Effect of pupal weight and insemination volume on oviposition of queen bees

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Introduction

A vulnerable period for a queen honey bee (*Apis mellifera* L.) is the time before she begins to lay eggs. During this period, she could be killed in her cell, killed by another queen, killed or injured by worker bees, or lost during her mating flights.

It is best for a colony to keep this preovipositional period as short as possible. Longer periods without egg laying not only increase the chance of death or injury to the queen, but diminish population growth by delaying egg laying in the colony.

From an economic viewpoint, the most critical period for an instrumentally inseminated (II) queen is between insemination and oviposition. This is because a queen triples in value after she is instrumentally inseminated. Labor costs (time) of producing a virgin queen to the point of insemination is about 6 minutes; drone production, semen collection, and insemination add about 13 minutes (Harbo 1985).

The period between insemination and egg laying is about 5 days for II queens that receive carbon dioxide (CO_2) narcoses and 2 days for naturally mated (NM) queens. However, without CO_2 narcoses, the 5 days for II queens extends to about 25, about when an unmated queen would begin to lay eggs (age 40 days, Mackensen 1947). In contrast, something in the natural mating process provides a strong stimulus to lay eggs. Koeniger (1981) found this stimulus to be contact with the male genitalia. The process may be chemical or physical, but Koeniger (1981) could not artificially induce virgin queens to lay by manually inserting the half-everted endophallus of a drone into the sting chamber of a queen.

My objective was to find stimuli other than CO_2 narcosis that would reduce the time between II and oviposition. Narcosis with nitrogen gas stimulates egg-laying in queens in a way very similar to CO_2 (Harbo 1986a), but cold narcosis has no apparent stimulative effect (Harbo unpublished). In this study, I looked for a natural stimulus that could complement or possibly replace CO_2 or nitrogen narcosis in stimulating oviposition. The experiment tested whether insemination volume or the pupal weight of a queen have a significant effect on how soon an instrumentally inseminated queen begins to lay eggs.

Materials and Methods

Experimental colonies

All the test colonies were established on 28 April 1987 with about 3,000 worker bees, a 2-day-old virgin queen, and 4 combs (one with honey, one with brood of all stages, and two empty). Each comb was 13 x 19 cm, and the hives could hold 11 of them. Each colony that had not produced eggs by 27 May was given one frame of capped brood (750 - 1050 cells). These combs contained no uncapped larvae or eggs.

Experimental queens

The queens (full sisters or supersisters) were grafted on 15 April and weighed as pupae on 23 April. The pupae were removed from their cells and weighed to the nearest milligram with an analytical balance. The cells were opened with a circular cut between the top of the cocoon and the excess jelly. The uncut section of about 3 mm kept the cell in one piece and served as a hinge to open the cell for queen removal. The hinge also guided my reclosing of the cell after the queen was returned. After weighing, a pupa was returned to her cell and to the incubator.

Abstract

Queens were weighed as pupae and inseminated with 0, 1, 6, or 12 µl of semen. The objective was to measure the effects of pupal weight and insemination volume on the time required for an instrumentally inseminated queen to begin to lay eggs. Cold (8°C) was used in place of carbon dioxide as a narcosis for insemination because cold does not stimulate egg laying as does carbon dioxide. Insemination volume did not have a significant effect, but pupal weight did. Heavier pupae were slower to begin egg laying when they became adults (3 days per 17 mg). The correlation was weak ($r = 0.38$), but the linear slope was significantly greater than zero ($P = 0.037$). In nature, this gives an advantage to larger queens, for when queens delay their oviposition because of adverse conditions, larger queens may delay longer and thus provide themselves with more days to achieve successful mating flights.

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Adult queens were marked with numbered tags and caged in the test colonies until 4 May when all queens were released and allowed to move freely in their colony. Excluder material over the entrances prevented the queens from leaving their colony, but as an added precaution, one wing was clipped to prevent any escaping queen from returning to her colony after a possible mating flight.

Insemination and narcosis

The queens were inseminated on 6 May when they were 10 days old. 200 µl of semen was collected from miscellaneous free-flying drones on the morning of 6 May, and the queens were inseminated in the afternoon. Queens were removed from their hive immediately before narcosis and returned after insemination.

Since CO_2 narcosis may mask the detection of other factors that stimulate oviposition, I eliminated CO_2 and used cold narcosis. Cold narcosis has very little or no stimulatory effect on the oviposition of queens.

The purpose of cold narcosis was to immobilize queens during insemination. The queens were kept at 8°C. for 28-38 minutes before insemination. Temperatures colder than this rendered queens too stiff to inseminate; warmer temperatures left queens too active. While in the cold, each queen was held in a queen holder as used during instrumental insemination. One by one, the queens were removed from the cold and quickly inseminated before regaining mobility (within 2 minutes). Queens were randomly assigned an insemination dose of 0, 1, 6, or 12 µl of undiluted semen, and inseminations were given in random sequence. The control group receiving no semen was treated with cold, but the insemination syringe was not inserted.

Data collection

Primary data were the age of each queen at the time of her initial oviposition. This time was calculated as 72 hours before the first eggs hatched. Colonies were checked at 3-day intervals. On 23 June, after all queens had oviposited, I used spectrophotometry to estimate the number of spermatozoa in the spermatheca of each queen (Harbo 1986b).

Analyses

Analysis of variance (complete randomized design) was used to evaluate the effects of insemination volume on the initial oviposition of queens. Pupal weight was a covariate in the analysis. When insemination volume and the interaction between insemination volume and pupal weight were found to be not significant, the analysis was reduced to simple regression with pupal weight as the independent variable and time to lay as the dependent variable. In a separate analysis of variance, I measured the effects of number of spermatozoa in the spermatheca on the time of initial oviposition.

Results and Discussion

Pupal weight had a significant effect ($P < 0.03$) on how rapidly a queen begins to lay eggs, whereas insemination volume and the interaction between insemination volume and pupal weight did not (Table 1). The relationship between number of spermatozoa in the spermatheca and insemination volume was similar to that reported by other investiga-

Table 1

Analysis of variance to evaluate the effects of insemination volume, pupal weight, and the interaction between pupal weight and insemination volume on the age at which a queen begins oviposition.

Source	df	mean square	F value	P
Insemination vol.	3	49.8	1.3	0.29
Pupal weight	1	193.5	5.2*	0.03
Pupal weight x insemination volume	3	52.1	1.4	0.27
Error	24	37.5		

Table 2

The time of initial egg laying by queens that were free-running with about 3000 workers in outdoor colonies. Queens received only a single, cold-induced (8° C) narcosis immediately before insemination. Data are means ± standard deviation. Ages at first oviposition were not significantly different among treatments (see Table 1).

Treatment (insem. vol.)	No. of queens	Age at first oviposition	No. of sperm in each spermatheca (X 10^6)
0 µl	8	34.8 ± 5.8	0
1 µl	7	28.7 ± 7.5	1.2 ± 0.5
6 µl	8	31.4 ± 6.8	2.8 ± 0.8
12 µl	9	31.7 ± 6.0	4.4 ± 0.7

tors (reviewed by Harbo 1985 and shown in Table 2). As with semen volume, number of spermatozoa had no effect on the onset of oviposition (F = 0.37; df = 1,19).

Queens that were heavier as pupae were slower to begin egg laying than queens that were lighter. The weights ranged from 245 - 290 mg. Regression analysis of weights alone produced the equation Y = - 17.74 + 0.183X, with pupal weight (in mg) as the independent variable (X) and age of initial oviposition (in days) as the dependent variable (Y). This means that a 17 mg increase in pupal weight resulted in an average delay of 3 days in initial oviposition. The effect of pupal weight was significant, but highly variable (R^2 = 0.136) indicating that pupal weight explained only 14% of the total variation in this experiment.

These results show that factors other than CO_2 narcosis can affect the initial oviposition of an unmated queen; queens that were lighter as pupae began oviposition sooner than queens that were heavier. Unfortunately, this has little practical significance because (1) beekeepers prefer larger queens and (2) the effect of queens size may be diminished by the CO_2 narcoses that queens receive during routine inseminations.

The results do not suggest that one should produce smaller queens. Although there is no conclusive

evidence that larger queens are more productive in a colony (cause a colony to produce more brood or honey) than smaller queens (Weiss 1983), larger queens have more ovarioles (Hoopingarner & Farrar 1959) and may be capable of laying more eggs per day.

The queens in this experiment are comparable to virgin queens that were free in a colony but not allowed to make mating flights. Since analysis showed that insemination had no apparent effect on oviposition time, the cold treatment (not known to affect behavior) was the only factor that would make the initial oviposition differ from a group of queens that had been put into these colonies to mate naturally.

In nature, queen size probably has no effect on oviposition unless mating is somehow delayed (weather conditions, lack of drones, etc.). In such cases, smaller queens would begin to lay unfertilized eggs sooner. The onset of egg laying indicates that a queen has ended her chance to mate naturally.

This earlier oviposition by smaller queens suggests that larger queens may have a higher probability of mating. Queens do not mate after they have begun to lay eggs, so when queens delay their oviposition because they are unable to mate, larger queens may delay longer and thus provide themselves with more days to achieve successful mating flights.

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