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CORRELATIONS BETWEEN THE AGE AT WHICH HONEYBEE BROOD WAS GRAFTED, CHARACTERISTICS OF THE RESULTANT QUEENS, AND RESULTS OF INSEMINATION*

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Summary

Queens were reared from eggs and from larvae 1, 2, 3 and 4 days old. They were then mated either naturally or instrumentally with 1 to 2×8 mm^3 of semen. Each increase of 1 day in the age of brood grafted decreased not only the body weight, the size of the spermatheca and the number of ovarioles of the virgin queens, but also the number of spermatozoa in the spermathecae of naturally and instrumentally inseminated queens. A given amount of semen injected into the oviducts resulted in different numbers but similar concentrations of spermatozoa in spermathecae of different sizes. A smaller number of spermatozoa entered the smaller spermathecae, despite a surplus of semen in the oviducts and plenty of space in the spermathecae. Correlation coefficients between different characters were significant only when queens reared from brood of different age were compared. Partial correlations showed that a direct correlation existed between the age of grafted brood and the number of spermatozoa in the spermathecae of mated queens.

The process of weighing virgin queens therefore helps in their selection.

Introduction

Only a small percentage of the spermatozoa injected into the queen's oviducts enters the spermatheca, and there is a great need to increase the percentage, especially when the queen must be inseminated with a small amount of semen (e.g. from one drone, or from diploid drones, which produce only small amounts). The common belief is that the number of spermatozoa in the spermatheca of naturally inseminated queens depends upon the number of drones with which the queens have mated. But Woyke (1966) showed that the number of spermatozoa in the spermatheca of naturally mated queens was correlated with the size of spermatheca. We do not know, however, whether the differences were caused by different volumes of semen being received in the oviducts, or by different numbers of spermatozoa (from a given amount of semen) reaching spermathecae of different sizes.

An investigation was, therefore, made to find out whether differences in the age of brood used for rearing queens influenced the results of natural and instrumental insemination, and to study certain morphological characters of the queens.

Eckert (1934) and Weiss (1969) found that the age of the grafted larvae did not influence the number of ovarioles or other parts of the body in the queens reared. Becker (1925) ascertained that queens reared from larvae of different ages up to 3–3·5 days did not differ, but after that age great changes occurred. Komarov (1935) and Gontarski (1941) reared individuals which were intermediate between queens

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and workers. Adult queens reared by Weaver (1957) from larvae grafted at 1 and 2 days old were very similar in most respects, but they differed from queens reared from larvae aged 3 days. According to Vagt (1955) the relation of the width to the length of spermatheca of adult queens decreased with an increase in the age of grafted larvae. Using the method of rearing queens from eggs described by Orösi-Pál (1960), Woyke (1960, 1964) and Jordan (1960) found that the weight and number of the ovarioles of queens reared decreased as the age of grafted brood increased (from eggs to larvae aged 3 days).

Eckert (1934) did not find a correlation between various characters of adult queens, but other authors found correlations, as follows: between the number of ovarioles and the weight of adults, $r = 0{\cdot}472$ (Weaver, 1957), $r = 0{\cdot}345$ (Avetisyan, 1961); between the number of ovarioles and the weight of pupae, $r = 0{\cdot}90$ (Hoopingarner & Farrar, 1959); between the number of ovarioles and the spermathecal diameter, $r = 0{\cdot}737$ (Weaver, 1957); between the number of ovarioles and brood production, $r = 0{\cdot}578$ (Avetisyan, 1961); between the weight of queens and brood production, $r = 0{\cdot}45$ (Makarov, 1969). Instrumental insemination of virgin queens with larger amounts of semen resulted in greater numbers of spermatozoa in their spermathecae (Woyke, 1960; Mackensen, 1964).

Correlation and regression coefficients have not previously been calculated for the age of brood grafted for queen rearing and characteristics of the adult queen, and for the results of insemination with different volumes of semen. A preliminary report of the present paper, without the calculated coefficients, was presented at the XXI International Apicultural Congress (Woyke, 1967).

Materials and Methods

The 114 virgin queens used originated from one hybrid queen (*A. mellifera* × *A. m. caucasica*) and, after she was lost, from her daughter. Rearing conditions were varied by transferring brood of different ages to the queen cups, which resulted in the larvae being fed with royal jelly for different periods of time. To obtain brood of known age, the queen was placed for 12 hours on a comb in a queen-excluder isolator in the middle of the brood nest. Eggs laid 3 days previously were grafted by the Orösi-Pál method, and larvae aged 1, 2, 3 and 4 days by the double grafting method. Every series consisted of brood of all these ages reared in the same colony.

All virgin queens were weighed soon after emergence.

Queens reared from the different ages of brood were divided into five groups:

(1) allowed to mate naturally in mating nuclei (5 replications)
(2) instrumentally inseminated twice with 8 mm^3 (5 replications)
(3) instrumentally inseminated once with 8 mm^3 (7 replications)
(4) instrumentally inseminated once with 4 mm^3 (5 replications)
(5) instrumentally inseminated once with 1 mm^3 (5 replications).

At least one replication of each insemination group was carried out on any given day, and each replication contained queens reared from brood of each age under consideration. Semen from drones of the same origin and age was used for all groups instrumentally inseminated on the same day.

The naturally mated queens were killed after they laid their first eggs, and the instrumentally inseminated queens 2 days after the last injection of semen. The spermatheca was then taken out and the tracheal covering removed before three measurements were made: (1) the length, (2) the breadth, (3) the diagonal midway

between (1) and (2). This diagonal was the basis of the calculation of spermathecal volume.

The contents of the spermatheca were then emptied into a drop of 1·5% saline solution, and the volume made up to 1 ml with saline solution; after further dispersion of spermatozoa 4 or 9 ml tap water was added. The spermatozoa in 2 mm^3 of solution were counted immediately in two Fuchs-Rosenthal counting chambers.

Before counting the number of ovarioles, the ovaries were put into 95% alcohol for about 15 minutes. Then a disc several mm thick was cut out of the middle region of the ovaries, and the number of ovarioles present was counted.

The statistical significance of the results was calculated by analysis of variance. Because of the small number of queens reared from larvae 4 days old, they have been omitted from Tables 3–7; results from this group were not included in the analyses of variance, but the statistical significances of differences between their means and those of other groups were calculated from individual standard errors.

Comparisons of different correlation coefficients (r) were made after conversion to z values.

Results

Results of rearing

Eggs transferred to the queen cups were not always accepted by the bees. When this failed, newly hatched larvae were grafted before they had been fed by the bees. Many larvae grafted at 4 days of age were not accepted, and queens reared from the remainder were small in size. Heavy losses of these queens occurred during natural mating flights, and great difficulties were encountered with instrumental insemination. Thus, although 27 individuals were investigated in each group of queens reared from brood of the different ages, only six queens reared from larvae 4 days old were available at the end of this investigation, and spermatozoa could be counted in only three of them.

Characteristics of queens

Table 1 shows that the weight of virgin queens reared from brood of different ages ranged from 105 to 256 mg, and the means from 119 to 209 mg. The greatest difference between virgins reared from brood differing in age by 1 day occurred in those reared from larvae 3 and 4 days old. The differences between all means were highly significant. Thus, each day's increase in the age of brood grafted for queen rearing decreased the mean weight of the queens produced.

The number of ovarioles in both ovaries in the virgin queens ranged from 177 to 340 (means: 224 to 317). The greatest difference between two adjacent groups again occurred between virgins reared from larvae aged 3 and 4 days, but the differences between each of the groups were significant. The average number of ovarioles therefore decreased with each day's increase in the age of brood used for queen rearing.

The diagonal diameter of spermatheca deprived of the tracheal cover ranged from 0·900 to 1·425 mm (means: 1·033 to 1·310 mm). The differences in the size of spermathecae are, however, shown better by the volume (means: 0·586 to 1·182 mm^3), the largest spermatheca being four times the size of the smallest. Here again the differences between queens reared from larvae aged 3 and 4 days were greater than those between any other adjacent groups. In each group the mean diameter and volume of the spermatheca differed significantly from its adjacent groups reared

TABLE 1. Characteristics of 114 queens reared from brood of different ages.

	Age of brood (eggs and larvae) used for rearing queens				
	Egg	*1 day*	*2 days*	*3 days*	*4 days*
No. queens	27	27	27	27	6
			Weight (mg)		
Range	196–256	182–201	156–190	129–182	105–127
Mean (± Standard Error)	209** ± 2·40	189** ± 1·00	172** ± 1·34	147** ± 2·79	119 ± 3·76
Coefficient of variation (%)	5·96	2·27	4·04	9·85	7·75
			No. ovarioles		
Range	295–340	290–325	263–324	221–301	177–263
Mean (± S.E.)	317* ± 2·40	308** ± 1·98	292** ± 2·57	272** ± 3·44	224 ± 12·60
Coefficient of variation (%)	3·927	3·347	4·568	6·577	13·781
			Diameter of spermatheca (mm)		
Range	1·250–1·425	1·225–1·375	1·150–1·300	1·050–1·250	0·900–1·125
Mean (± S.E.)	1·310** ± 0·009	1·276** ± 0·009	1·212** ± 0·009	1·159** ± 0·010	1·033 ± 0·032
Coefficient of variation (%)	0·366	0·376	0·371	0·440	0·764
			Volume of spermatheca (mm^3)		
Range	1·023–1·516	0·963–1·362	0·697–1·151	0·607–1·023	0·382–0·651
Mean (± S.E.)	1·182** ± 0·024	1·093** ± 0·024	0·936** ± 0·020	0·821** ± 0·020	0·586 ± 0·052
Coefficient of variation (%)	10·575	11·619	10·897	12·667	21·509

* $P<0·05$ for difference between this mean and subsequent one

** $P<0·01$ for difference between this mean and subsequent one

from brood of different ages, and the means decreased with an increase in the age of the brood grafted.

The variation in the weight of queens, number of ovarioles, and diameter of the spermatheca was greatest in queens reared from larvae aged 4 days. The high variability in the group of queens reared from the youngest brood was rather unexpected, but this may have been caused by rearing some of them from newly hatched larvae instead of from eggs.

Results of natural and instrumental insemination of queens reared from brood of different ages

To understand better the physiology of the spermatozoa entering the spermatheca, inseminations with various amounts of semen were made. Table 2 shows that in each insemination group the number of spermatozoa entering the spermatheca decreased (as the size of the spermatheca decreased) with the increasing age of the brood from which the queens were reared. The differences between all the means in the group inseminated with 8 mm³ semen (i.e. the largest group) were significant. In the other groups differences between queens reared from brood differing in age by 2 days were also significant.

TABLE 2. Volume of spermatheca and number of spermatozoa in spermathecae of naturally and instrumentally inseminated queens.

	Age of brood (eggs and larvae) used for rearing queens				
	Egg	*1 day*	*2 days*	*3 days*	*4 days*
No. queens		27	27	27	27	6
Mean volume of spermatheca (mm³)		1·182**	1·093**	0·936**	0·821**	0·586
Queens mated	*No. queens*	*Mean no. spermatozoa (millions) in spermatheca*				
Naturally	20+1	6·133	5·737	5·026	3·942	1·520
Instrumentally (2×8 mm³)	20	6·145	5·820	5·269	4·630	—
,, (8 mm³)	28+1	3·791*	3·511*	3·234**	2·631	0·140
,, (4 mm³)	20+1	2·746	2·440	2·335	1·955	1·288
,, (1 mm³)	20	1·585	1·507	1·299	1·141	—

* $P<0.05$ for difference between this mean and subsequent one
** $P<0.01$ for difference between this mean and subsequent one
In column 2, +1 indicates a mated queen reared from a larva 4 days old. Spermatozoa were counted in only 3 out of 6 queens; their mean spermathecal volume was 0·683 mm³.

Comparing queens reared from brood of the same age, the mean numbers of spermatozoa in the spermatheca were similar for the naturally mated queens and those which were inseminated with 2 × 8 mm³ semen, the only exception being queens reared from larvae aged 3 days. Here the naturally mated queens had fewer spermatozoa (Table 2). This suggests that they mated with fewer drones and therefore received less semen than queens reared from younger brood (using the queens inseminated with 2 × 8 mm³ semen as a measure of the optimum level).

When the queens were inseminated with smaller amounts of semen, the number of spermatozoa in the spermatheca also fell, as the age of the brood at grafting increased. This occurred despite the fact that there were plenty of spermatozoa in the oviducts of queens inseminated with even the lowest dose (7·0–7·7 million in 1 mm³), and that there was plenty of space in the spermatheca for additional spermatozoa.

Concentration of spermatozoa in the spermatheca

To ascertain why fewer spermatozoa entered the smaller spermathecae, the number of spermatozoa per mm³ of spermatheca was calculated (Table 3); the concentration of spermatozoa in the spermathecae of instrumentally inseminated queens fell as the amount of injected semen decreased.

Naturally mated queens reared from larvae 3 days old had lower concentrations than the other age groups, and it is possible that the sexual behaviour of such queens may have been different, or that their attraction for drones was less. The concentration of spermatozoa in the spermathecae of queens inseminated instrumentally with the same amounts of semen was very similar, and no statistically significant difference was found between queens reared from brood of different ages. It should be noted, therefore, that almost the same concentration of spermatozoa occurred in spermathecae of very different volumes (see effect of age on spermathecal size). It can, therefore, be concluded that from a given amount of semen injected, only the number of spermatozoa required to reach a certain concentration entered the spermatheca. Thus, a balance existed between the amount of injected semen and the concentration of spermatozoa in the spermatheca, and not between the amount of injected semen and the actual number of spermatozoa. As a result, less semen entered the smaller spermathecae, despite the fact that there was plenty of space, as well as a surplus of spermatozoa in the oviducts. This specific relationship applies only to a given volume of semen, and when a different amount of semen is injected the new value for the concentration of spermatozoa in the spermatheca cannot be calculated directly.

TABLE 3. Concentration of spermatozoa in spermathecae of different groups of queens.

		Age of brood (eggs and larvae) used for rearing queens				
	No. queens	*Egg*	*1 day*	*2 days*	*3 days*	*Overall mean*
No. queens	108	27	27	27	27	
Mean volume of spermatheca (mm³)		1·182**	1·093**	0·936**	0·821**	1·008
Queens mated		*Mean no. spermatozoa (millions) per mm³*				
Naturally	20	5·078	5·182	5·249	4·481	4·997
Instrumentally (2 × 8 mm³)	20	5·137	5·048	5·278	5·383	5·212
,, (8 mm³)	28	3·256	3·493	3·418	3·322	3·372
,, (4 mm³)	20	2·299	2·149	2·624	2·153	2·306
,, (1 mm³)	20	1·432	1·428	1·497	1·530	1·472

** P>0·05 between this mean and the subsequent one

Correlation and regression coefficients for different characters of queens reared from brood of different ages

Table 4 shows that a high, and highly significant, negative correlation existed between the age of brood used for queen rearing and various characteristics of the queens reared: body weight, number of ovarioles, diameter and volume of spermatheca, the number of spermatozoa in the spermatheca. It is, therefore, confirmed that the age of grafted brood determines to a great extent the number of spermatozoa which naturally or instrumentally inseminated queens will have.

TABLE 4. Correlation coefficients (r) and regression coefficients (b) for some characters of queens reared from brood of different ages (eggs to larvae 3 days old).

Character	No. queens	*Age of grafted brood*		*Weight of queen*		*Volume of spermatheca*	
		r	*b*	*r*	*b* (*for 10 mg*)	*r*	*b* (*for 1 mm³*)
Weight	108	–0·91	–20·2	—	—	—	—
No. ovarioles	108	–0·77**	–14·9	0·75**	6·49	0·71**	85·5
Diameter of spermatheca	108	–0·78**	–0·052	0·75**	0·02	—	—
Volume of spermatheca	108	–0·77**	–0·124	0·75**	0·054	—	—
No. spermatozoa (millions)	*No. queens*						
Queens mated							
Naturally	20	–0·83**	–0·728	0·72**	0·269	0·77**	4·261
Instrumentally (2 × 8 mm³)	20	–0·67**	–0·510	0·62**	0·181	0·89**	4·817
,, (8 mm³)	28	–0·90**	–0·376	0·92**	0·167	0·84**	2·486
,, (4 mm³)	20	–0·70**	–0·308	0·67**	0·160	0·58**	1·519
,, (1 mm³)	20	–0·64**	–0·154	0·63**	0·089	0·50*	0·702

* $P<0·05$
** $P<0·01$

Correlation coefficients for the age of grafted brood and the number of spermatozoa in inseminated queens were highest for queens inseminated once with 8 mm³ of semen, and lowest for those inseminated both with the largest amount (2 × 8 mm³) and with the smallest amount (1 mm³). Both the latter coefficients differed significantly from that of queens inseminated once with 8 mm³.

The regression coefficients given in Table 4 show that each increase of 1 day in the age of brood grafted to rear queens resulted in the following mean decreases: body weight, 20 mg; number of ovarioles, 15; volume of spermatheca, almost 0·125 mm³. An increase of 1 day in the age of the grafted brood decreased the mean number of spermatozoa in the spermatheca of naturally mated queens by 0·728 million, and of instrumentally inseminated queens by smaller amounts.

Table 4 also shows that highly significant correlations existed between the weight of the (virgin) queens reared and the number of ovarioles, size of spermatheca, and number of spermatozoa in the spermatheca. The correlation coefficient for the queen's weight and the number of spermatozoa in the spermatheca was lowest in queens inseminated with the largest (as well as with the smallest) amount of semen, and both were significantly lower than that for queens inseminated once with 8 mm³ of semen. In Table 4 it can be seen from the regression coefficient that a mean increase of 10 mg in body weight was related to a mean increase of 6·5 ovarioles. The greatest increase in the mean number of spermatozoa in relation to an increase in body weight was found in queens mated naturally.

Table 4 also shows that there was a significant correlation between the volume of the spermatheca and the number of spermatozoa, in all groups of queens. The highest coefficient was found in queens inseminated with the largest amount of semen (2 × 8 mm³); this was significantly higher than the coefficients for the groups inseminated with the smallest amounts (4 mm³, 1 mm³), but was not significantly higher than that for the naturally mated group or that for the group inseminated with 8 mm³. The regression coefficients show that, when the queens were inseminated with larger amounts of semen, an increase in the size of the spermatheca was related to a greater increase in the number of spermatozoa in it. All the above

results show that the number of spermatozoa in the spermatheca was closely correlated with its volume only when the queens were inseminated with the largest amount of semen. In the other cases the number of spermatozoa in the spermatheca was more closely correlated with the age of grafted larvae, and usually also with the weight of the queens.

The following explanation of these results is proposed. The maximum amount of semen injected fills the spermatheca completely and, consequently, the number of spermatozoa in the spermatheca depends on its size. Smaller doses result in a number of spermatozoa in the spermatheca which depends in the physiological condition of the queen, and this in turn is determined by the age at grafting of the brood from which she developed.

Correlation coefficients for different characters of queens reared from brood of the same age

To find out the influence of different ages of grafted brood on the relationship between different characters, correlation coefficients were calculated separately for queens reared from brood of the same age (Table 5). These correlation coefficients, in contrast to those for queens reared from brood of the complete age range, are low and (with one exception) are not statistically significant. Thus, it is necessary to rear queens from brood of different ages in order to demonstrate these correlations.

TABLE 5. Correlation coefficients (r) for different body parts of queens reared from brood of the same age.

Age of brood	*No. queens*	r *for weight and:* *No. ovarioles*	*Volume of spermatheca*	r *for diameter of spermatheca and no. ovarioles*
All ages (eggs to larvae 3 days old)	108	0·75**	0·75**	0·7**
Eggs	27	0·02	0·09	0·43*
Larvae 1 day old	27	0·13	0·22	0·17
Larvae 2 days old	27	0·16	0·19	0·33
Larvae 3 days old	27	0·26	0·27	0·22

* $P<0·05$
** $P<0·01$

Table 6 shows that the correlation coefficients for body weight and the number of spermatozoa in the spermathecae of inseminated queens reared from brood of the same age varied greatly, and on several occasions there were negative correlations; the small numbers of queens used in producing the figures for each coefficient may be partly responsible for this variability.

The relationship between the volume of the spermatheca and the number of spermatozoa in it is rather unexpected. Entirely positive correlation coefficients were found only in the group in which the queens were inseminated with the largest volume of semen (2×8 mm^3), but though these coefficients were large only one was statistically significant. In the other groups both positive and negative coefficients occurred. Thus it is confirmed that a close correlation between the volume of the spermatheca and the number of spermatozoa in it is found only when the spermatheca is completely filled with spermatozoa. If this is not so, some other factors influence the results.

TABLE 6. Correlation coefficients (r) for the number of spermatozoa in the spermatheca and two other characters of the queen, using queens reared from brood of the same age (5 queens for r value for each age, except for 8 mm³ semen, for which 7 queens were used).

Age of brood	*Amount of semen used*				
	Naturally mated	*2×8 mm³*	*8 mm³*	*4 mm³*	*1 mm³*
	r *for body weight and no. spermatozoa*				
All ages (eggs to larvae 3 days old)	0·72**	0·62**	0·92**	0·67**	0·63**
Eggs	–0·47	0·03	0·81	0·45	–0·16
Larvae 1 day old	0·44	–0·94	–0·21	–0·32	–0·07
Larvae 2 days old	0·11	–0·14	0·10	0·21	–0·51
Larvae 3 days old	0·60	0·34	–0·13	0·21	0·87
	r *for volume of spermatheca and no. spermatozoa*				
All ages (eggs to larvae 3 days old)	0·77**	0·89**	0·84**	0·58**	0·50**
Eggs	–0·06	0·79	–0·63	0·48	–0·67
Larvae 1 day old	0·07	0·71	0·51	–0·64	–0·39
Larvae 2 days old	0·22	0·84	0·61	0·68	0·88*
Larvae 4 days old	–0·18	0·95*	0·86*	–0·02	0·32

* $P<0·05$
** $P<0·01$

Partial correlations

A direct relationship between two variables may be found after the influence of other variables is excluded. Partial correlations were, therefore, calculated between two variables while a third variable remained constant.

Table 7 shows that, in general, there was no close relationship between body weight and other characters when the age of the brood grafted was constant. Thus, the high total correlation between weight and other characters of the queens shown in Table 4 appears to be largely a reflection of the close relation between the age of grafted brood and the characters measured in the queens reared.

Correlation coefficients for the volume of the spermatheca and the number of spermatozoa in it (with the age factor held constant, Table 7) were found to be highly significant in queens inseminated with the largest volume of semen. Relatively high coefficients were also found in queens inseminated with 8 mm³ of semen, and in those mated naturally.

Correlation coefficients for the age of the grafted brood and the number of spermatozoa in the spermatheca (with volume held constant, Table 7) were negative and were statistically significant, apart from that for the queens inseminated with 2×8 mm³ of semen.

Thus, a direct correlation between the volume of the spermatheca and the number of spermatozoa in it existed only when the queens were inseminated with the largest volume of semen. In the other queens a direct correlation existed between the age of the grafted larvae and the number of spermatozoa in the spermatheca, and therefore the high correlations between the volume of the spermatheca and the number of spermatozoa in it (shown in Table 4) are in great part a reflection of this relationship.

TABLE 7. Partial correlation coefficients (r) for two variables when a third is constant.

Character	*No. queens for each* r *value*	r *for weight and other characters when age constant*	r *for volume of spermatheca and other characters when age constant*	r *for age and no. spermatozoa when volume of spermatheca constant*
No. ovarioles	108	0·19	0·29**	—
Diameter of spermatheca	108	0·15	—	—
Volume of spermatheca	108	0·19	—	—
No. spermatozoa in spermatheca, after insemination with semen:				
naturally	20	–0·07	0·41	–0·61**
2 × 8 mm^3	20	0·12	0·80**	0·27
8 mm^3	28	0·44**	0·41*	–0·68**
4 mm^3	20	0·07	0·08	–0·49*
1 mm^3	20	0·07	–0·01	–0·46*

* $P<0·05$
** $P<0·01$

Discussion

Some authors whose work is quoted in the Introduction did not find correlations between characters of queens, whereas others did. The present investigation provides an explanation for these apparent contradictions, since it shows that variations exist in different characters of queens reared from brood grafted at the same age, whereas correlations were found when queens reared from brood grafted at different ages were investigated. It may well be that authors who failed to find correlations examined queens reared from brood grafted at the same age, whereas those who found correlations used brood grafted at different ages.

Conclusions

High correlations exist between certain characters of queens and the age at grafting of the brood from which they were reared. Correlation coefficients between different characters are significant when queens reared from brood grafted at different ages are considered together, but the correlations were not significant within the small groups of queens reared from brood of the same age.

A given amount of semen injected into the oviducts does not result in similar numbers of spermatozoa in different spermathecae, but in similar concentrations. Fewer spermatozoa enter smaller spermathecae, despite a surplus of semen in the oviducts and plenty of space in the spermathecae.

The partial correlations show thát there is a direct relation between the volume of the spermatheca and the number of spermatozoa in it, only after the queens are inseminated with the largest amount of semen. In the other cases the apparent relationship is only a reflection of direct relation between the age of grafted brood and the number of spermatozoa.

One fact that has been shown here which is of importance in practical bee-keeping is that the body weight and number of ovarioles of virgin queens, as well as the number of spermatozoa in the spermatheca of naturally or instrumentally inseminated queens, can be increased by rearing queens from brood grafted at the earliest possible age. As a criterion for selection of better queens, the weight of virgin queens can be used.

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