

ORIGINAL RESEARCH ARTICLE

Onset of oviposition by honey bee queens, mated either naturally or by various instrumental insemination methods, fits a lognormal distribution.

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Summary

It is commonly believed that instrumentally inseminated honey bee queens initiate oviposition much later than naturally mated queens. A large scale investigation was therefore conducted on 1675 queens. Naturally mated queens initiated oviposition 2 to 23 (mean 6.8) days after the start of mating flights and the mode occurred 2.8 days earlier. Queens inseminated instrumentally with 8mm^3 of semen initiated oviposition 3 to 21 (mean 9.1) days after insemination and the mode occurred 3.1 days earlier. Thus, the mean and the mode of the onset of oviposition occurred only 2.3 or 2 days later in instrumentally inseminated queens than in naturally mated queens. Different treatments of instrumentally inseminated queens accelerated or delayed the onset of oviposition by only one day in relation to instrumentally inseminated queens not additionally treated. The distribution of the onset of oviposition was skewed positively in both naturally mated queens and instrumentally inseminated queens and fitted a lognormal distribution. We therefore recommend presentation of the mode value in all future papers concerning the onset of oviposition by both naturally mated queens and instrumentally inseminated queens.

El comienzo de la puesta de reinas de abejas fecundadas naturalmente y por distintos tratamientos de inseminación artificial, se ajusta a una distribución lognormal.

Resumen

Comúnmente se cree que las reinas de abejas que han sido inseminadas instrumentalmente inician la puesta más tarde que las que se han apareado naturalmente. Por tanto se realizó una investigación a gran escala con 1675 reinas. Las reinas fecundadas naturalmente iniciaron la puesta de 2 a 23 días (media de 6,8) después del comienzo de los vuelos de fecundación y la moda ocurrió 2,8 días antes. Las reinas inseminadas instrumentalmente con 8mm^3 de semen iniciaron la puesta de 3 a 21 días (media 9,1) después de la inseminación y la moda ocurrió 3,1 días antes. Así, la media y la moda del comienzo de la puesta ocurrió sólo 2,3 o 2 días después en reinas inseminadas instrumentalmente respecto a las reinas fecundadas naturalmente. Distintos tratamientos de las reinas inseminadas instrumentalmente aceleraron o retrasaron el comienzo de la puesta solamente un día en relación con reinas inseminadas instrumentalmente no tratadas posteriormente. La distribución del comienzo de la puesta fue sesgada positivamente tanto en reinas fecundadas naturalmente como en reinas inseminadas instrumentalmente y sigue una distribución lognormal. Recomendamos por tanto presentar el valor de la moda en futuros trabajos relativos al comienzo de la puesta de reinas fecundadas naturalmente como en reinas inseminadas instrumentalmente.

Keywords: Instrumental insemination of queen bees, natural mating, mating flights, oviposition, *Apis mellifera*

Introduction

In recent years in Poland about 80,000 honey bee queens are instrumentally inseminated annually, several times more than in the whole of the rest of the world. It is commonly believed that instrumentally inseminated queens (IIqs) initiate oviposition much later than naturally mated queens (NMqs). Two questions therefore arise: how many days later do IIqs initiate oviposition than NMqs; and can the onset of oviposition (OofO) by IIqs be accelerated by different treatments of queens before or after instrumental insemination?

Beekeepers require queens with a large number of spermatozoa in the spermatheca, which will initiate early oviposition. Several factors affect those traits in NMqs as well as in IIqs. Young queens five to six days old start orientation flights from the hive, and the same, the next or another day, perform mating flights. During a single mating flight, queens mate with one to 18 drones (mean: 8) (Woyke, 1955, 1956, 1960). The semen is injected into lateral oviducts of the queen. The sting chamber of a mated queen is plugged with the "mating sign", which consists of part of the bulbus of the drone endophallus and the mucus of the mucus glands. After returning to the hive, the mating sign is removed and the semen enters into the spermatheca within 24h (Woyke, 1960). About 50% of queens perform a second mating flight within the next few days (Alber *et al*., 1955). The mating process was described by Woyke and Ruttner (1958). One to four days after the last mating, the queens start oviposition (Butler, 1954; Koeniger, 1986).

Artificial introduction of the mucus from drone mucus glands into the sting chamber of virgin queens did not induce OofO, but contact with drones during the flight of queens with a sealed sting chamber induced oviposition, despite sperms not having been transferred into the spermatheca (Koeniger, 1976). Queens allowed to fly on an island without drones also did not start oviposition (Koeniger, 1981).

Different factors affect the success of instrumental insemination (Inst Ins) of queens. Mackensen (1947) showed that InstIns without CO_2 treatment has little, if any, effect on OofO. While IIqs not treated with CO_2 initiated oviposition as late as 51 days after insemination, those treated once or twice with CO_2 reduced the OofO to 36 or 15 days respectively. The OofO occurred on average 5.3 days after the last treatment with CO_2.

Mackensen (1955) found that the age of queens inseminated with single drone semen, has, after the first day, little effect on the number of spermatozoa entering the spermatheca. Therefore, he suggested that queens from two to 10 days old could be inseminated with equal effectiveness. However, after Woyke and Jasiński (1976) inseminated one to three day old queens with a larger dose of 8mm^3 of semen, heavy losses occurred (survival 14-23%), and low number of spermatozoa entered the spermatheca (2.6million). Much better results in survival (75-100%) and the highest numbers of spermatozoa in the spermatheca (4.0 million) were obtained when queens five to 10 days old were inseminated.

Woyke and Jasiński (1990) showed that the number of worker bees that attend IIqs in the nuclei significantly affects the OofO. The authors recommended releasing the queens immediately after InstIns onto the comb in the nucleus or hive. However, several authors investigated OofO by IIqs kept for some time in cages in queen banks (Wilde, 1994; Siuda and Wilde, 2003), or in small boxes with few worker bees (Chuda-Mickiewicz and Prabucki, 1993; Chuda-Mickiewicz *et al*., 2003). Ebadi and Gary (1980) investigated the effect of diluted CO_2 and flight prior to Inst Ins on the OofO. Kaftanoglu and Peng (1982) compared the OofO by NMqs and by IIqs inseminated with semen or with washing fluid. All the positive results presented above were applied in the methods of the present investigation.

Until now, investigations on the OofO by IIqs have been conducted on small groups of 10–30 queens. We therefore conducted a large scale investigation on 1675 queens and the numbers in the overall groups ranged from 174 to 322. The investigations were also conducted in three places with different meteorological conditions, so we hope that the results are more general. Due to the large number of queens examined, we were able to describe the frequency distribution of the OofO by NMqs and IIqs.

Materials and Methods

The investigations were conducted in the three Bee Divisions of the two Agricultural Universities in Warsaw, and Szczecin and the Warmia-Mazury University in Olsztyn, Poland, during 2003 to 2005. Altogether 1675 queens were investigated¸ of which 269 mated naturally and 1406 were instrumentally inseminated, being treated differently before or after insemination. Three repetitions were conducted each year. Carniolan bees (*Apis mellifera carnica*) were used. Virgin queens were introduced into four comb (length 13 and 8 x height 9cm) trapezoid mating nuclei. Queen excluders were fixed at the entrance of the nucleus hives.

The nuclei with accepted queens were randomly divided into 6 groups of 15 nuclei in each replicate. The queens of each group were treated differently: 1. Queens mated naturally (NMqs) - control group; 2. Queens instrumentally inseminated (IIqs) without additional treatments (II-only); and queens treated differently before or after insemination: 3. IIqs, which flew for three minutes on a window inside a room before insemination (II+Flb); 4. IIqs, which flew before and after insemination (II+Flba); 5. IIqs, which flew after insemination (II+Fla); and 6. IIqs, whose sting chamber was plugged with mucus after insemination (II+Mu). In the control group of queens assigned for natural mating, queen excluders were removed in Szczecin and Olsztyn after the queens were accepted and in Warsaw in the morning when the queens reached the age of six days.

All IIqs were inseminated with 8mm^3 of semen. Two days before insemination, at the age of six days, they were treated with CO_2 for three minutes. The second CO_2 treatment was applied during insemination at the age of eight days. The flying of queens, and plugging of the sting chamber with mucus, were chosen as possible equivalents of natural factors, like mating flights and the mating sign in naturally mated queens. Queen excluders in nuclei with IIqs were removed from the entrances after the queens started oviposition.

The time of the appearance of eggs and larvae in the nuclei with NMqs and IIqs were examined daily during 21 days after insemination, up to the age of 29 days. One-Way and Two-Way ANOVA were applied and an LSD test was used to detect statistically significant differences between the means. Probability distribution options were examined to find the curve which would best fit the distribution of the data. For non-normally distributed data, a Kruskal-Wallis test was applied to find significant differences between the medians of the data. A Mann-Whitney test was used to compare two medians of non-normally distributed groups of naturally and instrumentally inseminated queens.

Results

Onset of oviposition by naturally mated queens
Results in Table 1 show that despite the flight prevention, up to the age of six days in Warsaw, the earliest OofO by NMqs occurred in all three places at the age of eight days. Thus, prevention of free flights during the first days of queen life in Warsaw did not delay the early OofO. This supports the reports that queens do not start mating flights before the age of six days. The latest OofO was performed in different places by queens 17 to 29 days old. Thus, the duration of the period within which NMqs initiated oviposition extended from nine days in Warsaw up to 21 days in Olsztyn.

The mean age, of the OofO varied in the three places between 11.7 to 15.3 days. Queens in Olsztyn started oviposition on average significantly later than in the two other places. The median OofO occurred in different places 0.3 to 0.7 days earlier and the mode OofO 1.7 to 5.3 days earlier than the mean start. The overall median OofO occurred 1.1 days earlier and the overall mode 3.1 days earlier than the mean start (13.1 days). This suggests some skewness in the data distribution.

Table 1 shows positive skewness (0.4 – 1.0) in all three places. (The right tail of the curve, with higher values is drawn out). The overall standard skewness (8.6) was higher than in the three places (1.7 to 3.5). The lowest coefficient of variation was observed in Warsaw and the highest in Olsztyn. However, no relation was found between the height of the skewness and the coefficient of variation (Table 1). Standard skewness above 2 indicates significant non-normality of the data. Three tests for normality of the overall data (χ^2 goodness-of-fit = 728.96, P = 0.0; Shapiro-Wilkis statistics W = 0.88, P = 0.0 and the score for skewness Z = 4.96, P < 0.001) showed significant deviation from normal distribution (all P < 0.01).

Investigation to find appropriate type of distribution revealed that of 22 types of distribution, the lognormal distribution best fitted the distribution of the OofO by NMqs in the three places as well as the overall distribution (Fig. 1). The lognormal distribution fits to data positively skewed. It is sufficient to only know the mean and standard deviation (even without having the data), to draw the lognormal curve by a computer supplied with statistical software. Fig. 1 showed that the curves fitted better to the data in two places and to the overall, after data mean, was replaced by the data median (1 bold line). All the graphs showed the mal/misinformation presented by the mean value.

Because the distribution was not normal, the StatAdvisor of the statistics software Statgraphics 4.1 advised to use the Kruskal-Wallis test to compare the medians instead of the means. The calculated Kruskal-Wallis test (H_2 = 39.5, P < 0.0001) indicated statistically significant difference between the medians of the OofO at the three sites. The results presented graphically in Fig. 2 show that the notches in the box for queens in Olsztyn did not overlap with those for queens in the two places. This indicates a four days significantly later median OofO by queens in Olsztyn (15 days) than in the two other places. Hence, the results concerning significant differences between the medians of the OofO in the three places (Fig. 2) were identical to differences between the means of the OofO presented in Table 1.

Overall, this showed that the middle 50% of all NMqs (perpendicular line between the notches) initiated oviposition within the age of 12 days and 75% up to the age of 15 days. Some queens started oviposition in two places (Szczecin and Olsztyn),very late. They are presented on the graph (Fig. 2) by the outside points, which lay more than 1.5 times the interquartile range. The mean value is affected by the extreme values and the mode is not. This therefore shows the importance of presentation of the mode value in results concerning the OofO by queen bees.

Table 1. Age (days) at which naturally mated queens initiated oviposition.

Place	No Queens	Min - Max	Range	Mean ± sd *	Median	Mode	Skewness	Standard skewness	Coeff. var.
Warsaw	92	8–17	9	11.7±2.1 a	11	10	0.4	1.7	18.2
Szczecin	78	8–20	12	11.7±2.4 a	11	10	1.0	3.5	20.3
Olsztyn	99	8–29	21	15.3±4.7 b	15	10	0.5	2.0	30.9
Overall	269	8–29	21	13.1±3.8	12	10	1.3	8.6	29.1

*Different letters indicate significant differences (P<0.05) between the means.

Onset of oviposition by instrumentally inseminated queens
The earliest OofO by IIqs occurred in different places 3 – 4 days after Inst Ins and the latest 18 – 21 days after insemination (Table 2, Inst Ins). Thus, the duration of the period within which IIqs initiated oviposition ranged in different places from 14 to 18 days. The mean OofO by IIqs, ranged in the three sites from 8.4 to 9.7 days after Inst Ins. The overall OofO occurred 9.1 days after InstIns. The median OofO occurred in different places 0.4 to 0.8

Table 2. Onset of oviposition (days) by naturally mated queens (Nat Mat) after the start of flights (age 6 days) and by instrumentally inseminated queens (Inst Ins) after insemination (age 8 days).

Groups	No Queens	Min - Max	Range	Mean ± sd *	Median	Mode	Skewness	Standard skewness	Coeff. var.
Warsaw									
Nat Mat	92	2–11	9	5.8±2.1 a*	5	4	0.4	1.7	37.3
Inst Ins	138	3–21	18	9.7±4.5 c	9	6	0.6	2.9	46.6
Szczecin									
Nat Mat	78	2–14	12	5.8±2.4 a	5	4	1.0	3.4	41.5
Inst Ins	80	4–18	14	8.4±3.1 b	8	6	1.2	4.3	37.4
Olsztyn									
Nat Mat	99	2–23	21	9.3±4.7 bc	9	5	0.5	2.0	50.8
Inst Ins	104	3–18	15	8.8±4.0 bc	8	7	0.6	2.5	45.2
Overall									
Nat Mat	269	2–23	21	7.1± 3.8 A	6	4	1.3	8.6	53.8
Inst Ins	322	3–21	18	9.1± 4.1 B	8	6	0.8	5.6	44.8

* Different small letters after means indicate significant differences ($P<0.05$) between all three places.
Capital letters concern overall means.

days earlier than the mean start, and the mode initiation even 1.8 to 3.7 days earlier. The overall median OofO occurred 1.1 days and the mode (6.0 days) even 3.1 days earlier than the overall mean of the OofO (9.1 days). This indicates skewness in the distribution of the OofO by IIqs.

Table 2 shows that the skewness ranged in the three places from 0.6 to 1.2 and the standard skewness from 2.5 to 4.3. Standard skewness above 2 indicates significant non-normality of the data. Three tests for normality of the distribution of the overall OofO by all IIqs (χ^2 goodness-of-fit statistic = 711.79, P = 0.0; Shapiro-Wilks *W* statistic = 0.92, P = 0.0 and the score for skewness Z = 3.59, P < 0.00) showed that the distribution differed significantly from normal with 99% confidence.

Examination revealed that lognormal distribution best fitted the distribution of the OofO by IIqs in the three places, as well as to the overall distribution (Fig. 3). However, the curve fitted better to the data when the mean applied to draw the curve was replaced by the median (bold curves). The above graphs showed the mal/misinformation presented by the mean value.

Because the OofO was not normally distributed, the Kruskal-Wallis test was used to compare the medians instead of the means. The test ($H_2 = 3.56$, P = 0.17) did not detect significant differences between the medians of the OofO by queens in the three sites. Thus, the two tests, for differences between the medians (no significant difference) or between the means (Table 2, difference between some means) differed.

The results presented graphically, in the Box and Whisker Plot (Fig. 4) showed that the notches in the boxes for IIqs in the three places overlapped. This indicates that the three medians (perpendicular lines between the notches) of 8, 8 and 9 days after which IIqs started oviposition did not differ significantly. Overall this showed that 50% of all Inst Ins queens started oviposition within 8 days after insemination and 75% of all IIqs initiated oviposition up to 12 days after Inst Ins.

All the above results showed that the positively skewed distribution of the OofO by IIqs was similar to the skewed distribution of the OofO by NMqs. The mean value is affected by the outside data and the mode is not. This shows the much more informative and valuable modal value, instead of the mean value, in presenting results concerning OofO by both, NMqs and IIqs.

Comparison of the onset of oviposition by naturally mated and instrumentally inseminated queens

The number of days after which NMqs initiated oviposition was counted from the time the queens started their first flights at the age of six days. However, the number of days after which IIqs initiated oviposition was counted from the time the queens were inseminated at the age of eight days.

Table 2 shows that the earliest OofO was observed in all the three places, 1 – 2 days later in IIqs than in NMqs. The latest OofO occurred in both types of queens after about three weeks. Thus, the range of days within which NMqs and IIqs started oviposition was similar. IIqs started oviposition in Warsaw or Szczecin significantly 3.9 or 2.6 days later than NMqs. However, no significant difference was found between mean OofO by NMqs and IIqs in Olsztyn. Thus, the mean OofO by NMqs and IIqs varied in different places. The overall mean OofO was significantly 2 days later in IIqs than in NMqs. The mode OofO was in the three places 2 – 3 days later in IIqs than NMqs, and the overall mode OofO was 2 days later in IIqs than in NMqs.

The OofO was skewed in both groups of queens; NMqs and IIqs (Table 2). The distribution of the OofO by queens of both groups fitted to the equation f(x) = lognormal distribution. The mean ($\bar{x}$) and standard deviation (SD) are necessary to draw (by the computer) the lognormal distribution curve. However, the curve fitted better to the observed data when the mean (X) was replaced by the median (Xmed ± SD) of the data (Figs 1 and 3). Thus, the curve for lognormal distribution of the overall number of days between first flights (age 6 days) and the OofO by NMqs is drawn, after Xmed ± SD = 6 ± 3.9 is applied. The curve for lognormal distribution of the number of days between insemination and the OofO is drawn after Xmed ± SD = 8 ± 4.1 is applied (Fig. 5).

The overall mean OofO by IIqs occurred significant 2 days later than by NMqs (Table 2). However, since the distribution was not normal, the two medians, 6 and 8 days of the OofO by NMqs and IIqs were compared. The Mann-Whitney test (W = 59061.0, N_1 = 269, N_2 = 322, P = 0.00) showed highly significant differences between those medians. Graphical presentation (Fig. 4) shows, that the notches in boxes for all Inst Ins and all Nat Mat queens did not overlap. This indicates that the median OofO by all IIqs (8 days) occurred significantly 2 days later than by all NMqs (6 days). All the overall results showed that the means, medians and modes of the OofO occurred two days later in IIqs than in NMqs.

Comparison of the onset of oviposition by queens treated additionally before or after instrumental insemination

All the queens treated additionally in different way before or after instrumental insemination initiated oviposition 3 to 21 days after insemination (Table 3). Thus the duration of the period within which queens started oviposition ranged up to 18 days.

The Two-Way ANOVA revealed that the factor; "different treatment" before or after Inst Ins significantly affected the OofO (F = 8.51, df = 4, P < 0.000). The effect of different treatment varied in different places (Table 3). The overall mean of the OofO by queens II+Flb and II+Flba (8.3 and 8.0 days) occurred significantly 0.8 and 1.1 days earlier respectively, than by IIqs not treated additionally (9.1 days). No significant difference was found between queens II+Fla and II-only. Thus, the flight after Inst Ins did not affect the OofO. However, queens plugged with mucus (II+Mu), initiated oviposition significantly 0.9 day later than II-only queens. Thus, the overall acceleration or delay was only about one day in relation to IIqs not treated additionally.

Positive standard skewness above 2 (4.1 - 7.4) occurred in all 5 groups of IIqs (Table 3). This indicated significant non-normality of the data. Lognormal distribution fitted the best to the data of all groups (not presented graphically), similarly to that, presented in Fig 3, for IIqs not treated additionally.

The Kruskal-Wallis test, for non-normal distribution (the statistics H_4 = 42.7, P < 0.0001), revealed statistically significant differences between the medians of the OofO in the five groups of differently treated queens. Fig. 6 shows that the median OofO by II+Flba and II+Flb queens (7 days) occurred significantly one day earlier than the OofO by queens not treated additionally (II-only, 8 days). No significant difference was found between median OofO by II+Fla and II-only queens. However, median OofO by II+Mu queens occurred significantly one day later (9 days) than that of II-only queens. Thus, the significances of the differences

Table 3. Onset of oviposition (days) after instrumental insemination by queens differently treated before or after insemination.

Group No & type of treatment*	Places of investigation									Overall 1406 queens		
	Warsaw			Szczecin			Olsztyn					
	No	Min-Max	Mean ** ME MO	No	Min-Max	Mean ME MO	No	Min-Max	Mean ME MO	No	Mean ME MO	Std skew
4.II+Flba	71	3–13	6.1 a#	41	4–16	8.1 a	62	5-20	10.2 c	174	8.0 a	5.3
ME MO			5 4			7 6			10 10		7 7	
3.II+Flb	129	3–20	8.0 b	74	4–17	8.6 ab	109	3–21	8.3 a	312	8.3 a	7.4
ME MO			7 5			8 7			8 6		7 7	
5.II+Fla	121	3–21	8.5 b	76	4–21	8.5 ab	107	3–17	8.6 ab	304	8.6 ab	5.6
ME MO			8 9			7 7			8 10		8 8	
2.II-only	138	3–21	9.7 c	80	4–18	8.4 ab	104	3–18	8.8 ab	322	9.1 b	5.5
ME MO			9 6			8 6			8 7		8 6	
6.II+Mu	116	4–21	10.7 d	70	4–20	9.6 b	108	3–21	9.5 bc	294	10.0 c	4.1
ME MO			10 9			8 6			9.5 3		9 9	

Table arranged according to increasing overall mean values.
* II – queens Instrumentally Inseminated; Flba – Flight before and after; Flb – Flight before; Fla – Flight after; II-only – instrumentally inseminated only; Mu – Mucus;
** ME – median, MO – mode; # different letters indicate significant differences between means in columns P < 0.05.

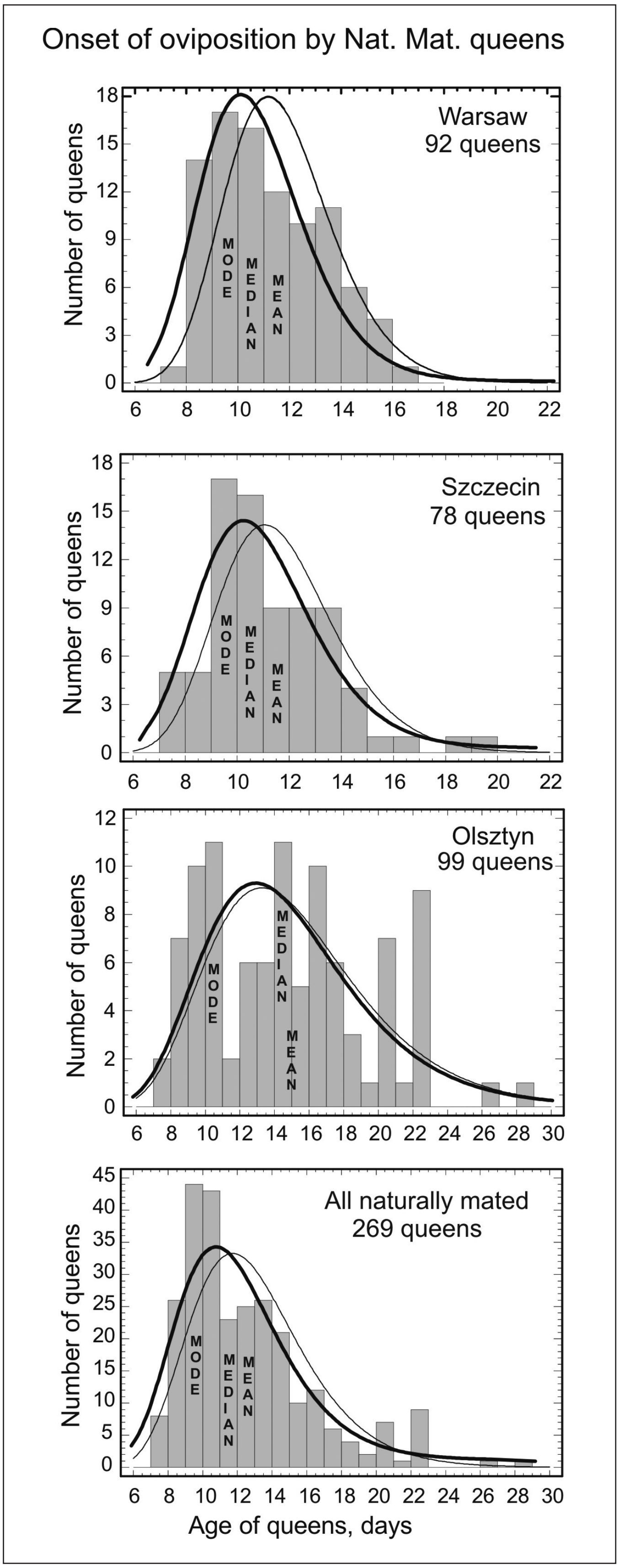

Fig 1. Distribution of the age at which naturally mated queens initiated oviposition. The curves present lognormal distribution. The thin line represents a curve in which its mean value is equal to the mean of the data. The bold line represents a curve in which the curve mean was replaced by the data median. The places of the mode, median and mean values are indicated on the bars.

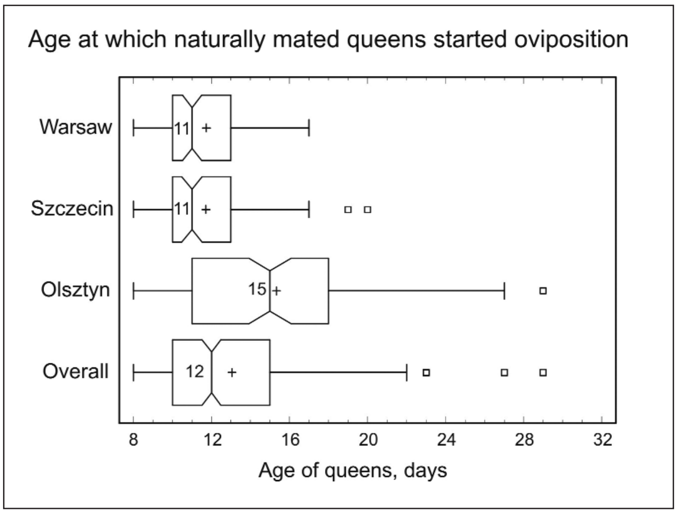

Fig 2. Distribution of the percentiles (0, 25, 50, 75 and 100%) of the age at which naturally mated queens initiated oviposition at the three sites, and overall. The cross in a box indicates the mean, the vertical line in the middle of the notches indicates the median, and the number its value, the notch indicates 95% confidence level for the median, If the two notches for any pair of medians do not overlap, there is statistically significant difference between them.

between the medians (Fig. 6) and the means (Table 3) of the OofO by queens in the five groups were the same.

The median 50% (interquartile range) of all differently treated queens started oviposition within a period of seven days (II+FIb) to nine days (II+Mu) after Inst Ins (Fig. 6), and the 75% of all IIqs within a period of 10 to 12 days after Inst Ins. Fig. 6 also shows that the median OofO by queens II+FIba and II+FIb (7 days) occurred significantly one day later and by queens II-only, two days later (8 days), than by NMqs (median NM, 6 days), after their start of first mating flights at the age of six days.

The Two-Way ANOVA did not detect significant effect of the factor; "place" on the factor; "different treatment" (F = 34.4. df = 2, P = 0.099). The grand overall means of the OofO by all IIqs were 8.6, 8.6 and 9.1 days, in the three places; Szczecin, Warsaw and Olsztyn, respectively. However, the interaction between the factor; "different treatment" and the factor; "place" was significant (F = 6.33, df = 8, P = 0.000). This indicates that particular treatments resulted in different OofO by queens in different places. Fig. 7 shows, that queens II+FIba reacted on the contrary, in Warsaw and Olsztyn. Because the distribution of the OofO by IIqs treated differently was not normal the informative importance of the mode value, which is not affected by outside data, is preferable over the mean value, which is affected by the extreme data.

Discussion

NMqs started oviposition in Olsztyn later (age 15.3 days) than in the two other places (11.7 days). We suppose that this was due to worse weather conditions (lower temperature and rain) occurring predominantly in this place. The late OofO by NMqs in Olsztyn presented now, is the same as found there earlier: 15.1 days (Wilde, 1994).

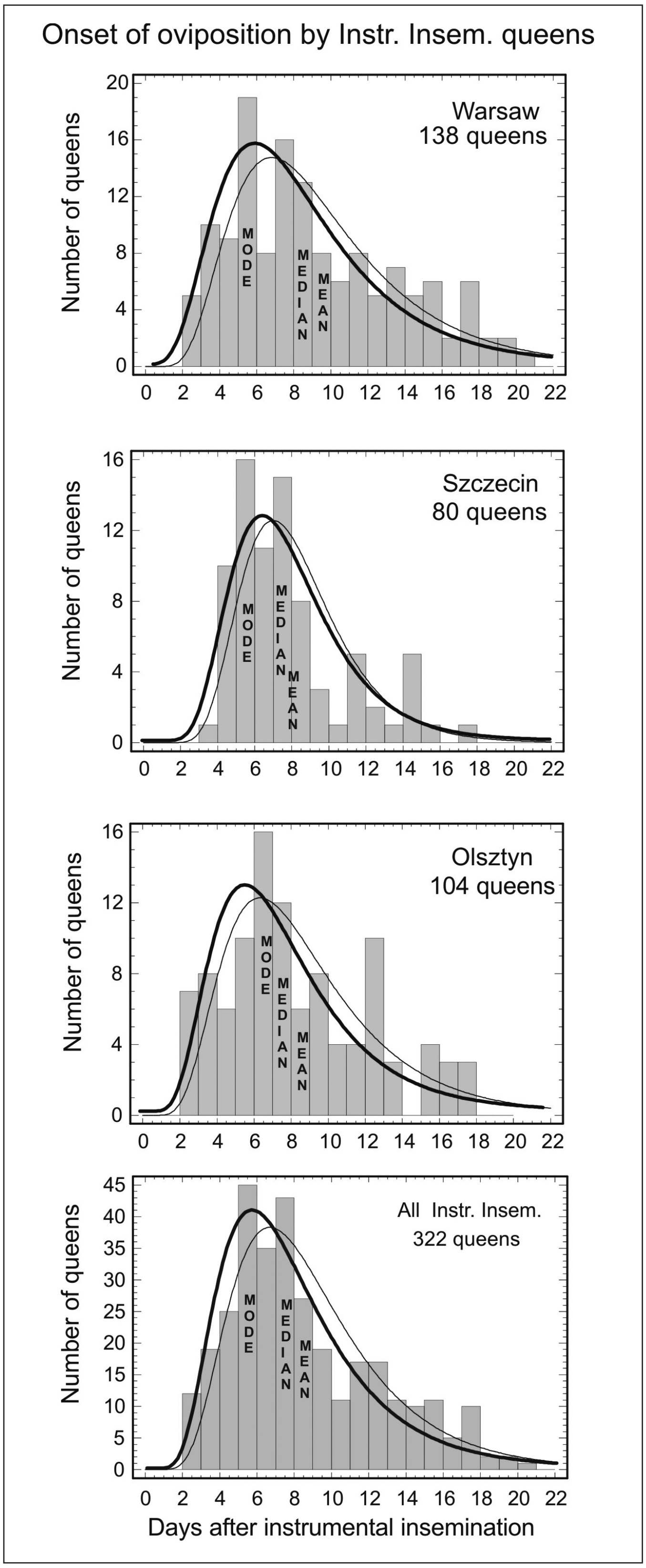

Fig 3. Distribution of the number of days after instrumental insemination when the queens initiated oviposition. For explanation see *Fig. 1*.

In our investigation, NMqs initiated oviposition at the age of 8 to 29 days. The mean age of the OofO in the three places was 11.7, 11.7 and 15.3 days, that is 5.7 to 9.3 days after first flights at the age of six days. The OofO in the two places is similar to the age of 10.3 days presented by Kaftanoglu and Peng (1982), nine days by Woyke *et al*. (2001) and 13.7 days by Czekońska *et al*. (2003). In our study, queens Inst Ins with 8mm^3 of semen and treated twice with CO_2 but not treated additionally, initiated oviposition 3 to 21, overall mean 9.1 days after Inst Ins. Similar results were obtained by several other authors: - 7.8 days (Kaftanoglu and Peng, 1982),- mean 9.9 days (Prabucki *et al*., 1987), – 12.0 days (Konopacka, 1991) – 8.9 days and 8.9 – 9.3 (Chuda-Mickiewicz and Prabucki, 1993, 2000), – 11.0 (Wilde, 1994) – 10.1 (Chuda-Mickiewicz *et al*., 2003).

Woyke and Jasinski (1990) showed, however, that the OofO by IIqs is affected by the number of bees attending the queens.

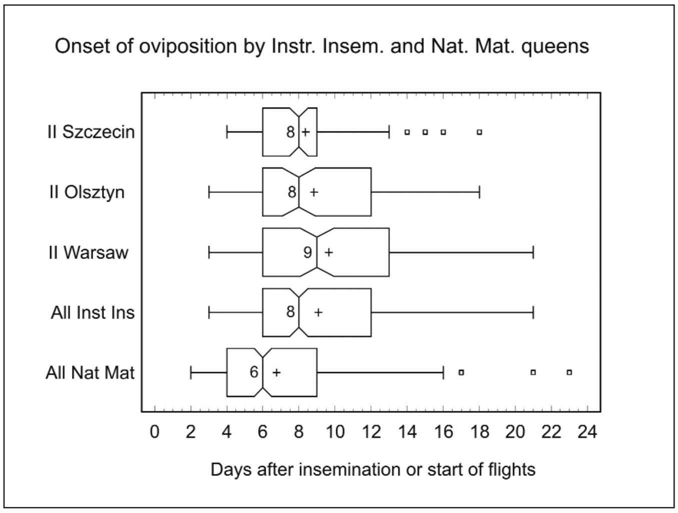

Fig 4. Distribution of the percentiles (0, 25, 50, 75 and 100%) of number of days after instrumental insemination when queens initiated oviposition and the number of days after first flights when naturally mated queens initiated oviposition. The groups of queens in the three locations are arranged according to increasing medians. For explanation see *Fig. 2*.

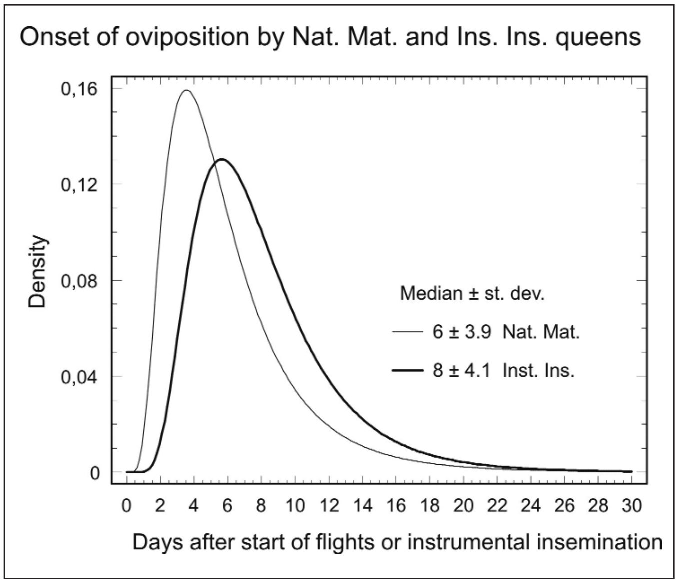

Fig 5. Curves of the lognormal distribution of the number of days between first flights (age 6 days) and the onset of oviposition by naturally mated queens (thin line) and between instrumental insemination (age 8 days) and the onset of oviposition by inseminated queens (bold line).

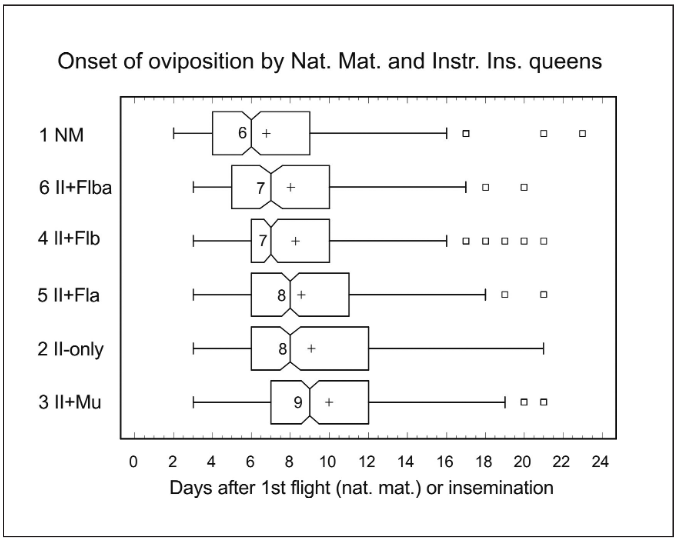

Fig 6. Distribution of the percentiles (0, 25, 50, 75 and 100%) of the number of days between first flights and OofO by naturally mated queens, and between instrumental insemination and the OofO by queens treated differently. The groups of queens are arranged according to increasing medians. For explanation see *Fig. 2* and queen groups *Table 3*.

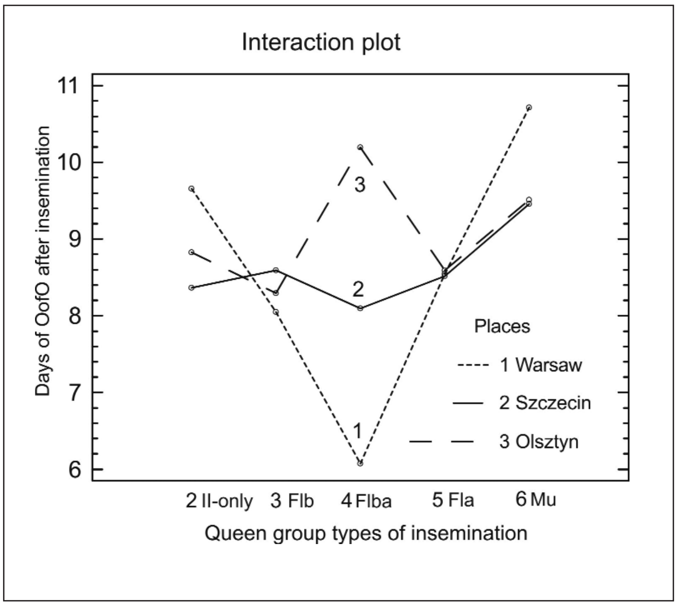

Fig 7. Interaction between the different treatments of queens before or after instrumental insemination and the onset of oviposition. The numbers inside the graph indicate the places. For explanation of queen groups see *Table 3*.

Queens kept in small mating nuclei with 750 worker bees, where the cluster temperature was 24°C, initiated oviposition 9.9 days after Inst Ins; similar to the present results, but queens kept in larger, 5 comb hive boxes with 9500 bees, where the cluster temperature was 31°C, initiated oviposition 6.9 days after Inst Ins, i.e. three days earlier.

In our investigation IIqs thus initiated egg laying on average only two days later after Inst Ins (mean 9, mode 6) than did the NMqs (mean 7, mode 4 days), after the start of flights (at the age of six days). Therefore, if IIqs were inseminated at the age of six days, the mode age of the OofO would be only two days later in IIqs than in NMqs.

The opinion that IIqs start oviposition much later than NMqs appears, therefore, to be for some other reason. Concerning natural mating, the modal value of 1 to 4 days of the OofO after last mating flight is usually reported, but some NMqs initiated oviposition at the age of 20 – 29 days, i.e. 14 – 23 days after the first mating flights. In IIqs, the mean value of 9 to 12 days of the OofO after Inst Ins is mostly taken into account.

Some authors inseminated older queens. After Chwałkowski (1969) inseminated queens 5-14 days, or above 14 days old, they initiated oviposition at the age of 17 and 29 days respectively. Prabucki *et al*. (1987) inseminated queens 7 and 14 days old, both groups started oviposition 11 days after insemination it is at the age of 18 and 25 days. Wilde and Bobrzecki (1994) also inseminated older queens, 7 – 11 (mean 9.0) days old, or Siuda and Wilde (2003) 7 – 11 (mean 9) days old. The queens started oviposit at the greater age of 19.9 days and 25.3 days respectively. Some queens were kept after insemination in cages in queen banks, and therefore they started oviposition at a greater age: 22.4 days (Wilde, 1994); 31.4 days (Siuda and Wilde, 2003). Other queens were kept in small boxes with few worker bees and they also started oviposition at the greater age of 23.5 days (Wilde and Bobrzecki, 1994; Chuda-Mickiewicz *et al*., 2003).

All of these results show that IIqs initiated oviposition later than NMqs, not because they were Inst Ins, but because older queens were Inst Ins, or the queens were kept after insemination in queen banks or small boxes. If queens were InstIns at the age of six days, IIqs would start oviposition only few days later than NMqs.

We showed that queens II+Fb or II+Flba accelerated the OofO by one day in relation to IIqs not treated additionally. However, II+Fla did not initiate oviposition significantly earlier than II-only. Thus, in reality, only the flight before Inst Ins accelerated OofO, but the acceleration was only by one day. It therefore does not repay the additional effort to achieve this result. Ebadi and Gary (1980) reported, however, that 10 mins flight prior to Inst Ins of queens with 8mm^3 of semen treated only once with 75% CO_2 decreased the OofO from 16.3 to 12.3 days after insemination, i.e. by four days. The OofO in both cases was later than in our investigation. It seems that the larger decrease of four days was due to using only a single CO_2 treatment instead of two, which decrease considerably the OofO.

None of these authors mentioned that the distribution of the OofO by both NMqs and IIqs is not normal. The large number of queens examined in our present study enabled us to prove that the distribution of the OofO is positively skewed and fits to the lognormal distribution. The biological result of the skewed lognormal distribution is that the majority of queens initiated oviposition earlier than if the distribution was normal. Our investigation showed that although IIqs started oviposition up to 21 days after insemination, the skewed distribution meant that as many as 50% and 75% of all Inst Ins queens initiated oviposition within eight and 12 days after insemination, respectively (Fig. 4). This justifies the queen rearing practice of waiting for OofO by NMqs and IIqs for only two weeks.

This skewed distribution of the OofO points out the misleading information presented by the mean value, which is influenced by extreme values and which depends upon the duration of the period during which OofO was studied, so that the means of the OofO presented by different authors are not directly comparable. We therefore recommend presenting the modal and median values in all further papers concerning OofO by naturally and instrumentally inseminated queens.

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