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Drones from Fertilized Eggs and the Biology of Sex-determination in the Honey Bee

by

J. WOYKE

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With the parthenogenetic origin of drones discovered by Dzierżon [1], drones are believed to this day to develop only from unfertilized eggs.

On the other hand, in some hymenoptera some diploid males are known to occur in addition to haploid ones, although the former have low viability [8], [9].

With regard to bees the problem was investigated by Mackensen [4], [5], Laidlaw, Gomes, and Kerr [3], Rothenbuhler [6], and Hachinohe and Jimbu [2], who have shown that after inseminations that might have led to homozygous combinations of alleles at locus *X*, the brood disappeared from the cells of the worker comb at the time of hatching. Hence, the conclusion was drawn that homozygous combinations of alleles at locus *X* are lethal, and the eggs do not hatch and are removed by the bees. Rothenbuhler obtained some mosaic drones, in whom certain patches in the eyes could have been diploid. He advanced the surmise that haploid tissues may make it possible also for diploid tissues to develop, which without such aid are lethal.

It appeared, however, that alleles at locus *X* may be somehow related with sex-determination in bees. This point is the subject of the present paper.

Method

Individual sibling mating by artificial insemination was carried on through two or three generations. Survival of the brood in comb cells was investigated by Mackensen's viability test [4], [5].

The hatching rate was determined in two ways. Either the eggs in tne comb cells were separated from bees by a screen and left in the hive for a subsequent check up of the percentage of hatchings, or they were placed together with the brood comb in an incubator, in either case the hatched larvae were counted afterwards.

To see when the brood disappears, the brood comb with the bees on it was withdrawn every 3 hrs from the hive and a detailed record was kept of the content of particular cells. The sex of the tiny larvae was determined histologically from microtome sections. To see why and how the

brood disappears, a close watch was kept for several days on the hive entrance so that bees carrying the brood outside might be noted.

Next, there was close inspection to see whether the brood is not removed and dropped to the hive bottom at night or day. Eventually, a special observation hive was built. In this hive, worker cells were cut off from the comb foundation and attached to a glass plate turned to face the observer. When a queen producing low-survival rate brood was put in the hive and deposited eggs in such a comb, it was possible to see through the window what happens to the brood.

To see whether the disappearance of brood is in any way related with the kind of cells, larvae hatched in worker cells kept in an incubator were transferred into queen or drone cells, and a record was kept of the time of their disappearance and sex of adult survivals.

To investigate the viability of low-survival brood and to eliminate bees as a factor, the brood was reared outside the hive in an incubator. Rearing was started from the egg stage. The larvae were fed either throughout with royal jelly, or initially with royal jelly and then with the mixed food of older worker larvae. All the individuals reared were sexed morphologically first at the age of five days, and next in the pupa or imago stage.

Results

Inbreeding through artificial insemination gave queens whose brood in worker cells survived in only 50 per cent. This was indicative of two-allele fraternity of the brood.

Observation of the eggs screened in the hive or placed in an incubator has shown that unlike previously believed the eggs of such queens hatched very much like those from queens mated naturally by any drone. Three-hourly observations of broods kept in hives without precautions demonstrated, however, that one-half of the larvae disappeared from worker cells within a few hours after hatching.

Sexing has shown that of all larvae hatched in worker bee cells in an incubator from eggs of queens producing low-survival rate brood about 50 per cent were males, and the rest, females. Thus the lethal character of homozygosity at locus *X* with regard to eggs has been disproved and shown to afford diploid drones, which soon disappear from the cells.

The next question was what happens to the disappearing diploid drone larvae. Observation of the hive entrance and inspections of the bottom supplied no evidence of their being carried outside or dropped to the bottom. What happened could be followed well in the combs attached to the glass plate. A part of the larvae were seen to be eaten up by the bees, but there was nothing to suggest that they were weaker or less viable than the others. They were eaten up alive by the bees and their movements were quite noticeable.

Hence, the question arose why they are eaten up if they are not less viable. One possible reason that suggested itself was that the wrong larvae were in the wrong cells, i.e., drone larvae in worker cells. Therefore, a comb with eggs was incubated until hatching, so that the bees could not devour drone larvae hatching in worker cells. All larvae were then transferred into drone cells and placed in the hive.

On the next day one-half of the larvae were found to have been eaten up. But whether the victims were larvae of drones or of workers was not certain. The brood was therefore screened and when the imagines emerged it turned out that here

again the bees had devoured the larvae of drones leaving those of worker bees, even though in drone cells.

The results were similar when the larvae had been transferred into queen cells. These results show that drone larvae hatched from fertilized eggs were eaten up not because they were in the wrong cells but for some other reason.

This conclusion is supported by further investigations still. When queens producing low-survival brood had no more sperm in the spermatheca, they laid in worker cells unfertilized as well as fertilized eggs. The bees, however, did not devour drone larvae hatched in worker cells from unfertilized eggs. In these cases, the scattered brood in worker combs included drone brood, and the emerging adult drones inherited only after the queen. So here again, wrong sex in wrong cells can be seen to have not been the cause of the cannibalism observed.

If lower viability is not the reason either, it should be possible to rear further the drone larvae hatched from fertilized eggs. This was done in an incubator, where the larvae were protected from bees. For this purpose a worker comb was taken in which eggs had been deposited by a queen producing low-survival brood. Repeatedly most of the hatched brood was successfully reared to the fifth day, when the live larvae could be sexed morphologically. The ratio of diploid drones to females was roughly balanced.

Next, the rearing was carried on successfully to the pupal stage, and eventually fully developed queens, worker bees, and drones were obtained. Thus, there is nothing to suggest lower viability for drone larvae hatched from fertilized eggs. Hence, the cause of cannibalism is neither the wrong sex in the wrong cells, nor lower viability, but some other, perhaps hormonal reason. Further investigations on this point are in progress.

The results here described show it is necessary to assume some new mechanism of sex determination in bees. Drones can develop from unfertilized as well as fertilized eggs. Normally, however, drones from fertilized eggs fail to appear in the hives because the larvae are devoured by bees shortly after hatching.

Statistical data on the progeny of queens producing low-survival brood demonstrate that those that disappear are larvae homozygous at locus *X* ,which have been shown to be male. Drones can therefore arise from unfertilized eggs. i.e., hemizygotes, as well as from fetrilized eggs that are homozygous at locus *X*. Females, however, develop from fertilized eggs heterozygous at locus *X*. In accord with the abbreviations of the names of genes and mutations by Rothenbuhler [7] it is proposed to name locus *X* in bees the sex determining locus, and the alleles *a*, *b*, *c*, ..., or 1, 2, 3, ... there, the sex alleles.

The experiments and results will be described in separate papers [10]—[15].

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DEPARTMENT OF APICULTURE, SKIERNIEWICE, CENTRAL COLLEGE OF AGRICULTURE
(ZAKŁAD PSZCZELNICTWA, SKIERNIEWICE, SGGW)

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