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THE RATE OF DEPLETION OF SPERMATOZOA IN THE QUEEN HONEYBEE SPERMATHECA *

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Summary

Fourteen uniformly inseminated hybrid queens were randomly divided into three groups. Spermatozoa in the spermatheca of the first group were counted before egg laying began (5.00 ± 0.26 (SE) million). Queens of the second group laid about 63 000, and of the third group about 123 000, eggs before spermatozoa in the spermathecae were counted (3.25 ± 0.06, 2.49 ± 0.18, respectively). The queens used half their spermatozoa between 16 June and mid-November—about half the egg-laying season in Louisiana. The data were negatively correlated with the predicted logarithmic loss of spermatozoa from the spermatheca ($r = -0.94$).

Introduction

A queen honeybee (*Apis mellifera*) controls egg fertilization by controlling the release of spermatozoa from her spermatheca. Her entire supply of spermatozoa is limited to those obtained before she starts egg laying, so she must dispense them gradually. Although their rate of depletion is unknown, many spermatozoa are apparently released each time an egg is laid: Adam (1912) estimated 10-12; Bresslau (1905) reported 50-100. The number entering each egg was reported by Nachtsheim (1913) as 3 to 7 with a maximum of 10; Woyke et al. (1966) found 1 to 8.

My hypothesis was that each time a queen lays an egg, she releases a specific volume of spermatozoa-containing fluid from her spermatheca. The spermatozoa plus fluid are then replaced by fluid alone, which reduces the concentration of the spermatozoa in the spermatheca. Therefore successive releases of the same volume gradually contain fewer and fewer spermatozoa. The hypothesis is expressed by the equation:

$$\ln a = \ln a_o - t(q/v),$$

where: a is the number of spermatozoa in the spermatheca after a queen has laid any number (t) of fertilized eggs; a_0 is the original number of sperm in the spermatheca after mating; q is the release volume; v is the spermatheca capacity. (The formula assumes that q and v remain constant, and that the spermatozoa are always distributed uniformly within the spermatheca).

My overall objective was to evaluate mathematically, and to predict the consequences of different sperm numbers in the spermatheca. It would then be possible to estimate the minimum insemination volumes for a queen, based on the performance required of her.

This study was designed to test my hypothesis with field data, by producing a uniform group of queens, inseminating them as uniformly as possible, and then counting the number of spermatozoa remaining in their spermathecae after they had laid 0, or 60 000, or 120 000 eggs.

Methods and Materials

Hybrid, supersister queens emerged as adults on 23 May and were instrumentally inseminated three times, each being given $2\frac{1}{2}$ μl of semen on 5 June, $1\frac{1}{2}$ μl on 6 June and

* In co-operation with Louisiana Agricultural Experiment Station.

4 μl on 7 June. The practice of multiple inseminations reduces the variation in the number of spermatozoa that enter the spermatheca (Mackensen & Roberts 1948). The semen was collected from drones of a single queen of unrelated stock. From emergence until 12 June all queens were kept caged in the same queenless colony.

On 12 June the queens were randomly divided into those whose spermatozoa would be counted before any eggs were laid, and those that would be allowed to lay 60 000 or 120 000 eggs. The queens chosen to lay were placed in small colonies (nuclei) and began laying about 16 June. On 21 June they were transferred to populous colonies, in which they were confined to the second (or the second and third) hive bodies by queen excluders. Each hive body contained 10 frames measuring 16 × 45 cm.

The number of fertilized eggs a queen laid was estimated by measuring the total area in cm^2 of worker brood, and multiplying by 3.9. Brood measurements were made about every 20 days (before a brood cycle could emerge). At that time the combs of brood were exchanged with the empty frames from the bottom hive body. These frames, and others from elsewhere if necessary, provided space for the queens to continue laying. An effort was made to move pollen frames with the empty frames, and when pollen supplies seems inadequate, a pollen supplement was fed to all colonies.

Sperm counts were made with a haemacytometer. First the spermatheca was immersed in 10 ml of 0.5-M NaCl; then it was punctured, and the spermatozoa dispersed by the sucking and expelling action of a pipette. Often the cells were further diluted to make counting easier. Spermatozoa in at least 1.8 μl of the final mixture were counted for each queen.

Results and Discussion

Table 1 gives the counts of spermatozoa and eggs for individual queens in the three groups. Since the queens started egg laying in mid-June, they had laid 60 000 eggs by August and 120 000 eggs by October or November. Thus they used about half their supply of spermatozoa while laying in a strong colony for about a half season.

The logarithmic decline in the number of spermatozoa in the spermatheca was negatively correlated with the arithmetic increase in the number of eggs laid ($r = -0.94$). The logarithmic curve that best fits the data is shown in Fig. 1. This curve supplies the constant needed for my hypothetical formula as follows: $\ln a = \ln a_0 - t(1/153\,000)$. Thus, if my hypothesis is correct, the (constant) release volume for each egg laid was about 1/153 000 of the total spermathecal volume (about 5000 μm^3).

Conclusions

The rapid decline in the number of spermatozoa in the spermatheca gave indirect evidence that the rate of depletion of spermatozoa is nonlinear. With a linear rate, a queen starting with 6 million spermatozoa could fertilize only 295 000 eggs before exhausting her supply of spermatozoa. This seems extremely unlikely, for a greater number would be laid in one good season. In contrast, with a logarithmic decline in spermatozoa similar to the curve shown in Fig. 1, the queen could fertilize 458 000 eggs and still retain 300 000 spermatozoa.

Although the hypothetical formula was quite compatible with the data, it makes two assumptions that may not be valid. First, the release volume may not be constant. In fact, a decreasing release volume would give a better fit in Fig. 1. Secondly, sperm distribution in the spermatheca may not be uniform. Because of these possible complications, and the insufficiency of data, the formula for sperm depletion remains a working hypothesis for which, however, the constant is known: ($q/v = 1/153\,000$).

TABLE 1. Numbers of spermatozoa remaining in the spermatheca of queens after they had laid 0, 60 000 and 120 000 eggs. See also Fig. 1.

Total no. eggs laid	*Mean daily rate of egg laying**	*No. spermatozoa in spermatheca (millions)*
Group 1		
0	—	6·18
0	—	5·18
0	—	4·83
0	—	4·74
0	—	4·71
0	—	4·38
Mean		5·00 ± 0·63 (SD)
Group 2		
60 600	1104	3·35
65 000	1202	3·25
62 200	938	3·14
Mean		3·25 ± 0·11 (SD)
Group 3		
124 200	1134	2·89
123 300	1100	2·80
121 400	1060	2·47
120 600	1013	2·40**
125 900	1234	1·90
Mean		2·49 ± 0·39 (SD)

*Between 22 June and 22 September. Rates in October and November were much lower (332 ± 172 (SD) after 10 October).

**Queen cells were present at July, August, September and October inspections.

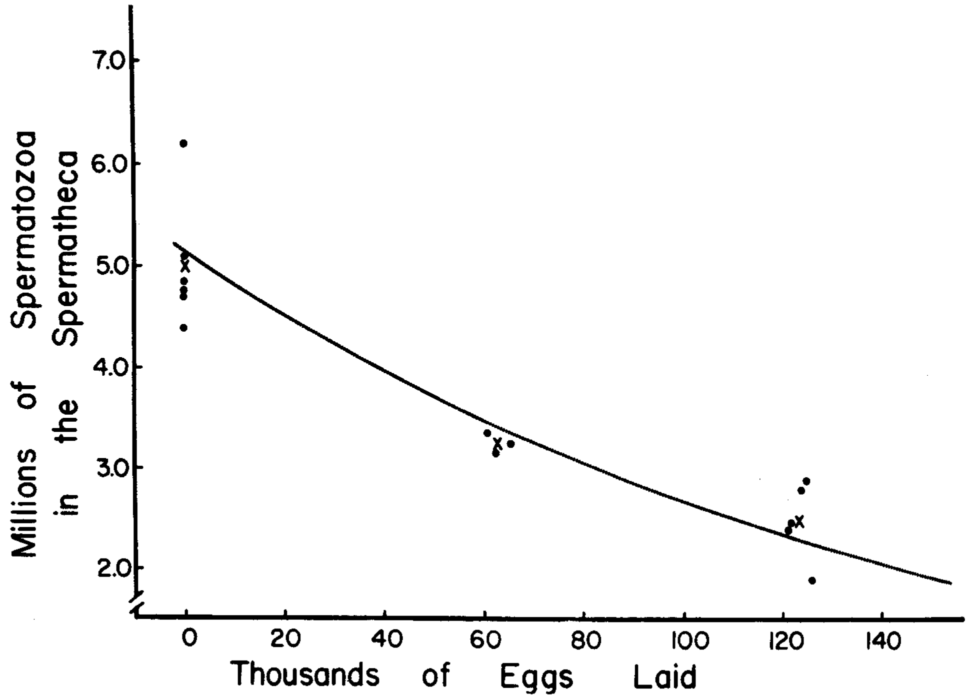

FIG. 1. Decrease in the number of spermatozoa in the queen's spermatheca in relation to the number of fertilized eggs laid. Each solid circle represents one queen of a group of uniformly inseminated queens, and x represents the mean for the group. The line is the logarithmic curve that best fits the depletion rate of spermatozoa.

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