CAUSES OF REPEATED MATING FLIGHTS BY QUEEN HONEYBEES

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SUMMARY

Observations were made on 2434 flights of 628 queens. Of 303 once-inseminated queens left alive for further observations, 63% flew again and 38% mated on a second flight; 8% of the queens flew yet again, and 6% were inseminated a third time. Sperms in the spermatheca were counted in 207 queens returning from a mating flight or departing on the next one.

The average number of sperms in the spermatheca of *all* queens that started to lay was 5·340 million; of those that mated on one flight only 5·057 million; of those mated on two and three flights 5·979 and 6·975 million respectively.

The average number of sperms from the *first* mating flight, in the spermatheca of queens mating on a *second* flight, was 3·462 million; for queens not *mating* again it was 4·628 million, and for queens not even *flying* again, 5·248 million. It is concluded that the amount of sperm from the first mating flight influences the likelihood of a second flight and successive matings.

INTRODUCTION

Queen honeybees (*Apis mellifera*) usually make one or more flights before an actual mating flight. Recent investigations have shown the queen mates with several drones in one flight (Tryasko, 1951, 1956; Taber, 1954, 1958; Woyke, 1955, 1956; Peer, 1956). In the act, the semen enters into the oviducts, and after the queen returns to the hive a small part of it passes into the spermatheca, the rest being ejected. A queen that has mated with one or more drones on one flight is referred to in this paper as once-inseminated; one that has mated on two mating flights to as twice-inseminated, and so on. Roughly half the queens studied by Roberts (1944), F. & S. Ruttner (1953/54), and Alber, Jordan & F. & H. Ruttner (1956) were found to leave the hive and mate again on another flight, when mating also occurs with several drones (Woyke, 1960).

Queens may mate on a single flight with widely different numbers of males; Woyke (1960) reported 1 to 17. Hence the question arises whether queens mated on two or three flights have more sperms in the spermatheca than once-inseminated queens.

In the course of studies on multiple mating of queens it was noted that some of the once-inseminated queens did not leave for another mating flight, but presently began oviposition; others left the hive again but did not mate; still others left to mate on second, third, and even fourth or fifth mating flights.

The object of the present work was to discover the causes of further flights and inseminations.

MATERIAL AND METHODS

The investigations extended over four years. Each queen was kept in a modified Zander nucleus. A small box with a movable queen excluder, fixed in front of the entrance, made it possible to observe the queen's exit and return. The total number of

queens under observations was 791; 628 made flights and 469 became inseminated. In all, 2434 flights were observed, and 590 of these were mating flights. Sperms in the spermatheca were counted in 207 queens, of which 24 were killed immediately on return from the first mating flight. For these queens the count represented the sperms that entered the spermatheca in the interval between mating and the removal of the spermatheca (roughly 10–15 minutes after the queen's return). Thirty queens were killed immediately after they had returned from a second successful mating flight. The semen from the second flight was in the oviducts of these queens, and that from the first in their spermatheca. Thus it could be established how many sperms from the first mating flight were in the spermatheca of queens reinseminated on the second flight. Finally, 153 queens were killed after they had begun laying.

The spermatheca of a queen was broken in a drop of Ringer's solution, the sperms were well dispersed, and tap water was added to make 10 c.c. The sperms in a total volume of 2 cu.mm. of liquid were counted simultaneously in two Fuchs-Rosenthal counting chambers. Direct counts were made of over 230 thousand sperms in all.* The significance of differences between group-averages was calculated by Fisher's analysis of variance.

RESULTS

Repeated mating flights

Of the 303 once-inseminated queens not killed on their return, 63% left for another flight, but only about two-thirds of them (38% of the 303) were reinseminated.

Of the twice-inseminated queens only 21% left on yet another flight; and 15%—again roughly two-thirds—became inseminated a third time. Using once-inseminated queens as a basis, the results are as follows: 63% of once-inseminated queens left the hive again, but only 38% became reinseminated; 8% made still further flights (having been inseminated twice), and 6% were inseminated a third time.

Number of sperms in spermatheca of laying queens, mated on one, two or three mating flights

Considering the data on queens that were inseminated a different number of times, and then killed immediately on commencement of laying, it will be shown that in the 153 (102)* queens now considered, the number of sperms in the spermatheca varied within the range 0·693–8·350 million, and averaged 5·340±0·121 (5·048)* million.

The distribution of the values according to the number of inseminations was as follows:

75 (112)* once-inseminated queens averaged 5·057±0·133 (4·721) million sperms in the spermatheca;

23 (36) twice-inseminated queens averaged 5·979±0·218 (5·834) million;

4 (5) thrice-inseminated queens averaged 6·975±0·499 (6·718) million.

Hence, twice- and thrice-inseminated queens had respectively one and two million sperms more than had once-inseminated queens. The average for thrice-inseminated queens was calculated from a very small number of queens and its standard error is therefore larger.

*In 1958, nosema disease depressed average counts, and results in which 1958 data are included are therefore given in parentheses.

TABLE 1. Average number of sperm in a queen's spermatheca

Year	*Queens with sperm in the spermatheca from 1st mating flight only, that mated again*		*Queens mated on one flight, that flew out (but did not mate) again*		*Queens mated on one flight, that did not fly out again*		*Queens mated on two flights*		*Queens mated on three flights*		*Total no. queens*
	Group A 1×+ (1×)		*Group B* 1×+		*Group C* 1×		*Group D* 2×		*Group E* 3×		
	No. queens	*No. sperm (million)*	*No. queens*	*No. sperm (million)*	*No. queens*	*No. sperm (million)*	*No. queens*	*No. sperm (million)*	*No. queens*	*No. sperm (million)*	
1956	—	—	9	4·368	12	5·203	8	5·408	—	—	29
1957	16	3·882	10	4·775	28	5·407	11	6·505	—	—	65
1958	5	3·277	11	3·897	26	4·100	13	5·575	1	5·690	56
1959	9	2·715	7	4·751	9	5·012	4	5·677	4	6·975	33
1956–1959	30	3·431 ± 0·310	37	4·410 ± 0·194	75	4·874 ± 0·150	36	5·834 ± 0·200	5	6·718 ± 0·464	183
1956,1957,1959	25	3·462 ± 0·303	26	4·628 ± 0·224	49	5·284 ± 0·158	23	5·979 ± 0·218	4	6·975 ± 0·499	127

Number of sperms in the spermatheca of queens behaving differently after insemination

At this point it is necessary to ask how many sperms twice-inseminated queens received from the first mating flight, and whether in once-inseminated queens there was a difference between the number in queens that began laying immediately and in those that left for further flights.

In 24 queens captured immediately on return from the first mating flight, the number of sperms in the spermatheca averaged 137 thousand. This figure is so small that it lies within the range of the standard error in the other groups of queens. In other groups we may therefore ignore any minor changes in the number of sperms in the spermatheca which may have taken place after the last mating but before the queens were killed (immediately they returned from the mating flight.)

The average number of sperms in the 183 queens of the other groups are given in Table 1, and the frequency distributions are shown in Fig. 1.

Twice inseminated queens, killed on return from the second mating flight (Group A), received from 0·287 to 5·825 million sperms from the first flight. This is a considerable range, and Fig. 1A shows that the class containing the largest number of queens is that containing not the average, but the *maximum* number. The 1957 data (+ in Fig. 1A) differ from those of other years; if they are ignored, the largest class is the more central one, queens with 2–3 million sperms. Twice-inseminated queens received on the average 3·462 (3·431)* sperms in the spermatheca (Table 1) from the first mating flight, about 2 million less than the average for all laying queens (5·340 million).

Once-inseminated queens were divided into two groups according to whether they did (B) or did not (C) leave for still other flights before beginning to lay. Once-inseminated queens that left for other *unsuccessful* flights (Table 1, Fig. 1B) received 0·695–6·960 million sperms in the spermatheca, average 4·628 (4·410) million, roughly one million more than the twice-inseminated queens had from the first mating flight.

On the other hand, once-inseminated queens that made no further flights (Group C) had 1·670–7·245 million sperms, average 5·284 (4·874) million, an average of 0·656 million more than had once-inseminated queens that made subsequent (unsuccessful) flights before laying, and 1·822 million more than the twice-inseminated queens received from the first mating flight.

The distribution of once-inseminated queens that made no further flights (Fig. 1C) has some binomial characteristics: the classes above the most numerous one contain only 25% of the total, and those below as many as 45%.

It is reasonable to suppose that other factors besides a large number of sperms in the spermatheca (and possibly a consequent subsidence of the sexual urge) may prevent the queen leaving the hive again. These factors include bad weather, a weak or diseased colony, robbing, and shortage of food. In 1958—when colonies were infected with nosema disease—most of the queens (in Fig. 1C) fell within the classes below the most numerous for all four years.

At the commencement of laying, the twice-inseminated queens (D) (Table 1, Fig. 1D) had from 2·535 million (or—ignoring two of them—from 4 million) to 7·915 million sperms, average 5·979 (5·834)* million. This was 695 (960) thousand more than the average for once-inseminated queens that made no further flights.

*See footnote on page 18

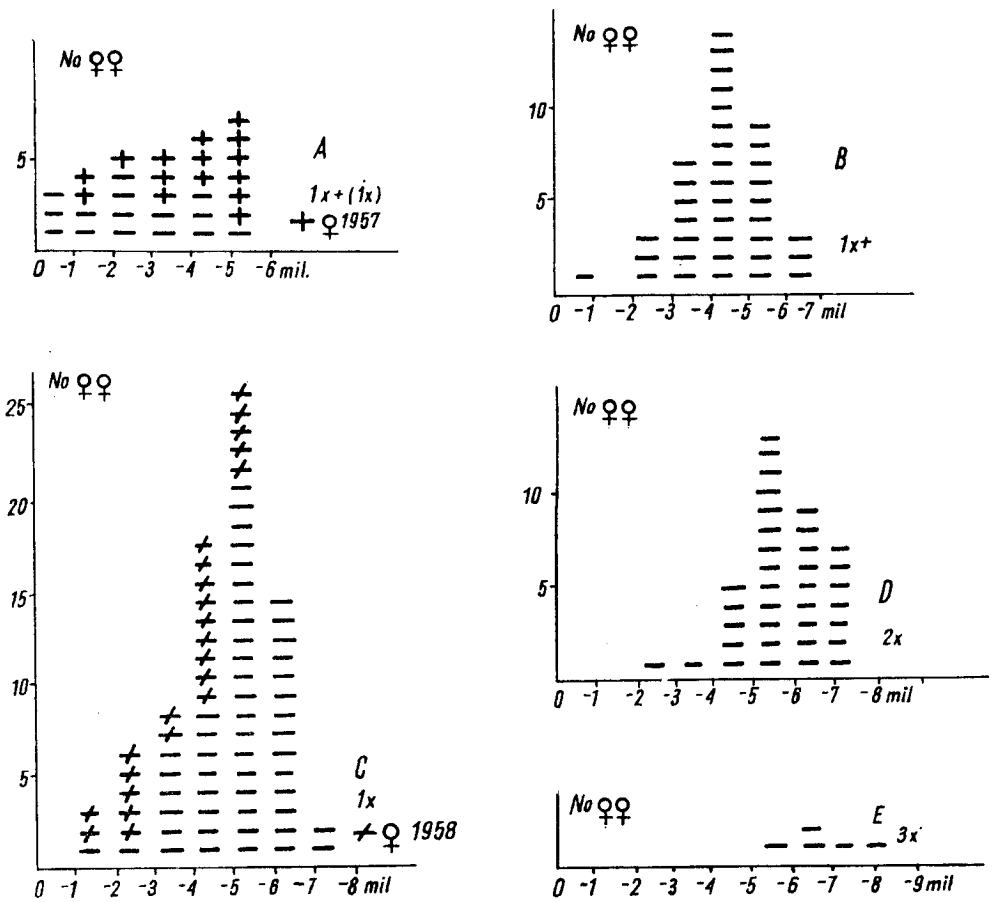

Fig. 1. Number of sperms in the spermatheca of individual queens of different groups :

A. Queens killed at the moment of returning from the second mating flight, whose spermatheca contained only sperms from the first mating flight.
B. Queens mated on one mating flight that flew, but did not mate again.
C. Queens mated on one mating flight, that did not fly again.
D. Queens mated on two mating flights.
E. Queens mated on three mating flights.

The most numerous class for twice-inseminated queens (D) is 5–6 million sperms, as for the once-inseminated (C) that remained in the hive afterwards (Fig. 1). Many of the twice-inseminated queens had increased their initial subnormal store of sperms on the second mating flight, to the level found in queens well inseminated on the first flight—queens that made no further attempts to mate. However, as many as 44% of the twice-inseminated queens ranked above their most numerous class; Fig. 1D shows clearly that larger numbers of sperms are more frequent in twice- than in once-inseminated queens.

The 5 thrice-inseminated queens (E) had from 5·690 million to 8·350 million sperms, average 6·975 (6·718)* million, (Table 1, Fig. 1E), roughly one million more than in the twice-inseminated queens.

The differences between the averages of the various groups were shown by Fisher's analysis of variance to be statistically significant, except that between twice- and thrice-inseminated queens. There would probably have been no exception if more thrice-inseminated queens had been available.

If averages of sperm numbers are rounded to 0·5 million, we can say that twice-inseminated queens had an average of 3·5 million from the first mating flight. Once-inseminated queens that made unsuccessful later flights averaged 4·5 million, roughly 1 million more. Once-inseminated queens that began laying without further flights had 5·5 million, yet another million more. Twice-inseminated queens averaged 6 million from the two flights together, a gain of only 0·5 million, and thrice-inseminated queens averaged 7 million sperms from the three flights together.

Behaviour of queens in relation to the number of sperms in the spermatheca after the first insemination

In further estimations, it was assumed that queens twice and thrice inseminated and not killed until they began to lay (Fig. 1D, E) did not differ from twice-inseminated queens killed on return from the second flight, with respect to their distribution according to the number of sperms they received from the first flight. The number of sperms these twice- and thrice-inseminated queens were likely to have received from the first mating flight could thus be calculated. The number of sperms entering the spermatheca after the first mating flight was therefore known with reasonable accuracy for all the queens in Table 1.

In the following final conclusions, 1958 data are ignored, because of the prevalence of disease that year. Of the remaining 127 queens (Table 1), 22 had under 3 million and 105 over 3 million sperms after the first mating flight. Of the former, 19 (86·4%) were reinseminated, whereas of the latter 33 (only 31·4%) mated again. Hence it may be concluded that queens whose spermathecae receive less than 3 million sperms from the first mating flight are usually reinseminated, whereas the majority of others are not.

CONCLUSIONS AND DISCUSSION

All the data discussed here bear out the idea that a queen's behaviour is related to the number of sperms in her spermatheca. This number is itself dependent on the amount

*see footnote on page 18

of semen in her oviducts, and it is not clear which of these two factors is the more directly responsible for the queen's behaviour and the onset of laying.

However, the data presented here show that the degree of insemination affects the queen's subsequent behaviour and beginning of laying, and that poor insemination leads to further flights and reinsemination, if conditions allow it.

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REFERENCES

ALBER, M., JORDAN, R., RUTTNER, F. & RUTTNER, H. (1955) Von der Paarung der Honigbiene. *Z. Bienenforsch.* 3(1) : 1–28

PEER, D. F. (1956) Multiple mating of queen honey bees. *J. econ. Ent.* 49(6) : 741–743

ROBERTS, W. C. (1944) Multiple mating of queen bees proved by progeny and flight tests. *Glean. Bee Cult.* 72(6) : 255–259, 303

RUTTNER, S. & RUTTNER, F. (1953/54) Ueber die Paarung der Bienenkönigin. *Oest. Imker* 3(12) : 206–211; 4 : 3–6, 27–30

TABER, S., III (1954) The frequency of multiple mating of queen honey bees. *J. econ. Ent.* 47(6) : 995–998

TABER, S., III & WENDEL, J. (1958) Concerning the number of times queen bees mate. *J. econ. Ent.* 51(6) : 786–789

TRYASKO, V. V. (1951) [Signs indicating the mating of queens.] *Pchelovodstvo* 28(11) : 25–31

—— (1956) [Repeated and multiple mating of queens.] *Pchelovodstvo* 33(1) : 43–50

WOYKE, J. (1955) Multiple mating of the honeybee queen (*Apis mellifica* L.) in one nuptial flight. *Bull. Acad. polon. Sci.* II 3(5) : 175–180

—— (1956) Anatomo-physiological changes in queen-bees returning from mating flights, and the process of multiple mating. *Bull. Acad. polon. Sci.* II 4(3) : 81–87

—— (1958) [The process of mating in the honeybee.] *Pszczel. Zesz. Nauk.* 2(1) : 1–42 *In Polish; English summary*

—— (1960) [Natural and artificial insemination of queen honeybees.] *Pszczel. Zesz. Nauk.* 4(3/4) : 183–275 *In Polish; English summary* Summarized also in *Bee World* 43(1) : 21–25 (1962)