seem to be especially susceptible, but the studies and experiments so far made suggest that she is resistant.

Little work has been done on amoeba disease in queens. According to Morgenthaler (1929), the Institute at Liebefeld in Switzerland found a small colony in 1928 infected with amoeba, but without *Nosema apis*, and the queen was healthy. Fyg (1954) has described 135 queens (received by the same Institute) which originated in colonies suffering from 'spring dwindling' (*Frühjahrsschwindsucht*). There were *Nosema apis* spores and amoeba cysts in the attendant workers of all queens. Of the queens themselves, 88 (65%) showed infection by *Nosema apis*. Only one had amoeba as well, and Fyg considered this single infection to be unproven (the queen was not dissected but ground up whole; in the pulp, a minute piece of a Malpighian tubule was found with some amoeba cysts, but the various samples of workers investigated at the same time could have caused an error in the finding).

Fyg had examined many queens for several decades without finding ameoba, and he believes that the results of his experiments confirm the resistance of the queens. In 1932–1945, he fed 7 healthy queens of various ages with sugar syrup mixed with *N. apis* spores and amoeba cysts. The queens were kept in 'small nursery colonies' in an incubator for 16–35 days. All 7 become heavily infected with *Nosema*, but none developed amoeba cysts. Worker bees fed in the same way became infected with both agents. Fyg assumes that the difference may be due to the different feeding habits of queens and workers. He points out that the pyloric area of the queen's small intestine is normally free of bacteria, whereas this is not so in workers. In a later paper (1961) Fyg again asserts that queens are resistant to amoeba disease.

Malpighamoeba mellificae is not rare in Hungary. I have found it in workers for more than thirty years, usually with *Nosema apis* but occasionally alone (Örösi-Pál,

1929). From 1954 to 1961, 8550 samples of adult bees were examined at Gödöllö for disease diagnosis. Of these, 115 (1·3%) were infected with *Malpighamoeba*, 87 with and 28 without *Nosema* infection in addition. During this period 516 queens were also examined; some were suspected of disease, the others were old or surplus to requirements. No amoeba cysts were found in any of these queens. Until recently, I had not seen *any* cysts in queens, but I have now found them in caged queens; a preliminary note on this has been published in Hungarian (Örösi-Pál, 1963).

METHODS

In autumn 1962 I experimented with overwintering queens outside the colony. After some trials by others several decades ago, a method was worked out by Foti in Rumania in 1956 (Foti, 1958). The queens winter in cages, with attendant workers (which are frequently changed for others) in an incubator or a room; liquid honey is provided for food. The ten cages for the present tests were of plastic, given by the Central Research Institute of Sericulture and Beekeeping in Bucharest. The cages were stocked on 17th October, each queen being given about 60 workers. (This number did not remain constant, since some workers always died between inspections). The cages were kept in an incubator, at 22·5°C. and 70% relative humidity. The worker bees were changed every 1–3 weeks, or sooner if the bees had defaecated excessively in the cages, or if many had died.

RESULTS

These are shown in Table 1. Between 17th October 1962 and 2nd April 1963, 7 queens died; all were infected with *Nosema apis*. After amoeba cysts had been found in the ground-up remains of two queens, on 31st January and 18th February, I dissected

TABLE 1. Infection of queens overwintered in cages

Queen no.	*Year reared*	*Date of death*	*Nosema present*	*Amoeba cysts*
1	?	Jan. 8	yes	no*
2	1962	Jan. 14	yes	no*
3	1962	Jan. 23	yes	no*
4	1961	Jan. 31	yes	yes*
5	1962	Feb. 18	yes	yes*
6	1962	Feb. 23	yes	yes
7	1961	Apr. 2	yes	no
8	1961	introduced to colonies on 5th April, and lived until July		
9	1962			
10	1962			

* queen ground up whole

the next queen found dead. Her Malpighian tubules were as full of amoeba cysts as are those of heavily infected workers; *Nosema* was also present. Amoeba was thus found

in 3 out of 7 queens that died by spring, from a total of 10 queens. No cysts were found in the workers accompanying any queen.

DISCUSSION

It is striking that the disease has been found only in caged queens, and one may assume that caging in winter favours infection. This effect can usefully be considered in relation to Kluge's work (1963) on the intestinal flora of healthy honeybees. The characteristic bacteria of the worker intestine are *Lactobacillus rigidus apis*, *L. constellatus*, and a third which is probably identical with *Bacillus influenzoides*. The hindgut of the laying queen in a colony contains no bacterial flora resembling that of the workers, but if the queen is caged with some attendants and kept in an incubator, her intestinal flora becomes similar to that of the workers. This phenomenon may be connected with the change in food. In Kluge's experiments, the intestinal flora of the workers depended largely on whether they fed mainly on protein or on carbohydrate.

It thus seems possible that a caged queen is sensitive to amoeba infection because of either the change in food or the modified intestinal flora. In Fyg's unsuccessful infection experiments, the queens were confined in 'small nursery colonies' for a much shorter time, and with more workers, than were mine.

Amoeba disease warrants special care when overwintering queens in cages outside the colony. Fumidil and Nosemack, used for treating nosema disease, have so far proved ineffective against amoeba, and no other remedy is known. The quality of the queen's food might be improved by changing the attendant workers frequently, taking them from colonies containing bees which have well developed hypopharyngeal glands, but which have not yet fed brood. Honey for use in the cages might perhaps be mixed with some protein food. A drug active against amoeba should also be sought.

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