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Cytological Proof of the Origin of Drones from Fertilized Eggs of the Honey Bee

by

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After Dzierzon [1] had shown bee drones to be produced parthenogenetically, Petrunkewitsch [3] and Nachtscheim [2] demonstrated by cytological research that normally mated queens deposit in worker combs fertilized eggs, each with nuclei of 32 chromosomes, from which queen and worker bees hatch. The eggs in drone combs are not inseminated, have nuclei of 16 chromosomes, and produce drones.

Woyke [4] has demonstrated that upon sibling-mating, eggs previously regarded as lethal do hatch. Some of the queens thus mated laid in worker combs eggs that gave broods with a 50 : 50 sex ratio [5]. The drones were eaten by worker bees soon after hatching [6]. In further research, genetic [8] as well as cytologic [9] methods have demonstrated that the drone larvae were not produced without a father. The investigations, however, do not exclude androgenesis. In the present work the object was to ascertain whether the drones in point hatch from eggs not merely inseminated but actually fertilized. We sought to settle this question by determining the number of chromosomes in the eggs.

Material and method

The investigations concerned 171 eggs from four sibling-mated queens and one non-inseminated. Ninety-five of the eggs were taken from worker combs, and the rest from drone combs.

The queens were inseminated artificially according to the system brother × sister. Survival of the brood was checked by the method described earlier [4], [5]. After four queens had been selected who produced brood surviving in the colony in 50 per cent only, anatomic [7] and histologic methods [5] were applied to make certain that it was indeed drone larvae that hatched from eggs deposited in worker combs and were eaten by worker bees.

The eggs were examined when 4—10 hrs. old; they had been laid by a queen caged for six hours under a queen excluder cover on a comb which was afterwards kept for four hours in an incubator.

After the eggs had been fixed in Petrunkewitsch's fluid, cross-sections were prepared, stained with Heidenhain's iron hematoxylin, and counterstained with eosin.

Results

It may be seen from Table I that out of the 76 eggs taken from drone combs 65 contained nuclei that were in the process of dividing. In 86 per cent of these eggs the nuclei had 16 chromosomes at the most. In 12 per cent of the eggs there were nuclei with 16 chromosomes together with others having 32. Presence of both in the same egg may be explained when we assume that the nuclei with an apparently single set of chromosomes were at the time of examination not in the stage of metaphase but of anaphase, with the split chromosomes not yet distributed between the poles.

TABLE I

Number of chromosomes in eggs deposited in drone combs

Sibling-mated queen No.		Eggs examined	Eggs with dividing nuclei	Eggs with a maximum of 16 chromosomes	Eggs with 16 and 32 chromosomes	Eggs with over 16 and up to 32 chromosomes
770		10	9	8	—	1
881		21	18	15	3	—
882		20	17	14	3	—
Non-inseminated queen		25	21	19	2	—
Total	absolute	76	65	56	8	1
	percents	—	100	86	12	2

A similar situation, i.e. nuclei with what appeared to be single and double sets of chromosomes together in the same egg, also was seen in eggs from the non-inseminated queen. Thus, numerical relations are here much like those in sibling--inseminated queens.

The fact that in 98 per cent of the examined eggs single sets of chromosomes were seen in all or some of the cells proves that, as was expected, the eggs taken from drone combs had not been fertilized.

In one of the eggs, i.e. in 2 per cent of those examined, there were over 16 chromosomes, about 32, in all nuclei. This egg, therefore, rather appears to have been fertilized, and the explanation of its presence in a drone comb may be that the queen had been artificially compelled in the experiment to lay eggs in a drone comb.

It may be seen from Table II that dividingn uclei were seen in 81 out of the 95 eggs taken from worker combs. Nuclei in one egg had 16 chromosomes, and in another 16 and 32. The latter, 2 per cent of the eggs in which chromosomes could be counted, must be regarded as unfertilized.

On the other hand, exclusively nuclei with over 16 chromosomes, about 32, were found in 93 per cent of eggs from sibling-mated queens. Finally, 32 and more

chromosomes were seen in 5 per cent of the eggs. Here a similar explanation may be sought as in the case of the eggs collected from drone combs, viz., that either an early anaphase or polyploidization was in point. At any rate, in the last-named group the eggs were positively not haploid. All in all, therefore, 32 or more chromosomes were seen in 98 per cent of eggs from worker combs.

TABLE II

Number of chromosomes in eggs deposited in worker combs by sibling-mated queens producing here 50 per cent of drone brood

Queen No.		Eggs examined	Eggs with dividing nuclei	Eggs with a maximum of 16 chromosomes	Eggs with 16 and 32 chromosomes	Eggs with over 16 up to 32 chromosomes	Eggs with 32 and 64 chromosomes
770		40	34	—	—	31	3
881		31	26	1	—	25	—
882		14	13	—	1	11	1
885		10	8	—	—	8	—
Total	absolute	95	81	1	1	75	4
	percent	—	100	1	1	93	5

Thus, 98 per cent of eggs from drone combs had 16 chromosomes, and 98 per cent of eggs deposited by sibling-mated queens in worker combs had 32 or more chromosomes in nuclei. It follows that the latter were fertilized. This must be taken to mean that queens producing scattered brood laid in worker combs eggs that were virtually all fertilized.

The brood of such queens is known to be 50 per cent male [5]. It hence follows that drone larvae accounting for 50 per cent of the brood hatching from eggs laid by sibling-mated queens in worker combs come from fertilized eggs.

Details of the investigations will be described in the Journal of Apicultural Research.

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