

## DOES THE DURATION OF THE PRE OVIPOSITION PERIOD OF HONEYBEE QUEENS AFFECT THE HONEY PRODUCTION OF COLONIES?

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### S u m m a r y

On the basis of data from The Breeding Evaluation Centre in Germany, data of the pre oviposition period (POP) of honey bee queens were collected from 3648 colonies over 19 years at 25 mating stations and from 64 bee breeders. Generally, the pre oviposition period of the tested queens lasted from 6 to 34 days with an average of 15.8 days. About 80.1% started egg laying 8 to 18 days after emergence. The length of POP varied significantly among years and months and among different queen bee breeders. Both, the type of mating and the mating location significantly affected POP. Artificially inseminated queens had the significant highest value (17.6 days) of POP in comparison with naturally mated queens at island- (15.4 days) and land-mating (14.9 days) stations. The relationship between POP and honey production of bee colonies was not found to be significant. Thus, the pre oviposition period is highly affected by environmental factors but provides no information concerning the potential of the queens.

**Keywords:** *Apis mellifera*, queen, Pre oviposition period, honey production, egg laying.

### INTRODUCTION

The common characteristics used to evaluate honeybee colonies are food-gathering activity and brood production. Many other characteristics can be used for this purpose, such as their overwintering ability, swarming tendency, resistance to diseases and gentleness (Rinderer et al., 1983; Hauser and Lensky, 1994; Olszewski, 2007). All the previously mentioned characteristics are partly heritable and respond to selection (Rinderer et al., 1983; Bienefeld and Pirchner, 1990; Willam and Essl, 1993) but are affected to various extents by environmental factors. Some of these factors affect colony traits externally and/or internally (Wille and Gerig, 1976; Laidlaw and Page, 1998). Population

size (Harbo, 1986; Chinh et al., 2005), the lifespan of workers (Eischen et al., 1982, 1983) and queen characteristics (Laidlaw and Page, 1998) represent internal factors, whereas weather conditions and the availability of fresh pollen and nectar are external factors that interact with these characteristics.

The factors relating to honeybee queens are a subject of a great deal of interest. The queen bee is the key to success of both the colony and the beekeeper. She is responsible, with her drones, for the heredity of the colony characteristics (Laidlaw and Page, 1998).

The mating of virgin queens is the last step in the production of young laying queens. These queens have to attain the reproductive capability of the colonies. The

queens have to be cared for during the breeding period and after their emergence.

During the rearing period of honeybee queens, the breeder has a significant impact on queen quality. The breeder plays a role, whether by choosing the right larval age for grafting or by establishing suitable situations in the rearing colony (Laidlaw and Page, 1998).

During the pre oviposition period (POP), honeybee queens undergo physiological changes from their emergence until oviposition. Many factors can influence the rate of these changes during this period. Genetic makeup, weight and the rearing season of the queens are factors affecting the duration of this period (Taranov, 1973; Ebadi and Gary, 1980; Szabo et al., 1987; Al-Ghzawi and Zaitoun, 2008; Kahya et al., 2008).

Wilde (1994) and Woyke et al. (2008) have reported a difference between naturally mated and artificially inseminated queens with regard to the onset of oviposition. The onset of oviposition is longer in instrumentally inseminated queens compared with naturally mated ones.

In beekeeping practice, a short POP is assumed to be linked with better vitality of the queen. Often beekeepers replace their virgin queens with other young ones, if the former do not mate early in their lives. The duration of honeybee queen POP has proven to be influenced by the physiology of the honeybee. Woyke (1960) and Zmarlicki and Morse (1962) have

observed that virgin queens older than 14 days tend to mate with fewer drones and store fewer sperm in their spermatheca than those mated at the normal mating time. Patricio and Cruz-Landim (2002, 2007) state that when the queens are prevented from leaving their colonies for the nuptial flight, the vitellaria do not differentiate, the cysts within the ovarioles begin to disorganize and the cells of the ovariole begin to degenerate.

Less is known with regard to the factors impacting POP and nothing is known about the relationship between POP and the characteristics of honeybee colonies. In the present work, we have studied the impact of POP of honeybee queens on colony characteristics.

## MATERIAL AND METHODS

In this study, the pre oviposition period (POP) of a queen is considered as the period between queen emergence and the beginning of oviposition. On the basis of data from performance tests of The German Beekeeping Association, data were collected from 3648 *Apis mellifera carnica* queens reared by 64 bee breeders during the years 1965-1984 at 25 mating stations or artificial insemination centres in Germany. The day of queen emergence was recorded by the breeders who reared the queens. The beginning of egg laying was monitored by the individuals responsible for the mating stations or the artificial insemination centres. These data and additional results from the performance

Table 1  
Parameters of honey production and post pre oviposition period (POP)  
of the observed *A. m. carnica* population

Characteristics	Number of queens	Mean	Standard deviation	Minimum	Maximum
Honey (kg)	2331	27.4	15.6	0.0	106.2
POP (days)	3648	15.8	5.0	6.0	34.0

tests (honey production) of the corresponding colonies were saved in the stud book of The Beekeeping Association ([www.beebreed.eu](http://www.beebreed.eu), Bienefeld et al. 2008). According to Ruttner (1972), honey yield was taken as the weight difference of the combs before and after extraction of the honey plus an estimate of the honey left in the brood nest (Tab. 1).

To obtain information on the impact of the factors influencing POP and the relationship between POP and honey production, two models were applied:

**Model 1:** Influence of assumed factors on POP

$$y_{ijkl} = \mu + a_i + b_j + c_k + d_l + f_m + e_{ijklm}$$

where:

$y_{ijklm}$  = estimated value of POP as the result of year effect, queen rearing condition, queen mating place and queen mating type,

$\mu$  = mean of population,

$a_i$  = influence of years (n = 19 years from 1965 till 1984),

$b_j$  = influence of the month of mating (n = 3, June, July, August)

$c_k$  = influence of queen rearing conditions (n = 64 bee breeders),

$d_l$  = Influence of mating place (n = 25 mating places),

$f_m$  = influence of mating type (662

artificially inseminated queens, 826 naturally mated queens on island-stations and 159 queens mated on land-mating stations),

$e_{ijklm}$  = error (residual).

**Model 2:** Relationship between honey production and POP

$$y_{ij} = \mu + a_i + b * POP_j + e_{ij}$$

where:

$y_{ij}$  = honey production of the colony,

$\mu$  = population mean,

$a_i$  = effect of apiary, within year and beekeeper,

$b * POP_j$  = regression of POP on honey production

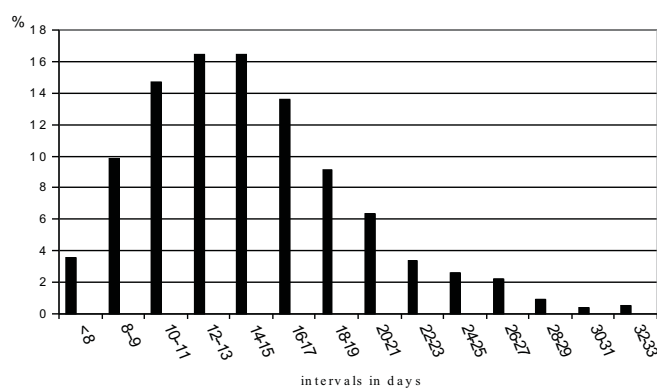
$e_{ij}$  = error (residual).

Because data of POP were not normally distributed, calculation in model 1 and 2 were carried out by log transformed values. The analysis was carried out by using the SAS statistical system (SAS Institute, 1988).

## RESULTS

### Frequency distribution of pre oviposition period

The duration of the pre oviposition period within the observed *A. m. carnica* population varied greatly. The values ranged between 6 and 34 days with an average of  $15.8 \pm 5.0$  days. The distribution



**Fig. 1.** Proportion of queens (in %) with various duration of pre oviposition period in the observed *A. m. carnica* population.

of POP was found to be right-skewed (skewness: + 0.79, mode 15.0) and not normally distributed (Kolmogorov-Smirnov D: 0.09,  $p < 0.01$ ). A few queens (3.6%) began oviposition quickly ( $< 8$  days) but the majority of the queens (80.1%) started oviposition between 8 to 18 days after emergence. Queens with a long POP ( $> 20$  days) accounted for only 16.4% of the population (Fig. 1).

#### Effect of environmental factors on pre oviposition period

The effect of all environmental factors investigated in Model 1 proved to be highly significant (Tab. 2). The length of POP varied significantly from year to year, from

month to month, from different locations and bee breeders (all  $P < 0.0001$ ).

Moreover, the mating type significantly affected the POP of the queens. The average duration of POP was  $17.6 \pm 4.4$  in instrumentally inseminated queens and  $15.4 \pm 5.06$  and  $14.9 \pm 5.6$  in queens naturally mated on island mating stations and on land-mating stations. The difference was significant between instrumentally inseminated queens and both of the naturally mated queen types ( $p < 0.02$ ). It was not significant, however, between the two naturally mated queen types ( $p = 0.24$ , fig. 2).

Table 2

Analysis of variance (Model 1) of the log-transformed pre oviposition period (POP) in the observed *A. m. carnica* population.

Variables	df	Mean squares	F-value	P-value
Year	18	1.29	17.2	$< 0.0001$
Month	2	2.25	29.9	$< 0.0001$
Rearing apiary	63	0.82	10.8	$< 0.0001$
Mating place	24	0.67	8.8	$< 0.0001$
Mating type	1	0.43	5.7	0.017

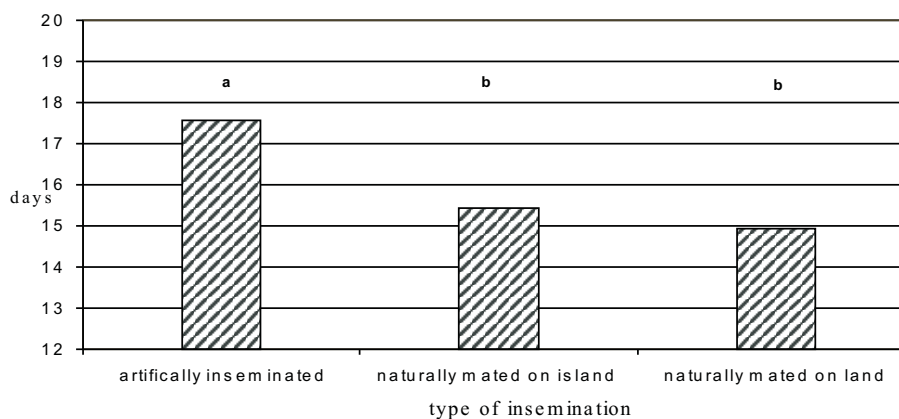


Fig. 2. Average duration of pre oviposition period in queens of different mating types. Different letters indicate significant differences ( $p < 0.05$ ) between the means.

### Effect of pre oviposition period on honey production

Honey production was strongly affected by apiary/year conditions ( $F = 14.2$ ,  $P < 0.0001$ ). However, when adjusted for this apiary/year effect, the honey production of the colonies was not significantly affected by the POP of its queen ( $F = 0.02$ ,  $P = 0.89$ ). The regression of POP on honey production was estimated to be  $b = -0.11 \pm 0.85$  (Tab. 3).

observation times but should nevertheless provide insights into any variation and involved factors.

In our study, with data generated from 3648 queens, over 20 years and several breeders with large environmental variation, POP ranges from 6 to 34 days, with a mean of 15.8 days. Confirming the results of Woyke et al. (2008) our data are also found to be right-skewed. Root and Root (1923) state that the maximum time

Table 3

Regression analysis (Model 2) of pre oviposition period on honey production adjusted for the apiary/year effect

Source of variation	df	Mean Square	F-value	P-value
log-pre oviposition period	1	1.26	0.02	0.89
Apiary	418	1022.7	14.17	<0.0001
Intercept: $98.1 \pm 3.5$ , Regression coefficient = $-0.11 \pm 0.85$				

### DISCUSSION

The life of a honeybee queen can be divided into four phases: pre-emergence, pre-mating, post-mating and post-oviposition. During each phase, some requirements have to be met in order to obtain a fully fertile egg-laying queen. The pre- and post-mating periods are critical phases in the adult life of a queen. During these periods, the queens reach their sexual maturity, mate with the drones and shortly after start oviposition. Any factor that influences one or both of these periods will also affect the duration of the two periods taken together. In this study, we have dealt with the period between queen emergence until the beginning of egg laying. Other studies have considered different periods during the early life of the queen, e.g. the period between mating and egg laying. However, all of these periods are affected by similar factors and governed by similar physiological pathways. Consequently, a direct comparison of means in the various studies has to consider the different

known to elapse between the emergence of a queen and egg laying is 25 days. Szabo et al. (1987) have shown in their study of 1396 queens during a 2 year time period that the onset of oviposition after queen emergence lies between 4 and 22 days with a mean of 10.6 days. They have also found a relationship between the maximum daily temperature and the time of oviposition. Skowronek et al. (2002) have observed, in a total of 1289 queens during 1995-2001, that the period between queen introduction and oviposition varies from 3 to 36 days with an average of 10 days. They interpret the highly significant year effects for the pre-oviposition period as being influenced by ecological factors such as the abundance of nectar flow, temperature and air humidity. Moritz and Kühnert (1984) mention that the beginning of oviposition of artificially inseminated queens after insemination is delayed into the late season ( $14.3 \pm 0.9$  days) in comparison with those inseminated early in the season

( $5.7 \pm 0.3$  days). We have confirmed the presence of significant year and month effects on the beginning of oviposition, but compared with the previously published data, we have found the range and the variability of the trait to be much larger. In addition to the different definitions of the parameter in some of the cited studies, the larger variability in our study is likely to be resulted from the structure of our data set. Our data set is characterized by data from queens reared at several beekeepers and mated at many mating stations over a period of 20 years. Indeed, all factors, such as season, year, mating station, breeder and mating type significantly influence POP. Wilde (1994) has shown that, in addition to queen rearing, the storage of queens influences the onset of oviposition. Queens stored in queen banks are assumed to receive less care by the worker bees. We have no data on this possible impact, because this information was not indicated in the data set, since queen storage in queen banks is not a common practice in Germany. We have also confirmed the result obtained by Wilde (1994) that artificial insemination delays the onset of oviposition. Mating flight and the act of mating probably stimulate the physiological development of a queen, resulting in an earlier onset of oviposition (Königer, 1976).

Honey production, which is often assumed to represent colony vitality, is not significantly affected by POP in our study. Bienefeld and Pirchner (1990) have demonstrated that honey production is affected both by the phenotype of workers and the queen. If we assume that honey production is linked with queen vitality, then POP does not seem to provide an insight into the quality of the queen effect on honey production. Other studies support this finding. A long pre oviposition period of a queen is often assumed to be caused by delayed mating. Woyke (1960) and

Zmarlicki and Morse (1962) have stated that queens mating late tend to mate with a smaller number of drones and store fewer spermatozoa than those mating at the normal time. Richard et al. (2007) have found that the insemination quantity significantly affects mandibular gland chemical profiles and queen-worker interaction. Patricio and Curz-Landim (2002, 2007) report that, if mating does not occur at the correct age (about 6 days after emergence) queen ovarioles begin to degenerate. This result is likely to evoke a significant negative correlation between POP and colony traits. We have been unable to establish any significant relationship between POP and the honey yield of bee colonies which may also reflect the vitality of the queens. The data of De Souza et al. (2007) indicate that a queen may compensate for prolonged oviposition. In their study of stingless bees (*Melipona quadrifasciata anthidioides*), they have shown that, even when mating is delayed, the queen may compensate. Their study shows that even when mating-delay interrupts oogenesis, prevents vitellogenesis and causes ovary degeneration, these queens provide normal ovariole activation after mating.

The results of this study have demonstrated that honey production, which is assumed to characterize queen vitality, is not affected by POP but that POP is highly affected by several environmental factors during the period before the start of egg laying. Consequently, the pre oviposition period of honeybee queens should not be used as a tool to pre-evaluate honeybee queens.

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## CZY DŁUGOŚĆ OKRESU POPRZEDZAJĄCEGO SKŁADANIE JAJ U MATEK PSZCZOŁY MIODNEJ WPŁYWA NA PRODUKCJĘ MIODU PRZEZ ICH RODZINY?

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### S t r e s z c z e n i e

Na podstawie danych z Breeding Evaluation Centre w Niemczech, zebrano informacje dotyczące okresu poprzedzającego składanie jaj (POP) u matek pszczoły miodnej z 3648 rodzin pszczelich na przestrzeni 19 lat z 25 stacji kojarzenia i od 64 hodowców pszczoł. Okres poprzedzający składanie jaj (preowipozycyjny) u badanych matek wahał się od 6 do 34 dni, wynosząc średnio 15,8 dni. Około 80,7% matek rozpoczęło składanie jaj pomiędzy 8 a 18 dniem od wygryzienia. Długość okresu preowipozycyjnego (POP) wahała się znacznie pomiędzy latami i miesiącami badań oraz pomiędzy hodowcami pszczoł. Zarówno rodzaj kojarzenia jak i lokalizacja kojarzenia wpływały znacząco na długość okresu preowipozycyjnego. Długość POP była statystycznie znacząco najwyższa u matek zapłodnionych sztucznie (17,6 dni) w porównaniu z matkami zapłodnionymi naturalnie w stacjach kojarzenia zlokalizowanych na wyspach (15,4 dni) i na lądzie (14,9 dni). Nie wykazano istotności korelacji pomiędzy długością POP, a produkcją miodu w rodzinach pszczelich. Reasumując, okres poprzedzający składanie jaj jest wysoce zależny od warunków środowiskowych, jednak nie dostarcza informacji odnośnie potencjału matek.

**Słowa kluczowe:** *Apis mellifera*, matka, okres poprzedzający składanie jaj (przedowipozycyjny), produkcja miodu, składanie jaj.