Studies on the Effect of Infection with *Nosema apis* on the Physiology of the Queen Honey-bee

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**SUMMARY**

When a queen honey-bee becomes infected with *Nosema apis*, the result can be very serious indeed for her colony. The metabolic processes are disturbed by the damage done by the parasite to the epithelial cells of the mid-gut, and this apparently leads to severe damage to the ovaries, at first by the production of a high proportion of eggs that fail to hatch, and ultimately by complete cessation of oviposition and supersession or death of the queen. In the case of *Nosema bombycis*, which attacks the silk moth, infection is carried from the female to her offspring via the egg; but there is no evidence that this ever occurs in the case of *Nosema apis*. Although large numbers of eggs, larvae, and pupae produced by infected queen honey-bees were examined, none was found to be infected with any stage of *Nosema apis*.

**INTRODUCTION**

It has been known for some time that the queen honey-bee is liable to contract most of the diseases that attack her offspring. One of the diseases to which she is subject is that caused by *Nosema apis*. It seems probable that her special mode of feeding and the fact that she does not assist her workers in the task of comb-cleaning before the brood is reared, reduces the risk of her becoming infected with protozoan diseases. The way in which she is most likely to become infected with such diseases is by eating contaminated food.

It is important to know whether the queen honey-bee can act as an agent in spreading infection amongst the members of her colony, more particularly since many of the bees in a colony come into close contact with her; and when for some reason, possibly on account of the presence of some disease, a colony becomes weak and perhaps eventually dies out, the queen often remains alive to the last in close company with a small number of her worker bees.

Experiments were, therefore, carried out in order to determine the effects of infection with *Nosema apis* on the queen.

**EXPERIMENT I**

To determine the Rate of Development of *Nosema apis* in Queen Honey-bees

During 1948 eight queens and during 1949 twelve more queens were removed from healthy colonies of bees and fed on dilute sugar syrup. After feeding they were released on to a closed window in a warm room and allowed
to defaecate. Microscopical examination of their excreta showed no sign of Nosema spores and it was concluded that they were free from this disease.

Each queen was subsequently allowed to feed for 1 hour on syrup in which viable spores of Nosema apis were suspended. At the end of this period they were transferred to sterile cages and fed thenceforth on sterile syrup. The cages were kept in an incubator at a temperature of 32° C. and at a relative humidity of about 40 per cent. The eight 1948 queens were subsequently examined for the presence of Nosema at various intervals after they had been fed on the contaminated syrup, as were two of the 1949 queens.

Results

The results of these infection experiments are shown in the following table (Table I).

**Table I. The Rate of Infection of Queen Honey-bees when fed upon Sugar Syrup containing Viable Spores of Nosema apis**

<table>
<thead>
<tr>
<th>Number of queen</th>
<th>Number of hours that elapsed after feeding with contaminated syrup before the queen was examined</th>
<th>Result of examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>No infection.</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>No infection.</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>Young schizonts in epithelial cells of mid-gut.</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>Young schizonts in epithelial cells of mid-gut.</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>Schizonts present; some multiplication of schizonts by division.</td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>Schizonts present. No ripe spores found.</td>
</tr>
<tr>
<td>7</td>
<td>96</td>
<td>Schizonts present. No ripe spores found.</td>
</tr>
<tr>
<td>8</td>
<td>120</td>
<td>Young spores present.</td>
</tr>
<tr>
<td>9</td>
<td>144</td>
<td>Young spores present.</td>
</tr>
<tr>
<td>10</td>
<td>168</td>
<td>Ripe spores present.</td>
</tr>
</tbody>
</table>

It will be seen from Table I that young schizonts were found in the epithelial cells of the mid-gut of the queen honey-bee 12 hours after infection; that multiplication of schizonts was taking place after 48 hours; that young spores were not produced until some 96–120 hours after infection; and that ripe spores were not present until about 28 hours later.

**EXPERIMENT II**

*To determine the Effect of Nosema apis on the Epithelial Cells of the Mid-gut of the Queen Honey-bee*

**Method**

In 1949 four of the queen bees that were infected with Nosema apis in the manner described in Experiment I above were killed, and their mid-gut and
on the Physiology of the Queen Honey-bee

adjacent parts removed, fixed, dehydrated, embedded in paraffin wax, and sectioned in order to study the effect of the parasite on the host.

Results

The infected epithelial cells of the mid-gut were found to have proliferated; the peritrophic membrane was degenerating. The epithelial cells of the Malpighian tubules were also found to be infected with the parasite.

Conclusions

It appears from the results of these experiments that the queen honey-bee becomes infected with *Nosema apis* as readily as the worker bee when fed on syrup containing viable spores of this parasite. In both cases the parasite feeds in the epithelial cells of the mid-gut of its host.

*Effect of Nosema Infection on the Ovaries of the Queen Honey-bee*

Introduction

In his work on queen supersession Farrar (1947) reached the conclusion that in many cases the abnormally high degree of supersession of queens in ‘packages’ was due to infection of the queens, which were found to lay normally for about 2 months and then suddenly ceased to lay and became sluggish; most of the last lot of eggs they laid shrivelled up and failed to hatch.

It was considered to be desirable to study this matter further by investigating the effect of *Nosema apis* on the ovaries of a queen. This was done by comparing ovaries taken from infected queens with those of healthy queens.

The Healthy Ovary

The ovaries of a fertilized queen honey-bee that has recently been laying normally are large, pear-shaped organs. Each consists of about 150–180 ovarioles loosely attached to one another.

Each individual ovariole consists at its anterior end of a somewhat coiled, slender filament, which is formed of a row of cells not surrounding a lumen. Posteriorly the ovariole becomes thicker and possesses a lumen which gradually widens out to form an egg canal; this unites with the egg canals of the other ovarioles to form a cup-shaped ovarian bursa, which still more posteriorly contracts to form a funnel and passes without any obvious boundary into the oviduct (fig. 1).

According to Paulcke (1901) the germ cells of each primary oogonium in the ovarioles first form four cells, by two consecutive divisions. One of these cells becomes an oocyte, whilst the other three, by four consecutive divisions, produce forty-eight nurse cells.

The distribution of food cells amongst the egg cells gives a bead-like appearance to the ovarioles. This characteristic bead-like appearance is lost only in the lowest part of each ovariole, where the nutritive cells have degenerated after fulfilling their function. The nutritive cells produce fatty yolk substances that nourish the developing egg cells. It is known that at the height of her
reproductive power a queen honey-bee can lay between 1,500 and 2,000 eggs daily. When this occurs, each ovariole must be producing from 5 to 8 eggs every 24 hours. It is clear that such high rates of egg-production must demand active intestinal metabolism to provide nutriment for the nurse-cells.

**The Ovary of a Queen infected with Nosema apis.**

In studying the effect of *Nosema* on the ovaries of queen honey-bees, material of two kinds was used:

(a) Naturally-infected queens that were sent by beekeepers in different parts of England and Wales to the Bee Advisory Section of the National Agriculture Advisory Service.

(b) Queens infected in the laboratory by feeding them on a suspension of viable spores of *Nosema apis* in syrup for 1 hour, followed by sterile syrup for 1 week. Three queens infected in this manner were subsequently introduced into small colonies ('nuclei') in an apiary and allowed to remain there for 3 or 4 weeks before being killed and examined.
During 1949 seventeen queens were received from bee-keepers; and of these, six sent in during April and May were found to be heavily infected with *Nosema apis*.

**The Infected Ovary**

In fig. 2 the effect of *Nosema* disease on the ovaries of the queen honey-bee is shown. The most striking effect is that the ovaries of an infected queen are smaller than those of a healthy queen, owing to disturbance of ovarian development. The anterior portion of each ovary appears to be normal but posteriorly growth appears to cease abruptly and the egg and nutritive cells break up and disappear. The ovarioles become greatly reduced in size and at their point of fusion at the ovarian bursa appear as thin empty tubes; this agrees with Fyg (1945).

In the case of the three queens that were deliberately infected with *Nosema* in the laboratory, the appearance of the ovarioles gave the impression that
development of the eggs had been inhibited completely and that destruction
and absorption of the egg and nutritive cells had occurred.

It would appear that the ovaries of these diseased queens were entirely
functionless and one was impressed by their markedly shrunken condition as
compared with normal, healthy ovaries. This is well seen in fig. 2.

In the more advanced stages of ovarian degeneration the egg tubes col-
lapsed. Usually the first part of the ovary to show injury is that which is
posterior to the second well-formed egg in the ovariole.

In the eggs themselves injury is marked chiefly by the disappearance of
yolk granules from the oocytes.

Discussion

The manner in which infection with *Nosema apis* causes the reproductive
system of the queen honey-bee to degenerate is not clear. One must, pre-
sumably, assume that disturbance of the epithelial cells lining the mid-gut
affects particularly all those organs of the adult bee that require continual and
abundant food, and in the case of the queen honey-bee the ovaries are amongst
the most important of the organs thus affected.

In the case of the females of the moth *Bombyx mori* it is known that when
infection with *Nosema bombycis* occurs, the oocytes and eggs themselves can
become infected. Examination of the oocytes and eggs of queen honey-bees
that were infected with *Nosema apis*, however, did not show the presence of
any of the live stages of this protozoon. It would therefore appear from the
above observations that *Nosema apis* has only an indirect effect on the ovaries
and eggs of the queen honey-bee, through the upset of intestinal absorption.
In order to reach a more definite conclusion on this point a further series of
experiments was carried out.

**EXPERIMENT III**

*To determine whether Nosema apis can be transmitted via the
Eggs of the Honey-bee*

**Method**

Five hitherto healthy queen honey-bees were infected with *Nosema* by
allowing them to feed in cages on sugar syrup containing viable spores. They
were subsequently introduced into healthy colonies of bees. At intervals
thereafter sample eggs, larvae, and pupae produced by these queens were
taken and smears made for microscopical examination. Approximately twenty-
five eggs, larvae, and pupae were examined in this way.

**Results**

In no case was any stage of *Nosema apis* found in smears prepared from
eggs, larvae, or pupae of queen honey-bees known to be infected with *Nosema
apis*. That the queens were indeed infected with *Nosema* was checked at the
end of these observations by dissection and microscopical examination.
ACKNOWLEDGEMENTS

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BOOKS AND PAPERS CONSULTED