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THE CHROMOSOME NUMBER AS PROOF THAT DRONES CAN ARISE FROM FERTILIZED EGGS OF THE HONEYBEE*

J. WOYKE & A. KNYTEL

Zakład Pszczelnictwa, Szkoła Główna Gospodarstwa Wiejskiego, Warszawa-Ursynów, Poland

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SUMMARY

Four sibling-mated queens producing brood in worker cells of which 50% survived were selected; controls were one unmated queen and one mated naturally at random. The number of chromosomes was investigated in eggs 4 – 10 hours old.

It was confirmed that the 'disappearing' larvae in worker cells from the sibling-mated queens were drones. Of eggs laid by these queens in drone cells 97·6% showed 16 chromosomes, whereas 32 chromosomes were found in 95·8% of eggs laid by the same queens in worker cells. So the sibling-mated queens producing brood of 50% survival rate lay eggs in worker cells which are virtually all fertilized, and the 50% of these larvae which are normally eaten by the workers, develop from fertilized eggs; they are males, but diploid in origin. Drones can thus develop not only from unfertilized but also from fertilized eggs.

INTRODUCTION

From Dzierzon's time (1845) until a few years ago it was generally accepted that drone honeybees (*Apis mellifera*) develop only from unfertilized eggs. In a series of earlier papers the author has however presented data which also support a completely zygotic origin of drone larvae (Woyke, 1963*a*, *b*). Later data showed that these drones developed from eggs into which spermatozoa entered (Woyke, Knytel & Bergandy, 1966*a*, *b*), and that these males inherited characters of their father (Woyke, 1965*b*). A more detailed history of investigations on drones developing from fertilized eggs has been given by Woyke *et al.* (1966*b*).

Drones of this new type were reared after their mothers had been mated to a closely related partner. But the process of fertilization of an egg by spermatozoa in inbred crosses of the honeybee is not known to occur. One can therefore suppose that the male tissue of the new drones was not the result of fertilization, but that it derived either from a female pronucleus which remained unfertilized in spite of the entry of a spermatozoon, or from both male and female (ununited) pronuclei, or from the pronucleus of a spermatozoon which entered the egg but did not unite with the egg pronucleus. Several examples of this last phenomenon have been described (Rothenbuhler, Gowen & Park, 1952; Drescher & Rothenbuhler, 1963, 1964).

It would seem that a good way to test the zygotic origin of the drone in question would be to count the number of chromosomes in eggs from which the

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new drones hatch, as described in the preliminary report of part of the material presented here (Woyke & Knytel, 1966). After the maturation process is finished (Nachtsheim, 1913), 16 chromosomes can be seen in unfertilized eggs and 32 in fertilized eggs. Sanderson and Hall (1948), using improved modern smear technique, and fixation and staining in aceto-carmine or aceto-orcein, found in male and female eggs in all stages exclusively 16 and 32 chromosomes respectively. In blastoderm (Petrunkewitsch, 1901) and in larvae (Risler, 1954), polyploidization takes place and a greater number of chromosomes can be found. Merriam and Ris (1954) found high degrees of polyploidy in various tissues of queen, drone and worker. Since no distinguishing cytological differences can be seen in somatic tissues of individuals from fertilized and unfertilized eggs, it appears that eggs in early cleavage would be best for the present study.

Complete or partial diploids might occur in the honeybee not only as a result of the union of egg and sperm pronucleus. Therefore eggs with the diploid number of chromosomes can be referred to as fertilized only in relation to the genetic investigations preliminarily reported (Woyke, 1965*c*; Woyke & Adamska, 1966).

MATERIAL AND METHODS

Eggs were collected from four sibling-mated queens producing scattered brood, from one queen mated naturally at random and producing normal brood, and from one unmated queen producing only drone brood. Altogether 375 eggs were investigated, 125 from drone cells, and 250 from worker cells of which 200 were laid by the sibling-mated queens. More than 70 000 chromosomes were counted.

Several queens which are discussed in the previous paper (Woyke, Knytel & Bergandy, 1966*b*) were artificially sibling-mated. After testing for survival rate (Woyke, 1963*a*) four queens producing brood of 50% survival rate were chosen; two (770 and 881) were of the first generation of sibling-mated and were each inseminated with semen from one drone only (1·2 and 0·9 cu. mm. respectively). The two others (882 and 885) represented the second generation of queens producing brood of low survival rate; they were inseminated with 2·5 cu. mm. of semen from several brothers. The test for survival rate was conducted on an average of more than 650 eggs of each queen.

To establish once again that the 50% of larvae (those normally eaten by workers) are drones, the eggs laid by three queens—two of the first inbred generation (770, 881) and one of the second (885)—were hatched in an incubator. The larvae were reared further in the incubator (queen 770—Woyke, 1963*b*), in queen cells in the colony (881—Woyke, 1965*d*), or in drone cells (885—Woyke, 1965*c*). The larvae of the three queens were sexed when one day old (Woyke, 1963*a*), when 4 – 5 days old (Woyke, 1963*c*), or after the cells were sealed.

To find the best stage of eggs, those of 4 – 6, 6 – 8 and 8 – 10 hours old were investigated. Since mitosis was observed in all the eggs, eggs at any age between 4 and 10 hours were collected for further investigation. Queens were covered under a queen-excluder cage on a comb for 6 hours, then the combs with eggs were put for 4 hours into an incubator at 34·5°C. and high humidity. Cytological investigations were carried out on 25 eggs laid in drone cells by each of the sibling-mated queens and the virgin queen, and on 50 eggs laid in worker cells by each of the same inbred queens and by the naturally mated one.

The eggs were fixed in Petrunkewitsch's fluid, and the sections stained in Heidenhain's iron haemotoxilin and counterstained with eosin. Chromosomes were counted, usually in 8 metaphase plates of each egg.

RESULTS

Characteristics of brood produced by the experimental queens

The queen mated naturally at random produced brood of which 94% survived. In the worker combs in the colony only compact worker brood was visible. The four instrumentally sibling-mated queens 770, 881, 882 and 885 produced brood in worker combs which survived in the colony in the proportions 43·4%; 49·4%; 52·0% and 43·4% respectively. The sex alleles were thus reduced here to two only.

Histological examination (Woyke, 1963*a*) of 22 larvae of queen 770, hatched in worker cells in an incubator and reared there for one day showed 10 males and 12 females. Rearing such larvae of queen 881 in queen cells in a colony gave 11 males and 16 females 4 – 5 days old, and rearing the larvae of queen 885 in drone cells (Woyke, 1965*c*), gave 168 sealed drones and 98 workers. Imaginal drones were reared from eggs laid in worker cells by this sibling-mated queen. The results obtained by the last two methods at the time the cells are sealed do not reflect the sex ratio in hatching larvae. Nevertheless, both examinations showed that queens producing brood of low survival rate also laid in worker cells eggs from which females as well as males developed, as in earlier experiments (Woyke, 1963*a*, *b*, 1965*a*).

The chromosomes

The chromosomes in honeybee eggs are very small, and it is difficult to count their exact number. It can only be established with great difficulty whether there are 15 or 16 chromosomes in the metaphase plate, or 31 or 32. But it is very easy to distinguish between the haploid and diploid numbers (16, 32).

Number of chromosomes in eggs from drone cells

The haploid number of chromosomes was found in all metaphase plates of 80·9% – 100% of the eggs laid by the four individual sibling-mated queens (Table 1). The total percentage of such eggs (89·4%) is very close to that for eggs laid by the unmated queen (90·5%). In 3 eggs laid by queen 881 and in 4 laid by 882 (entries 13·6% and 19·0%) 16 chromosomes were counted in some metaphase plates and 32 in others of the same egg. The total percentage of such eggs laid by all the sibling-mated queens (8·2%) is similar to that for the uninseminated queen (9·5%).

Since the diploid number of chromosomes was found only in some plates, and the uninseminated queen could not lay fertilized eggs, it may be assumed that here either early anaphases were investigated in some plates instead of the metaphases, or that polyploidization had occurred. The first alternative was confirmed by the observation of the neighbouring spindles where two separated plates of chromosomes were already visible.

Thus all the above eggs should be regarded as possessing the haploid set of chromosomes, and thus as unfertilized. This leads to totals of 97·6% for unfertilized eggs laid by the sibling-mated queens, and 100% for the uninseminated queen.

TABLE 1. Number of chromosomes in eggs laid in drone cells by an unmated and the sibling-mated queens

Sibling-mated queen no.	*No. eggs examined*	*No. eggs with mitosis*	*% eggs with* n *chromosomes*	*% eggs with* n *and* 2n *chromosomes*	*% eggs with* 2n *chromosomes*
770	25	22	95·5	0·0	4·5
881	25	22	81·9	13·6	4·5
882	25	21	80·9	19·0	0·0
885	25	20	100·0	0·0	0·0
Total sibling-mated	100	85	89·4	8·2	2·4
Unmated queen	25	21	90·5	9·5	0·0
Total	125	106	89·6	8·5	1·9

One egg laid by each of two sibling-mated queens (Table 1, entries 4·5%) was found in which many more than 16 (about 32) chromosomes were seen in all the metaphase plates. These two eggs, representing 2·4% of all those laid by the four sibling-mated queens, must be assumed to have the diploid set of chromosomes, i.e. these two eggs could be fertilized. This unexpected result may be due to the fact that the queens were artificially forced to lay the eggs in drone combs by being caged under a queen-excluder cover. In circumstances like this it may even happen that a queen 'unwilling' to lay unfertilized eggs lays fertilized eggs in *all* the drone cells, but this was not so in our experiment.

Another possibility is the union of two egg pronuclei.

We may assume that the eggs laid in drone cells by the sibling-mated queens were practically all unfertilized, with a single set of chromosomes, like those from the uninseminated queen.

Number of chromosomes in eggs from worker cells

Among the eggs laid by individual sibling-mated queens in worker cells, 0, 1, 1, 2 (giving entries 0%, 2·5%, 2·4%, 4·9% in Table 2) were found in which 16 chromosomes could be counted. In all 2·4% of these eggs were found to have the haploid set of chromosomes. In addition, in one egg laid by each of three sibling-mated queens (entries 2·3%, 2·4%), 16 chromosomes could be counted in some plates and 32 in others. In accordance with the explanation given earlier, these three eggs (1·8% of all laid by the sibling-mated queens) must be taken as unfertilized. These three eggs, together with those containing exclusively the haploid set of chromosomes, give a total of 4·2% of all eggs laid by the sibling-mated queens which must be accounted unfertilized. This percentage is far too low for the eggs to be those from which develop the 50% of the larvae which are eaten by the workers.

On the other hand no metaphase plate with 16 chromosomes was found in eggs laid by the naturally mated queen, i.e. no unfertilized eggs were found. This suggests that lack of fertilization of some of the eggs laid by the sibling-mated queens was caused by the small amount of semen used in the insemination (0·9 – 2·5 cu. mm.), and that it has nothing to do with inbreeding.

The diploid number of chromosomes was found altogether in 92·8% of the eggs laid by all the sibling-mated queens (Table 2), a percentage very similar to that for the naturally mated queen (94·7%). All these eggs were fertilized.

A few eggs were found with 32 chromosomes in some metaphase plates and 64 in others of the same egg (Table 2); these led to a relatively lower percentage of

TABLE 2. Number of chromosomes in eggs laid in worker cells by a queen mated at random, and by those sibling-mated producing 50% of drone brood (eaten by the workers)

Sibling-mated queen no.	*No. eggs examined*	*No. eggs with mitosis*	*% eggs with* n *chromosomes*	*% eggs with* n *and* 2n *chromosomes*	*% eggs with* 2n *chromosomes*	*% eggs with* 2n *and* 4n *chromosomes*
770	50	43	0·0	2·3	90·7	7·0
881	50	40	2·5	0·0	97·5	0·0
882	50	42	2·4	2·4	90·5	4·7
885	50	41	4·9	2·4	92·7	0·0
Total sibling-mated	200	166	2·4	1·8	92·8	3·0
Naturally mated queen	50	38	0·0	0·0	94·7	5·3
Total	250	204	2·0	1·5	93·1	3·4

eggs with 32 chromosomes from queens 770 and 882. Since eggs with a high chromosome number were also laid by the queen mated naturally at random, the phenomenon can have nothing to do with inbreeding. It was probably due to observation at early anaphase, or to polyploidization. At any rate the eggs were certainly not haploid and must be regarded as fertilized. Thus the queen mated naturally at random laid eggs in worker cells 100·0% of which had the diploid number of chromosomes; of the eggs laid by instrumentally sibling-mated queens in worker cells, 92·7% to 97·7% (total 95·8%) had the diploid number of chromosomes (last two columns of Table 2). We may assume that all these eggs were fertilized.

CONCLUSIONS

It is known that sibling-mated queens produce brood in worker cells of which 50% consists of drone larvae, which are eaten by the worker bees. It is shown here that eggs laid in drone cells by 4 such queens had 16 chromosomes, whereas 95.8% of their eggs laid in worker cells had 32 chromosomes, i.e. were fertilized.

Whether we regard all the eggs laid in worker cells by these queens as being fertilized, or only the observed 95.8%, the percentage is about twice as high as the 50% of larvae which survive in the hive (females). Consequently the other half of the brood (consisting of male larvae normally eaten by the worker bees) must also develop from fertilized eggs, and these drones are thus diploid in origin.

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REFERENCES

DZIERZON, J. (1945) *Eichstädter Bienenztg* 1(11) : 113

DRESCHER, W. & ROTHENBUHLER, W. C. (1963) Gynandromorph production by egg chilling. *J. Hered.* 54(5) : 195–201

—— (1964) Sex determination in the honey bee. *J. Hered.* 55(3) : 91–96

MERRIAM, R. W. & RIS, H. (1954) Size and DNA content of nuclei in various tissues of male, female, and worker honeybees. *Chromosoma* 6(6/7) : 522–538

NACHTSHEIM, H. (1913) Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica* L.) *Arch. Zellforsch.* 11(2) : 169–241

PETRUNKEWITSCH, A. (1901) Richtungskörper und ihr Schicksal im befruchteten und unbefruchteten Bienenei. *Zool. Jb. Anat. Ontog. Tiere* 14(4) : 573–605

RISLER, H. (1954) Die somatische Polyploide in der Entwicklung der Honigbiene und die Wiederherstellung der Diploidie bei den Drohnen. *Z. Zellforsch. mikrosk. Anat.* 41 : 1–78

ROTHENBUHLER, W. C., GOWEN, J. W. & PARK, O. W. (1952) Androgenesis with zygogenesis in gynandromorphic honeybees (*Apis mellifera* L.) *Science, N.Y.* 115 : 637–638

SANDERSON, A. R. & HALL, D. W. (1948) The cytology of the honey bee, *Apis mellifica* L. *Nature, Lond.* 162 : 34 only

—— (1951) Sex in the honeybee. *Endeavour* 10(37) : 33–39

WOYKE, J. (1962) Geneza powstawania niezwykłych pszczół. [The origin of unusual bees.] *Pszczel. Zesz. Nauk.* 6(2) : 49–63.

—— (1963*a*) Drone larvae from fertilized eggs of the honeybee. *J. apic. Res.* 2(1) : 19–24

—— (1963*b*) Rearing and viability of diploid drone larvae. *J. apic. Res.* 2(2) : 77–84

—— (1963*c*) Rozpoznawanie płci żywych larw pszczelich. [How to determine the sex of live honeybee larvae.] *Pszczel. Zesz. Nauk.* 7(1) : 23–31

—— (1965*a*) Study on the comparative viability of diploid and haploid larval drone honeybees. *J. apic. Res.* 4(1) : 12–16

—— (1965*b*) Genetic proof of the origin of drones from fertilized eggs of the honeybee. *J. apic. Res.* 4(1) : 7–11

—— (1965*c*) The diploid drones. *XX Int. Beekeep. Congr.* : 4 pages

—— (1965*d*) Rearing diploid drone larvae in queen cells in a colony. *J. apic. Res.* 4(3) : 143–148

WOYKE, J. & ADAMSKA, Z. (1966) Genetic evidence of biparental origin of adult honey bee drones. *Bull. Acad. pol. Sci.* V 14(1) : 73–74

WOYKE, J. & KNYTEL, A. (1966) Cytological proof of the origin of drones from fertilized eggs of the honey bee. *Bull. Acad. pol. Sci.* V 14(1) : 69–72

WOYKE, J., KNYTEL, A. & BERGANDY, K. (1966a) Cytological proof of the origin of drones from inseminated eggs of the honeybee. *Bull. Acad. pol. Sci.* V 14(1) : 65–67

—— (1966*b*) The presence of sperms in eggs as proof for the development of drones from inseminated eggs of the honeybee. *J. apic. Res.* 5(2) : 71–78