NATURAL AND ARTIFICIAL INSEMINATION OF QUEEN HONEYBEES

JERZY WOYKE

Dr. Jerzy Woyke has recently published a detailed account of some of his work in Pszczel. Zes. Nauk. 4(3/4) : 183–275 (1960), *in Polish. The results he obtained are of great interest to queen breeders and to all other beekeepers concerned with the mating of their queens, and include the following :*

On more than half the mating flights the queens mated with between 7 and 10 drones.

The less semen a queen received, the more the eggs in her ovaries developed.

The queens which went out on a second mating flight were those which received the least semen on the first flight.

The longer the interval between two flights on the same day, the shorter the second flight, and the less likely the queen was to mate effectively on it.

Mating flights from an apiary with plenty of drones were in no way more effective than those from another apiary where no drones were supplied, and with no other colonies within 2·5 km. [1½ miles].

In instrumental insemination, no advantage was found in using a double dose, unless the volume of semen given in each dose was 6 cu. mm. or more.

The following account summarizes most of the numerical results. Tables and diagrams in the original paper have English headings as well as Polish, so those interested in following up any of the detailed results can do so.

Dr. Woyke is one of the youngest of the Polish bee research workers, and he has specialized in studying mating behaviour in honeybees; many of his other papers have already been abstracted [A.A. 73, 236/56; 16, 21/57; 285, 387/58]. *He also collaborated with Dr. F. Ruttner in writing a paper for* Bee World *on their anatomical study of the mating process in the honeybee. Dr. Woyke has spent two years in America, continuing his research there in the U.S.D.A. and other laboratories.*

The investigations described here were made in 1952–60, those on artificial insemination being carried out in the U.S.A. in 1958–59. A total of about 1300 queens were examined, and over 1000 were put out in mating apiaries and their mating flights studied. About 800 of the queens flew, and they made between them nearly 3000 flights.

SEMEN PRODUCED BY THE DRONES

Direct counts were made of 464 thousand spermatozoa; they showed that the sperm concentration in the oviducts of queens returned from mating flights is the same as in the semen of a drone's ejaculate (about 7·5 million per cu. mm.). The results were 7·312 ± 0·171 for the oviducts of 28 queens and 7·478 ± 0·127 for 30 samples of drone ejaculate, both

measured in millions of sperm per cu. mm. of semen*. In determining these data, a syringe for artificial insemination was used for measuring semen volume. After each use, the tip of the syringe was rinsed to recover the residue adhering to the inner walls. (In artificial insemination this residue always remains in the syringe, and queens therefore receive only about 7·0 million sperm from each cu. mm.)

Drones usually yielded 1·5 - 1·75 cu. mm. semen; an average of 1·7 cu. mm. was obtained for a total of 78. But it is not possible to get more than 1 – 1·25 cu. mm. semen into the tip of the syringe from one drone, or exceptionally up to 1·5 cu. mm. The amount of mucus and semen together ejaculated by one drone averaged 7·3 cu. mm., and one drone had on the average about 11 million spermatozoa.

SEMEN RECEIVED BY THE QUEEN

The oviducts of queens returning from mating flights contained virtually only semen, its volume ranging from 0·600 to 28·234 cu. mm. for 123 individuals — an average of 11·579 ± 0·498 cu. mm. The highest volume (28·234 cu. mm.) was 17 times the average volume of semen produced by one drone, so the queen concerned must have mated during one flight with up to 17 drones. Taking into account the volume of semen found in the other parts of the genital tract of queens, it can be assumed that the queens mated with an average of 8–9 drones in one mating flight, and that more than half of them mated with 7, 8, 9 or 10 drones. The oviducts of returning queens contained an average of 87 million sperm, and the entire genital tract about 100 million. Some queens returned with even 200 million.

It was found that the oviducts of naturally inseminated queens were emptied of semen within 10 to 20 hours of mating. Queens whose oviducts were emptied after less, or little more, than 10 hours usually had fewer sperm in the spermatheca than queens whose oviducts were still filled 10 hours after mating. On the other hand queens whose oviducts took longest to empty had by no means the most sperm in the spermatheca.

HOW THIS IS AFFECTED BY THE QUEEN'S EGGS

The length of the most highly developed eggs in the ovaries was found to be correlated with the volume of semen received by a queen. For 84 queens in the mating period it ranged from virtually 0 mm. up to 1·2 mm., the average being 0·4 mm., and the commonest size 0·1 mm. The correlation coefficient was –0·6 and was highly significant statistically: the smaller the volume of semen brought home by the queen, the larger the eggs in her ovaries. The largest eggs were 0·1 mm. shorter on the average for each cu. mm. more semen in the oviducts.

WHAT DECIDES THE NUMBER OF MATING FLIGHTS ?

Of 303 queens which had made one mating flight, 63% *flew* again, and 38% *mated* on a further flight; 8% of the queens flew yet again, 6%

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* The figure after ± is the 'standard error' of the average which precedes it. Two averages are *significantly* different only if they differ by more than several times the standard error. Here the difference (7·478–7·312) is only 0·166, so the averages are effectively the same.

on a third mating flight. Measurements showed that queens returned from the second mating flight with the same average volume of semen as from the first.

The sperm in the spermathecae of queens were counted in 1956–59. The 1958 averages were lower because of nosema disease, and are omitted from the totals given here. The 1956, 1957 and 1959 results concern 201 queens, of which 24 were killed immediately after the first mating flight, 25 immediately after the second, and 102 after egg laying had started. The average number of sperm in the spermathecae of the 102 queens which had started to lay was $5 \cdot 340 \pm 0 \cdot 121$ million. Of these, the 75 queens which mated on one flight only contained $5 \cdot 057 \pm 0 \cdot 133$ million; the 23 which mated on two flights contained $5 \cdot 979 \pm 0 \cdot 218$ million, and the 4 inseminated in three flights contained $6 \cdot 975 \pm 0 \cdot 499$ million.

Of the queens which had *less* that 3 million sperm in the spermatheca after the first mating flight, 86% mated a second or even third time. Of those which had more than 3 million sperm after the first mating flight, only 31% mated again. (The sperm content after the *first* mating flight was obtained by killing the queens immediately after their return from the *second* flight, before the semen from that mating had entered the spermatheca.) Those queens examined which mated on a second flight had an average of $3 \cdot 462 \pm 0 \cdot 303$ million sperm in the spermatheca from the first mating flight; queens which flew again but did not remate contained $4 \cdot 628 \pm 0 \cdot 224$ million, compared with $5 \cdot 284 \pm 0 \cdot 158$ million for the queens which did not fly again after their single mating flight.

All these data are evidence that the degree of insemination determines a queen's subsequent behaviour: whether she flies again, and whether she mates again on later flight.

WHEN ARE MULTIPLE MATING FLIGHTS MADE ?

Of 1327 observed flights, only 23 (1·7% of the total) were made after an earlier mating flight on the same day. Only 8·7% of the mated queens flew again on the same day, but most of them mated again on the subsequent flight. The first mating flight of these queens was rather short (average 21 min.), and the queens brought home an average of 10 cu. mm. of semen. Their oviducts were not emptied of semen when they set out for the second flight, and contained on examination 2·36 – 14·66 cu. mm. (average 9·08 cu. mm.) semen. The spermatheca already contained an average of 0·803 million sperm, so copulation on the first mating flight, and transfer of the semen into the spermatheca, were normal. Mated queens occasionally made two more mating flights on another day, but this was only when the first insemination was very slight (only about 1 million sperm in the spermatheca). It could also happen that a queen which made two mating flights on the same day made a further flight on another day and became inseminated for the third time. One such queen which had 4·110 million sperm in the spermatheca from two inseminations on the previous day, brought a further 5·71 cu. mm. from the third flight.

Successful second mating flights on the same day averaged 20 min.— as much as the first. On returning from the second mating flight, the queens had in the oviducts an average of 15·99 cu. mm. of semen, (6·91 cu. mm. more than after the first). So during the two flights the queens

were inseminated by an average of ten drones : six on the first, and four on the second flight.

The interval between the end of the first mating flight and the beginning of the next flight varied from 19 minutes to 2 hours, and averaged 52 minutes. The interval between two successful *mating flights* (average 47 min.) was however shorter than the interval between the first mating flight and an *unsuccessful* later flight (average 77 min.).

A clear correlation was found between the interval between the two flights and the queen's subsequent history. The longer this interval, the shorter the second flight (correlation coefficient −0·71), the smaller the total volume of semen brought home, and the smaller the number of sperm which reached the spermatheca. On the average, the second flight became 2 minutes shorter for every 10 minutes added to the interval (regression coefficient – 12 sec.); when this interval exceeded an hour the second flight was so short (average 10·5 min.) that no second insemination took place.

EFFECT ON MATINGS OF THE NUMBER OF DRONES IN THE APIARY

In 1956, at a mating station (A) with no other colonies within a radius of 2·5 km. and with no drones supplied, a total of 100 queens were released in three replications. Another 300 queens were released in a normal apiary (B). About the same number of queens flew (in all three replications) in both apiaries: 86 in A and 81 in B. At A 73 actually mated, but only 50 in B. The queens averaged 2·9 pre-mating flights at A, and 2·6 at B, but the most common number of pre-mating flights was 3 at A and only 1 at B. The queens at A mated earlier than those at B. The average duration of the mating flights was between 25 and 26 min. at both A and B, the most common duration being rather less (15–20 min.) at A than at B (20–25 min.).

Queens at A returned from a mating flight with an average of 13·8 cu. mm. semen in the oviducts, compared with only 10·8 cu. mm. at B. We can deduce from this the queens in the ' droneless ' apiary mated on one flight with an average of 8 drones, compared with 7 in the normal apiary, where there was an excess of drones. The highest volumes of semen (25·7 and 28·3 cu. mm.) were recorded from A, and these queens must have mated with 15 or even 17 drones in one flight. The queens examined received on the average 0·47 cu. mm. of semen per minute of flight at B, and as much as 0·66 cu. mm. at A. Copulation with one drone took an average of 3·3 min. at B and only 2·6 min. at A: the flights of the queens were thus more effective at the ' droneless ' mating station than in the apiary. When egg laying started, the queens at B had an average of 4·757 million sperm in the spermatheca, and those at A 5·215 million.

Thus an abundance of drones in close proximity to the queens did *not* increase the amount of semen they received; the queens at A presumably mated with drones from further away.

INSTRUMENTAL INSEMINATION

Seventy queens were inseminated instrumentally. Single inseminations were made with 1, 2, 3, 4, 6, 8, 10, 12, 16 and 20 cu. mm. semen, and double inseminations with 3, 4, 6 and 8 cu. mm. each. With a single insemination, as the dose was increased from 1 to 20 cu. mm., the average

number of sperm reaching the spermatheca increased correspondingly, from 1·390 million to 5·845 million. The percentage of the sperm which penetrated from the semen into the spermatheca decreased from 20% for 1 cu. mm. to 10% for 6 and 4% for 20 cu. mm. A 2-, 3- and 4-fold increase in the number of sperm in the spermatheca thus required a 3-, 6- and 12-fold increase of the single dose. Increasing the dose to 8–10 cu. mm. rapidly increased the number of sperm in the spermatheca, but a further increase up to 20 cu. mm. was substantially ineffective.

Results of single and double insemination with the same total volume of semen were compared. With a single insemination, an increase of the dosage from 6 to 16 cu. mm. increased the number of sperm in the spermatheca from 4·1 to 5·8 million, compared with an increase from 4·3 to 7·4 million with a double insemination in which the same total volume was given. With small volumes of semen, up to 8–10 cu. mm., there was no statistically significant difference whether they were given in one or two doses, but the difference increased as the total dosage increased.

Reinsemination was usually less effective than the first; for instance, insemination with 3 cu. mm. gave 2·8 million spermatozoa in the spermatheca, and reinsemination with the same volume added only 1·5 million; insemination with 8 cu. mm. gave 5·4 million, and reinsemination added only 2·0 million. The more intensive the first insemination, the less effective the second. Nevertheless, reinsemination was more effective than increasing the dosage in a single insemination. For instance, doubling of the 3, 4, 6 or 8 cu. mm. total dose increased the number of sperm in the spermatheca (in single and double insemination respectively) by 1·3 and 1·5, 1·7 and 1·9, 1·5 and 2·1, and 0·4 and 2·0 million. This superiority of reinsemination over a simple increase of single-insemination dosage was especially pronounced with larger doses, and commenced with the doubling of the 6 cu. mm. dose. For instance, two inseminations at 6 cu. mm. each gave even better results than one insemination with 20 cu. mm. Double insemination with small doses (less than 4 cu. mm. each, total volume 8 cu. mm.) is thus virtually useless, since much the same effect may may be obtained by increasing the dose in a single insemination. There is certainly nothing to recommend the common practice of inseminating queens with two consecutive 2·5 cu. mm. doses, for the same result may be obtained with one insemination with 5 cu. mm. semen. On the other hand, there is no reason to inseminate queens with a single dose larger than 8–10 cu. mm., since better results may be obtained with two inseminations.

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