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INFLUENCE OF EXTERNAL CONDITIONS ON THE NUMBER OF SPERMATOZOA ENTERING THE SPERMATHECA OF INSTRUMENTALLY INSEMINATED HONEYBEE QUEENS*

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Summary

Queens were inseminated with 8 mm³ semen and kept for 2 days in nursery cages containing 10 workers. Some of the caged queens were put into queenless colonies and the rest were kept in incubators at 24° or 34°C. The rearing conditions, as well as the insemination conditions, influenced the number of spermatozoa entering the spermatheca. Queens kept at 34° after insemination had 808 thousand more spermatozoa in the spermatheca than those kept at 24° or in colonies.

Introduction

After the technique of introducing the semen into the queen's oviducts was worked out, investigations were started to increase the number of spermatozoa entering the spermatheca.

Mackensen (1955) studied several factors operative during the process of instrumental insemination. Woyke (1960) and Mackensen (1964) investigated the volume of semen necessary for satisfactory insemination of a queen; Woyke recommended two inseminations with 4 mm³ or one insemination with 8 mm³ of semen, and Mackensen two inseminations with 3 mm³. Woyke (1971) showed that the conditions before mating, as early as the grafting time of the larva, influenced the results of insemination. The number of spermatozoa in the spermatheca increased when queens inseminated were reared from younger brood.

Laidlaw (1954) suggested that, after insemination, queens must be kept at a high temperature during the time the sperms are migrating to the spermatheca, and that the queens need the care of the workers during this time. The effect of temperature was studied in detail by Mackensen (1969) in queens inseminated with 2 mm³ of semen, and the necessity of the workers' care was shown by Veselý (1970; 1971).

Woyke (1971) showed that the relationship between the size of the spermatheca and its content of spermatozoa was closer when queens were inseminated with the largest amount of semen (8 mm³). Accordingly, in the present work on the effect of external conditions upon the results of insemination, queens inseminated with this amount of semen were used.

Materials and Methods

A total of 81 queens were investigated (60 in the first experiment and 21 in the second). These queens were reared from very young grafted larvae, but their exact age was not known.

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Each unmated queen was inseminated with 8 mm^3 semen when about 8 days old, and placed in a Zander nursery cage in a nursery frame. Each cage contained 10 workers and honey in wax cups. In the first experiment, 33 inseminated queens in cages were placed between two central brood combs in colonies from which the queens had been removed, and 27 queens were kept in an incubator at 34°C. Three series of tests were made, beginning on 25th July, 15th August and 27th August, respectively. In the second experiment 11 inseminated queens in cages were kept in incubator at 24° and 10 in an incubator at 34°.

All the queens were killed two days after insemination. After dissection, the diameter of the spermatheca without the tracheal sac was measured, and the number of spermatozoa in the spermatheca was counted by the method described earlier (Woyke, 1971).

Results

Experiment 1. Influence of conditions in a colony and in an incubator at 34°

Dissection of the queens 2 days after the start of the experiment showed that not all had their oviducts free from injected semen. Both oviducts were completely filled in 1 queen in each group, and partly filled oviducts were found in 3 queens kept in the colony and in 1 queen in the incubator; one oviduct was partly filled in 5 queens of each group. Thus, 9 queens out of 33 kept in the colony, and 7 out of 27 in the incubator, had incompletely emptied oviducts two days after insemination. It is possible that, with more space and more workers in the cages with the queens, the result would have been different.

Table 1 shows that there were considerable variations in both the number and the concentration of spermatozoa in the spermatheca. For both, the overall mean result for test 1 was the highest of the three. The overall mean number in test 1 was significantly higher than that in test 2 and in test 3 ($P<0{\cdot}001$ and $P<0{\cdot}01$, respectively); the overall mean concentration in test 1 was significantly higher than that in test 3 ($P<0{\cdot}05$). Other means were not significantly different ($P>0{\cdot}05$). For both number and concentration the overall mean for the three tests combined was significantly higher, by 1·292 and 1·262 million/mm^3 respectively, when queens were kept in the incubator than when they were in the colony ($P<0{\cdot}001$). On the basis of 7·026 million spermatozoa per mm^3 of injected semen (Woyke, 1960), the number of spermatozoa entering the spermatheca was 5·3% of the total injected in queens kept in the colony, and 7·6% in those kept in the incubator.

The overall mean volume of the spermatheca (Table 1) was significantly smaller ($P<0{\cdot}001$) in the second test than that in the first or third. A small spermatheca in a virgin queen results from poor rearing conditions, among which the higher age of the grafted larva is one of the most important factors (Woyke, 1971). It is suggested, therefore, that the low number of spermatozoa found in the spermatheca in the second period was related to poorer rearing conditions of the queens in this period. On the other hand, there was no significant difference between the mean volume of the spermatheca of queens according to whether they were kept in the colony or in the incubator after insemination. This indicates that the difference between the mean numbers of spermatozoa in the two groups was caused not by difference in spermathecal volume but by the difference in conditions after insemination. The low concentration of spermatozoa in the third test in both groups of queens suggests that the insemination conditions were poorer here (age and origin of drones).

TABLE 1. Results of instrumental insemination (with 8 mm³ semen) of queens kept subsequently in a colony or in an incubator at 34° for 2 days.

	Queens in colony			*Queens in incubator at 34°*			
Start of test	*No. queens*	*Range*	*Mean ± SE*	*No. queens*	*Range*	*Mean ± SE*	*Overall mean ± SE*
			No. spermatozoa in spermatheca (millions)				
25 July	14	1·980–4·835	3·424 ± 0·230	14	3·416–6·023	4·720 ± 0·206	4·072 ± 0·200
15 Aug.	7	1·755–3·168	2·256 ± 0·221	5	2·475–5·154	3·477 ± 0·475	2·765 ± 0·287
27 Aug.	12	1·820–4·120	2·828 ± 0·208	8	3·372–4·378	3·913 ± 0·115	3·262 ± 0·179
25 July–27 Aug.	33	1·755–4·835	2·959 ± 0·151	27	2·475–6·023	4·251 ± 0·169	
			Concentration of spermatozoa in spermatheca (millions/mm³)				
25 July	14	2·034–4·790	3·511 ± 0·224	14	3·116–6·190	4·680 ± 0·208	4·096 ± 0·187
15 Aug.	7	2·521–4·028	3·326 ± 0·200	5	3·956–5·297	4·695 ± 0·231	3·896 ± 0·252
27 Aug.	12	1·710–4·160	2·951 ± 0·234	8	3·302–5·135	4·171 ± 0·274	3·439 ± 0·221
25 July–27 Aug.	33	1·710–4·790	3·268 ± 0·138	27	3·116–6·190	4·530 ± 0·145	
			Volume of spermatheca (mm³)				
25 July	14	0·754–1·149	0·977 ± 0·030	14	0·837–1·096	1·012 ± 0·072	0·995 ± 0·018
15 Aug.	7	0·523–0·754	0·629 ± 0·033	5	0·555–0·973	0·744 ± 0·094	0·676 ± 0·042
27 Aug.	12	0·754–1·286	0·974 ± 0·037	8	0·903–1·149	0·959 ± 0·054	0·968 ± 0·032
25 July–27 Aug.	33	0·523–1·286	0·902 ± 0·033	27	0·555–1·149	0·946 ± 0·031	

Experiment 2. Influence of temperature

It was assumed that the temperature in the nursery cages in the colony was lower than that in a normal brood nest (34°), and the influence of different temperatures was therefore investigated. Table 2 shows that the mean number and the mean concentration of spermatozoa in the spermatheca were significantly higher in queens kept at 34° than in those kept at 24° after insemination ($P<0{\cdot}001$ and $P<0{\cdot}01$, respectively). The proportion of spermatozoa injected that entered the spermatheca of queens kept at 24° and 34° was 5·6% and 7·0% respectively.

TABLE 2. Results of instrumental insemination (with 8 mm³ semen) of queens kept subsequently in incubators at 24° or 34°.

	No. queens	*Range*	*Mean ± SE*	*Temp.*
No. spermatozoa in spermatheca (millions)	11	2·057–3.751	3·151 ± 0·147	24°
	10	3·372–4.378	3·959 ± 0·102	34°
Volume of spermatheca (mm³)	11	0·754–1·149	0·977 ± 0·034	24°
	10	0·754–1·149	0·962 ± 0·049	34°
Concentration of spermatozoa in spermatheca (millions/mm³)	11	2·587–4·975	3·258 ± 0·200	24°
	10	3·303–5·485	4·220 ± 0·249	34°

There was no significant difference between the mean volumes of the spermatheca in these two groups of queens, indicating that the rearing conditions of the queens could not have caused the above differences.

Thus, an increase of 10° in the temperature at which queens were kept after insemination resulted in a mean increase of 808 thousand in the number of spermatozoa entering the spermatheca.

Discussion

Previous estimates of the number of spermatozoa in the spermathecae of instrumentally inseminated queens (which were subsequently kept in a colony for a few days) have varied. Using queens inseminated with 8 mm^3 semen, Woyke obtained a mean figure of 5·374 million (1960), and 3·234–3·791 million in queens reared from eggs and larvae up to 2 days old (1971). Mackensen (1964), injecting 6 mm^3 semen, recorded a mean of 3·16 million spermatozoa in the spermatheca.

In the present paper, the mean number in queens subsequently kept in a colony (2·959 million) was lower than any of the earlier estimates. When the queens were kept at 24° after insemination the mean number was somewhat higher (3·151 million), and very similar to Mackensen's figure. Keeping the queens at 34° resulted in considerably higher numbers (4·251 million in Experiment 1 and 3·959 million in Experiment 2), which were greater than one recorded by Woyke (1971) but less than his earlier figure (1960).

The percentage of the total number of injected spermatozoa which entered the spermatheca (5·3–7·6%) was lower in the present experiments than the 9·6% found by Woyke (1960) but rather higher than Mackensen's 5·2% (1964). Nevertheless, all these results would have been more alike if the basis of calculation had been the same.

Mackensen (1969) studied the influence of temperature on the number of spermatozoa entering the spermatheca in queens inseminated with 2 mm^3 semen, and came to the opposite conclusion to that presented here. However, his coefficients of variance were large (25% for 25°, 34% for 34°) compared with those in the present work (15·6% for 24°, 7·7% for 34°), in which the queens were inseminated with 8 mm^3 semen. Furthermore, Woyke (1971) showed that there is only a very small correlation between the volume of the spermatheca and the number of spermatozoa entering it after the queen is inseminated with a small dose of semen ($r = 0{\cdot}50$ for 1 mm^3), but a high correlation after the queen is inseminated with a large dose ($r = 0{\cdot}84$ for 8 mm^3). The relatively large variances and small differences between means in Mackensen's work can be related to the small doses of semen injected and were not found when large doses were given (as in the present work); here temperature was shown to have a significant (positive) effect. Veselý (1970) also found higher mean numbers of spermatozoa in the spermatheca of queens kept at 34° than of those kept at room temperature. The differences between these means were not significant, but this may have been due to the fairly small numbers investigated.

Conclusion

When queens are inseminated with large doses of semen (8 mm^3), external conditions significantly influence the number of spermatozoa entering the spermatheca.

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