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BIOLOGY

Anatomo-Physiological Changes in Queen-Bees Returning from Mating Flights, and the Process of Multiple Mating

by

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Controlled selection of parent couples in bees is possible only by way of artificial insemination which, to be successful, calls for knowledge of the process of natural mating.

In 1955, we presented the results of our researches on multiple mating of queens [12], [13], [14]. We advanced the hypothesis that of all the drones which mate with the queen during a mating flight, it is only the last one that leaves the bulb of endophallus in the sting chamber of the queen.

The most serious objection to this hypothesis is expressed in the question:- What would happen if that last drone failed to appear? Would the queen return without the mating sign?

Methods

Young virgin queens were kept in nuclei. Some queens were kept for a time in cages in one queenless colony for purposes of more distinct differentiation as regards age and, possibly, sexual maturity. A special device was set up at the entrance to the nucleus, enabling us to catch queens immediately upon their return from the mating flight. The caught queens were carefully examined. Some of them were released into the nuclei for further observation, others were killed, for anatomical and histological examination.

The volume of semen was measured by the method mentioned earlier [12], [14].

Results and Conclusions

The volume of semen measured in this year vacillated in GO drones from 1.0 to 3.0 c. mm., the average being 1.8 c. mm., though in most drones it was between 1.6 to 1.8 c. mm.

In the course of investigations conducted in 1955, 44 queens were fertilised. Of these, 20 were dissected; 10 of the remaining 24, after the

first mating, repeated the flight Corn 1 to 4 times, and of these 4 mated for a second time.

Considerable differences were found as between individual queens returning from a mating flight: the ovaries of the queens in question were differently developed, the oviducts were filled in varying degrees and, finally, there were differences in the contents of the sting chambers. It would thus be interesting to detect any regularities of or interdependencies between the subsequent changes.

The results of examinations of queens dissected immediately on return from a mating flight are recorded in Table I.

TABLE I

Results of anatomic investigations on queens returning from mating flights (1955)

No. of queen	Volume of oviducts filled c. mm.	Presence or absence of the bulb of endophallus	Length of largest eggs in ovary mm.	Duration of flight min.	Difference in volume of oviducts c. mm.	Age of queen days	No probable matings
1	2	3	4	5	fi	7	8
124	1.13	+	1.1	16	0.26	15	1
107	2.32	+	0.7	10	0.65	13	2
112	3.75	—	1.2	20	0.12	21	3
122	4.36	+	0.0	17	1.77	10	3
127	4.66	—	1.1	12	0.00	32	3
110	5.62	+	1.0	12	3.66	25	4
123	6.22	+	0.5	31	1.56	. 12	4
121	7.26	+	0.4	15	1,53	11	5
117	9.97	+	0.6	30	1.28	25	6
126	10,20	+	0.4	23	3,75	22	6
106	10.84	+	0.6	13	0.97	13	7
120	11036	—	1.1	24	1.31	25	7
115	12.35	—	0.5	3i	1.37	11	7
125	13.41		0.6	21	4.57	26	8
109	14.84	+	0.0	20	0.21	10	9
114	17.44	+	0.0	32	3.62	11	10
113	18.03	—	0.3	45	0.20	11	11
119	19.54	—	0.2	45	1027	14	11
111	20.76	—	0.0	30	0.95	11	12
108	22.39	—	0.0 ,	(20) + 10	0.44	7	13

It follows from Table I (column 2) that the contents of the oviducts of queens returning from mating flights increase fairly consistently in volume firom 1.13 to 22.39 c. mm.

Both the maximum 22.39 c. mm. and the mean 10.82 c. mm. volumes of sperm contained in the oviducts of returning queens several times exceed the total volume carried by single drones — 1.8 c. mm. It follows that the queens in question, listed in the table, mated with a growing number of drones Tom 1 to above 10.

The duration of the mating flight (col. 5) of queens returning with lesser quantities of sperm (up. to 11 c. mm.) is usually shorter (on the average 18 min.) than in the case of queens returning with greater quantities (above 11 c. mm. — aver. 29 min.) The duration of the flight does not, however, increase with such consistent regularity as the amount of sperm in the oviducts of mated queens. This can be readily understood since the duration of the flight is affected not only by the number of consecutive matings but also by the number of drones in the apiary, by weather, by the time of day, etc. Queen No. 108, after a mating flight lasting only 10 min., returned with the largest quantity of sperm 22.39 c. mm. but she took flight twice on the same day at an interval of 10 min. and it is therefore reasonable to suppose that she mated during both the first and the second flights.

Where a queen has scantily filled oviducts on her return from a mating flight, differences in volume between the contents of the two oviducts (col. 6) are relatively small. This difference increases in the case of queens returning with better filled oviducts, and declines again for queens with the most amply filled oviducts.

The greatest difference established thus far was 4.57 c. mm., that is to say, it was in excess of the total volume of sperm from two drones. The difference increases and diminishes, however, without ascertainable regularity. A certain correlation here existing is not very consistent.

A closer interdependence may be found to exist between the volume of the contents of the two oviducts and the development of the ovaries. It can be seen from Table I, col. 4, that, with increasing size of eggs in the ovaries, the volume of sperm contained in the oviducts shows a tendency to decline. In addition to anatomical changes, there are physiological changes which also constitute a factor determining the volume of the contents of oviducts.

It is significant, on the other hand, that no close dependence has been recorded as between the development of eggs in the ovaries and the age of the queen at the moment of fertilisation (col. 7).

Some queens have well developed eggs in their ovaries (Fig. 5) and can, therefore, begin to lay immediately after fertilisation. But complete maturity of the eggs in the ovaries of unfertilised queens may, after their insemination, cause some queens to lay first unfertilised eggs.

Particular attention was devoted to the contents of the sting chamber in queens returning from mating flights. The results of these observations are recorded in Table II.

It follows from Table II that the majority of queens returned carrying a drone s bulb of endophallus in the sting chamber. The tip of the abdomen of such a queen is shown in Fig. 1. Almost 30 per cent of queens, however, returned with nothing more than a mass of mucus (Fig. 2.), without the bulb of endophallus. Finally, there were 5 queens revealing

no signs of mating on return from the flight. It was not until they began to lay that they were found to have been fertilised.

Table I, col. 3, illustrates a peculiarly interesting fact. The majority of queens returning from a mating flight with small quantities (1 to 11 c. mm.) of sperm carried a drone s bulb of endophallus in the sting chamber (Fig. 3). On the other hand, queens returning with a large volume (11 to 22.4 c. mm.) of sperm, returned in most cases without the bulb (Fig. 4). This is all the more interesting because reasons can be cited

TABLE II

Contents of sting chamber of queens returning from mating flights

	number	percentage
Queens mated	44	100.0
returned with bulb of endophallus	26	59.1
returned with mucus without bulb of endophallus	13	29.5
returned with no signs revealed	5	11.4

for the different behaviour of certain queens from that of the majority. The most marked divergencies from the rules referred to above were revealed in queens Nos. 112 and 127, which despite the very small volume of sperm contained in their oviducts, returned without a bulb of endophallus. Queen No. 112 had the best developed eggs and most markedly developed ovaries (Fig. 5), in fact the greatest we have ever encountered in non-laying queens. The other queen, No. 127, not only had well developed eggs but was also the oldest (32 days) to be mated.

Whether the above mentioned 11 c. mm. volume constitutes the ultimate limit separating queens returning from mating flights with a bulb of endophallus from those returning without, is as yet too early to say. It is, however, symptomatic that this limit approaches very closely the mean volume — 10.82 c. mm. — of sperm contained in the oviducts of all the queens in question.

Table III illustrates the presence or absence of a drone s bulb of endophallus in the sting chamber of 4 queens mated in the course of two different flights.

TABLE III

Data relating to queens mated in the course of two different mating flights

Queen No.	Bulb of endophallus		Number of flights from the 1-st to the 2-nd mating flight	Number of days from the 1-st to the 2-nd mating flight
	1-st mating flight	2-nd mating flight		
126	+	+	1	1
157	+		3	1
105	+		3	3
79			4	3

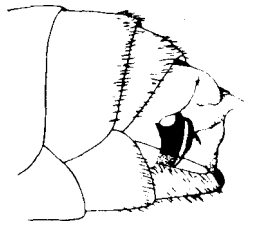

Fig. 1. Distal end of abdomen of a queen returned from a mating flight. Drone s bulb of endophallus lodged in sting chamber

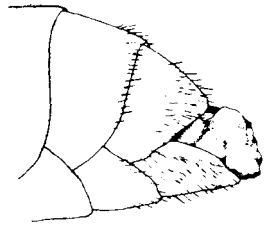

Fig. 2. Distal end of abdomen of a queen returned from a mating flight with mass of mucus but without drone s bulb of endophallus

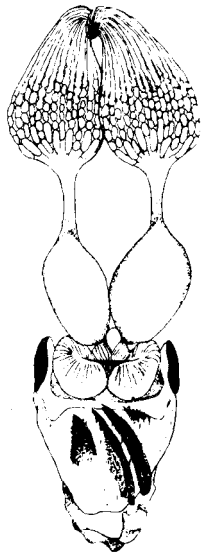

Fig. 3. Reproductive organs of a queen returned from a mating flight with drone s bulb of endophallus lodged in sting chamber (ventral view)

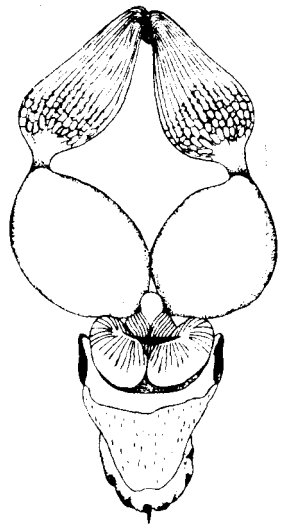

Fig. 4. Reproductive organs of a queen returned from a mating flight with mass of mucus only in the sting chamber (ventral view)

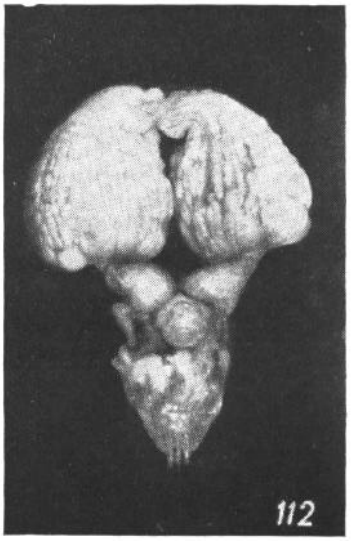

Fig. 5

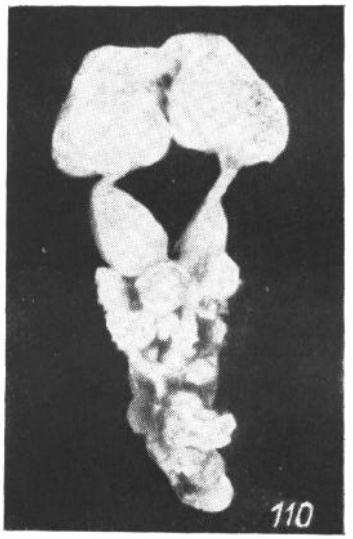

Fig. 6

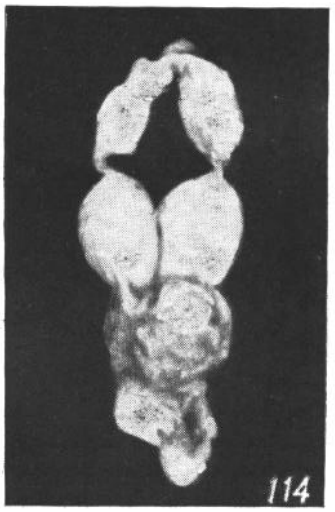

Fig. 7

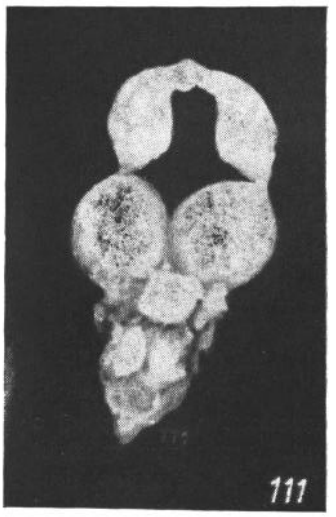

Fig. 8

Figs. 3-8. Reproductive organs of mated queens Nos. 112, 110, 114, 111 (dorsal view)

It can be seen from Table III that three queens returned from the first mating flight carrying a drone s bulb of endophallus, and one without. After the second flight the proportion was reversed; one queen returned with a bulb and three without. It is likely that subsequent flights were undertaken by queens which carried a lesser amount of semen, and the *receptaculum seminis* of which was not completely filled on return from the previous flight.

The question now arises:- Did the queens returning from a mating flight without a bulb diminish, owing to fatigue following several matings, the probable pressure of the distal plates of the abdomen, and of the sting, or was it rather the increased volume of well filled oviducts and other parts of the reproductive organs that caused the queens to return without the bulb of endophallus?

Examination of queen No. 112 gave findings somewhat favourable to the second view. The queen returned to the hive without the bulb and with little sperm in the oviducts; the ovaries, however, were extremely well developed (Fig. 5). It is thus likely that, in such conditions, the queen had a freedom of movement analogous to that of a queen with well-filled oviducts (Fig. 8), and, consequently, also behaved similarly.

The latter explanation is supported also by the results of examination of queens Nos. 109 and 114, both of which returned with a bulb of endophallus despite relatively well filled oviducts (Fig. 7). The ovaries of these queens, however, were only slightly developed and, as can be seen from Table I, column 4, contained completely immature eggs. It was very likely that these queens would have a freedom of movement similar to that of queens with poorly filled oviducts (Fig. 6).

Thus, an explanation of the mating process should not be sought in a purely schematic mechanism. Active participation of the queen in the whole process must be assumed. On the other hand, anatomical changes seriously affect the behaviour of the queen during the mating flight.

It seems that the results referred to above, together provide a sufficient basis for supplementing further details to the hypothesis advanced earlier [13],[14], relating to the progress of the mating process.

The bulb of endophallus is introduced into the sting chamber of the queen during the mating process. The queen compresses the drone s copulatory organ with the terminal plates of her abdomen and with her sting. If the pressure exerted by the queen is slight, ejaculation of the sperm is automatically followed by extrusion of the male copulatory organ from the sting chamber, and the queen then mates with other drones. If the pressure is stronger, the bulb of endophallus is severed and can be removed by the queen and/or the drone in the course of further mating. When, however, the bulb of endophallus is firmly lodged, the queen, incapable of further mating, returns to the hive. As the volume of sperm contained in the oviducts increases, contraction of the sting

chamber becomes increasingly difficult. The result is that, in the case of queens carrying larger amounts of semen, the bulb of endophallus is not severed or is easily lost. Therefore, queens carrying greater quantities of sperm in their oviducts tend to return from the mating flights without a drone s bulb of endophallus.

It can thus be seen that queens return from mating flights for two main reasons:

1) a drone's bulb of endophallus is lodged in the sting chamber of the queen and the queen returns irrespective of the volume of sperm contained in the oviducts;

2) the oviducts have been largely filled with sperm and the queen returns irrespective of the presence or absence of the bulb of endophallus in the sting chamber.

As regards practical apiculture, the results described in this and the earlier papers provide a basis for a conclusion differing from view> hitherto prevailing. To give artificial insemination of the queen the maximum resemblance to natural fertilisation, it is not sufficient to inject her with either two 2.5 c. mm. doses of sperm, as was practised till 1953, or a single 5 c. mm. dose [3]. Much larger single doses — up to and even above 20 c. mm. — must be used, and the operation repeated several times.

Discussion

Although the queen, and/or the drone, may remove the bulb of endophallus from the sting chamber in flight, and the differences in volume of semen contained in the oviducts of mated queens may be explained by various degrees of development of the queen s eggs and ovaries, we nevertheless assume that not every drone leaves the bulb of endophallus in the sting chamber to be subsequently removed by the queen and/or the next drone. If removal of the bulb in mid-air were the rule, then there would be more queens returning with well filled oviducts, since the observations were carried out in ordinary apiaries where drones sbounded. Also a closer interrelation would be noticeable between the development of the ovaries and the volume of semen they contain. Besides, it should be (precisely the queens carrying larger amounts of semen in the oviducts, i. e. those not wanting to mate any longer, which should return with a bulb of endophallus. It is difficult to assume that as many as 40 per cent of queens easily loose the bulb of endophallus in mid-air, while others have difficulties in removing it in the hive.

The results referred to above will be published in "Pszczelarstwo" [15], [16] and a full account of this work — in Folia Biologica .

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