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STUDY ON DIPLOID DRONE HONEY BEES

by Jerzy WOYKE,

Bee Department, Agricultural College, Warsaw 25 — Ursynów (Poland).

Since Dzierzon time (1945) it was generally accepted that the female honey bees develope from fertilized eggs, and the drones from the unfertilized ones.

It was presented at the XIX International Beekeeping Congress in Prague, that after sibling mating some queens produced scatterd brood. It was believed at first, that 50 % of the eggs laid by these queens to the worker cells do not hatch. But we have found that all the eggs hatch, and next 50 % of the very young larvae are eaten by the worker bees within a few hours after hatching.

Histological and anatomical investigation of all the larvae hatched and reared from the eggs stage in an incubator showed, that the 50 % of larvae which were eaten by the workers, were drones.

But direct proof of the diploid character of these drones, as well as an easy methode of their rearing has so far been lacking.

CYTOLOGICAL EVIDENCE OF THE ORIGIN OF DRONES FROM FERTILIZED EGGS

Search for sperms was carried on in 230 eggs taken from worker and drone cells. The 1-3 hrs. old eggs originated from one naturally — and four sibling — mated queens producing in worker cells 50 % of males which were eaten by the worker bees.

Sperms was found only in one, out of the 29 eggs taken from the drone cells. on the other hand sperms were found in 88 % out of the 178 eggs laid by the sibling-mated queens to the worker cells. The sperms were also found in similar percentage of eggs laid in the same kind of cells by the naturally mated queen.

It can be therefore concluded, that the male larvae originating from sibling-mated queens hatch in the worker cells from eggs which the sperms entered.

To exclude the possibility of androgenesis and to obtain an evidence of the diploid character of these drones, the number of chromosomes in eggs were counted. 171 eggs 4-10 hrs. old taken from worker and drone cells were investigated. They originated from similar queens as was given above. Altogether about 35 000 chromosomes were counted.

The chromosomes could be counted in 65 eggs taken from the drone cell. 16 chromosomes were found in 98 % of the eggs. In one egg exclusively more than 16 chromosomes were stated.

Entirely more than 16 chromosomes — 32 or more were found in 98 % of the 81 eggs taken from worker cells where the sibling-mated queens produced 50 % drone larvae.

Thus it can be concluded, that the drone larvae originating from sibling-mated queens and creating the 50 % of brood being eaten by the worker bees are diploid in their origin.

GENETIC EVIDENCE OF THE ORIGIN OF DRONE LARVAE FROM FERTILIZED EGGS

At first, characteristics were sought by which the bees could be identified genetically in earlier stages of development, 725 wild or mutant bees were investigated of different development stages. It was stated that wild (black) eye colour can be distinguished from brick, chartreuse and buff mutants from 4 days before pupation. Cordovan body colour can be distinguished from the wild type only one day before emergence from the cell.

Next homozygous cordovan queens and homozygous chartreuse ones were inseminated each to one wild (black) brother. Four queens producing low survival brood were selected. 321 larvae hatched in an incubator, in drone and worker cells were reared in that incubator further. Drone prepupae and pupae produced by those queens in drone cells showed only genetic character of mother like it was expected. But drone prepupae and pupae produced by the same queens in worker cells and reared from the egg stage in the incubator showed genetic character of the father like the workers of these queens did. This indicate that the drone larvae which hatched in worker cells from eggs laid by sibling-mated queens develope from the fertilized eggs.

VIABILITY OF DIPLOID DRONE BROOD

It was shown (Woyke 1963) that the viability of diploid drone larvae is till the age of 5 days the same as for the females. Nevertheless only three diploid drones were reared to the imago stage.

To state whether it would be possible to reare greater number of diploid drones, the viability of diploid and haploid drone was compared.

The brood of four sibling-mated queens was taken from worker and drone cells and was reared from the egg stage in the incubator.

60 % of 89 haploid larvae and 63 % of 128 diploid reached the age of 5 days. Roughly one half of the diploids were drones. Of the five

days old diploid drone larvae, 43 % reached the age of 9 days against 36 % of the haploid. The average weight of a drone larvae at the time of transference to pupation was 358 mg. for diploids and 298 for haploids.

Thus the viability of diploid drone larvae is not lower and may be even higher, than that of the haploid ones. By using the right technique of rearing, it should be possible to rear greater number of diploid drones to the imago.

DO WORKER HONEYBEES EAT DIPLOID DRONE LARVAE BECAUSE THEY ARE IN WORKER CELLS INSTEAD OF DRONE CELLS ?

The diploid drone larvae hatch in the colony in the worker cells, but normally the haploid drones developpe in drone cells. In search for the cause of eating the diploid drone larvae, this phenomenon was investigated.

457 diploid larvae of a low survival rate, were hatched in an incubator. Next they were transferred from the worker cells into drone cells to queenless colonies. A control group of 75 haploids was also included.

The greatest part of the diploid larvae was eaten during the first hours after transferring, leaving 48 % survivals till the next day, against 92 % of the control haploids. 65 % haploids were sealed, against 19 % originating from worker cells, of which 2,8 % were drones. But after emerging it turned out, that only one was diploid.

The diploid drone larvae were eaten in the drone cells in similar way as they were in worker cells.

Thus the sex of the brood uncorresponding to the right cells, is not the cause of eating the diploid drone larvae.

A METHOD OF REARING DIPLOID DRONE LARVAE IN THE COLONY

Due to difficulties in rearing any drones in the incubator, investigations were undertaken to work out a method of rearing the diploid drones in the colony. Two questions were investigated at first : 1) whether the diploid drones can be reared in the colony in the queen cells? 2) is there a critical period in the life of the diploid drone larvae after which they are not eaten in the comb cells by the workers?

1 608 larvae hatched in an incubator were transferred into colony. 1 332 of them were of law survival rate. All the larvae originated from 17 queens, 12 of which produced brood of low survival rate.

Out of 265 larvae of low survival rate, grafted into queen cells to mearing colonies, about 50 % reached the age of 5 days. The most surprising fact was, that about half of them were females and half males. But adult diploid drones were not reared in the queen cells. Thus the presence of larvae on royal jelly in queen cells provide against the eating of young diploid drone larvae, and special substance must be responsible for the eating phenomenon.

The diploid drone larvae can be reared in the queen cells longer than in comb cells, but it is also difficult to reare here any adults.

To answer the second question, the larvae were hatched and reared on royal jelly in an incubator for different period of time. Next they were transferred to comb cells in a colony.

Out of 144 larvae of low survival rate kept in an incubator from 0 till 3 days, 40 to 65 % reached the age of 5 days, after being transferred to worker cells. No drone larvae were found in series transferred to the colony at the day of hatching. In the other series reared in an incubator for 1 to 3 days, from 10 to 35 % of drones survived. The highest percentage was found in series kept in an incubator for 2 days. But no adult diploid drones emerged from worker cells.

The worker bees do not eat the older diploid drone larvae so fast as the younger ones, but the worker cells are to small for the diploid drones.

Out of 368 low survival larvae reared in an incubator from 0 to 4 days, and transferred to drone cells in a colony, 0 to 73 % reached the age of 5 days. 10 to 50 % of drone larvae were found among series being previously kept in an incubator for 2,3 and 4 days.

The most important result is, that 52 adult diploid drones were here reared. The most efficient were those series that has been previously kept in an incubator for 2 and 3 days.

The results show that after the diploid drone larvae are kept at least for two days on the royal jelly, they can be easy reared further by the worker bees in the comb in a colony.

The diploid drones can be also reared in the colony from the first day of life. For that purpose, they should be hatched in an incubator, grafted into the queen cells in a queenless colony, and after 2-3 days transferred to the drone cells.

To make the method more efficient, investigations were undertaken, which showed that the bees can reare in the colony also more than one larva per one queen cell. Three to four larvae per cell are recommended. In a series 50 % males were obtained, out of 8 larvae, reared by this method. Together 8 more diploid drones were reared.

Positive reasult were also obtained in rearing the diploid drones late in the season.

Thus an easy method of rearing the diploid drones in a colony was worked out. The low survival brood should be hatched in an incubator and then the larvae grafted into the royal jelly, in an incubator or in a colony. After two or three days it should be transferred to the drone cells in a colony.

GENETIC EVIDENCE OF ADULT DIPLOID DRONES

Three homozygous chartreuse (ch) eyed queens producing low survival brood were selected. Two of them were mated to a dominant wild (black) brother, and one to a wild eyed but recessive cordovan body coloured drone. All the queens produced chartreuse eyed haploid drones and phenotypic wild females.

The diploid drones were reared by the previously described method.

22 adult, phenotypical wild drones were reared from the brood of the two first queens. This indicates that the drones had to develope with the participation of the father.

To exclude androgenesis, drones of the third queen were reared. Again 22 eye and body wild adult drones were obtained. They could not be haploid neither of maternal origin (they would carry chartreuse genes) nor of paternal (they would be cordovan). These drones are the best evidence not only to indicate their origin, with the participation of the father but also to exclude androgenesis. They prove really their origin from both parents.

The adult diploid drones were reared to sexual maturity, their biology, morphology and anatomy was investigated.

REPRODUCTIVE ORGANS OF DIPLOID DRONES

The sexual mature diploid drones produced very small amount of semen. Examination of the reproductive organs of 0-3 days old diploid drones showed which follows. Except the testes all the other reproductive organs looked like in the haploids. The testes of the diploids were 1.5-3.0 mm. long against 4.0-5.5 mm for the haploids. The diploids had about 1.5 million sperms against 10-11 million in haploids. Spermatogenesis of the diploid drones has been investigated. The sperms were viable.

Analysis of the obtained results indicated an existence of sex limited genes which influence the size of testes. Basing on this diploid drones with greater testes were reared. Research work in now being undertaken to inseminate the queens with the semen of diploid drones and to test the progeny.

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Intervention de M. Christensen.

May I ask you : « Have you made squash preparations of the diploid drones? »

Réponse de M. Woyke.

Yes, and the spermatogenesis of the diploid drones is different from that what anybody could think about it.

Intervention de M^lle^ Pain.

Avez-vous constaté une différence quant à la mobilité des spermatozoïdes répartis dans les testicules des mâles diploïdes?

Réponse de M. Woyke.

We investigated the activity of the sperms in the ejaculated semen. It appared, that the sperms of the diploid drones were less active.