The Effect of Insemination on the Egg-laying Behavior of Honey Bees[1,2]

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ABSTRACT

When laying in worker comb, queen honey bees, *Apis mellifera* L., that were inseminated with live semen laid eggs in 3.0–9.6 times more cells than did queens that were not inseminated or were inseminated with frozen (nonfunctional) semen. Also when queens inseminated with live semen laid in a small colony, 0–7% of the cells with eggs had an egg positioned sideways or on the side of the cell. In contrast, this percentage rose to 33–94% when queens were not inseminated or were inseminated with frozen semen. These differences in behavior may arise from a disruption of the egg fertilizing phase of egg laying.

In insects, mated females commonly lay eggs at a faster rate than unmated females (Hunter and Hinds 1905, Davey 1965, Riddiford and Ashenhurst 1973, Nielsen and Cantwell 1973, and Gupta 1973). In addition, mated females often exhibit ovipositional patterns that differ from unmated females. Engelmann (1975) cited many examples. The objective of my study was to determine if such behavior occurs in honey bees (*Apis mellifera* L.) and, if so, to gain insight into possible causes.

My approach was to compare the effects of 3 different treatments (insemination with live semen, insemination with frozen semen, and no insemination) on the rate of egg laying and the egg positioning of queens. On the basis of preliminary tests made in 1973, I formulated 2 hypotheses. (1) Queens inseminated with live semen will lay eggs in more cells than uninseminated queens or queens inseminated with previously frozen semen. (2) Queens inseminated with live semen will lay eggs sideways in $<10\%$ of the cells in which they lay eggs; uninseminated queens or queens inseminated with frozen semen will lay eggs sideways in $>15\%$ of the cells in which they lay eggs.

METHODS AND MATERIALS

On June 11, each queen in a group of hybrid (YDGk) sisters was inseminated with 7 μl of live semen, inseminated with 7 μl of previously frozen semen, or not inseminated. The semen was collected 12 h before insemination and stored in 4 capillary tubes (Harbo 1974), 2 at 14°C (live semen) and 2 at −19°C (frozen semen). The uninseminated queens were held in an insemination holder to receive CO_2 anesthesia that was equal to that received by the inseminated queens (ca. 3–4 min), but no syringe tip was inserted. One June 13 all queens were given a 10-min CO_2 anesthesia to stimulate egg laying (Mackensen 1947).

On June 24, each queen was introduced into a small colony containing ca. 2500 bees. The colonies had small frames with 13×19 cm of comb.

After all the queens had been laying about 5 days (July 2), I removed all the combs and put one frame of capped worker brood, one frame of honey, 1 or 2 frames of foundation, and ca. 450 ml of sugar syrup into each colony. I did this so that all colonies would have a fresh supply of worker bees and none would be exposed only to drone brood for an extended period.

On July 13, I again exchanged the combs, this time to begin the test routine. Each colony then had one frame of solid capped honey, one empty worker comb that was darkened by use (5 g of pollen were placed in an upper corner of each empty comb) and 1 or 2 frames of foundation.

The test period began at 8:30 AM, July 14, and ended at 8:30 AM, July 17. At 8:30 AM, July 14, and at 24-h intervals thereafter, I rotated the queens, collected the comb of eggs from each colony, and gave 1 new comb (with pollen) to each colony. Queens were transferred without an introduction device.

Thus each queen laid for 24 h in each colony in its group. This equalized the effect of minor differences that existed among the bees and the populations of the colonies.

Each day, as soon as the darkened combs were removed from the colonies, the number of cells containing eggs and the positioning of the eggs were recorded. Egg positioning was divided into 2 categories: uniform (a single egg erect at the base) and nonuniform (Fig. 1). The nonuniform cells consisted mainly of a single egg sideways at the base or on the side of the cell; about 10% had multiple eggs with one or more eggs sideways.

In a 2nd test designed to measure the effect of drone cells on egg laying behavior, I set up a daily queen and comb rotation scheme similar to that described above. I used 4 unmated and 5 mated queens (sister KbYD hybrids). The test procedure differed in that no frames of honey were in the colonies, no pollen was fed, and no foundation was used during the 2-day test period. Instead, I gave each colony 2 frames of comb containing drone and worker cells and 15 ml of sugar syrup per day.

[1] Hymenoptera: Apidae.

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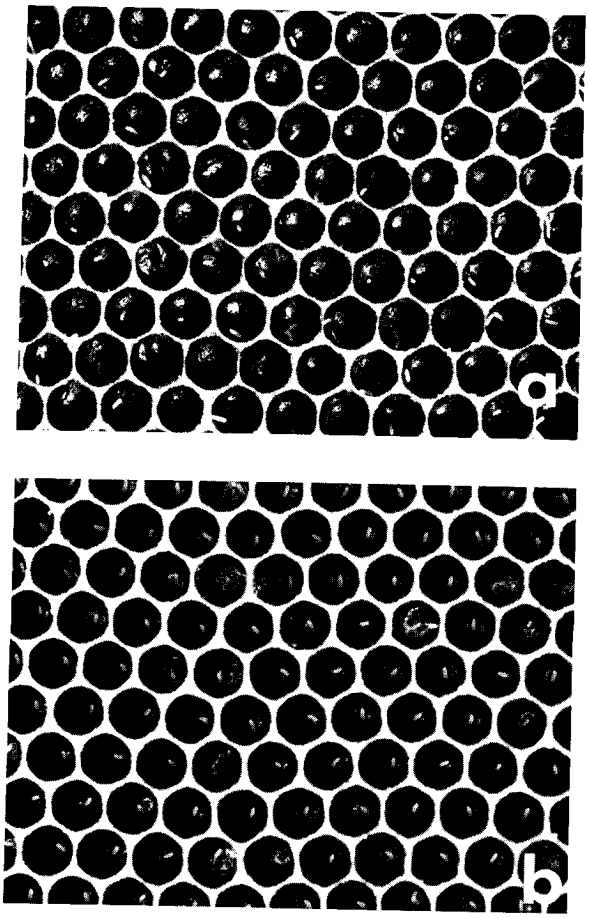

FIG. 1.—Typical egg laying patterns: a, nonuniform egg positioning by uninseminated queen no. 8; b, uniform egg positioning by mated queen no. 7 in the same colony on the following day.

RESULTS

My experimental hypotheses were supported. The queens inseminated with 7 μl of live semen placed eggs in significantly more cells and positioned significantly fewer eggs sideways than uninseminated queens [$P < 0.01$ with the Kolmogorov-Smirnov test (Siegel 1956)]. Queens inseminated with frozen semen were not significantly different from uninseminated queens in these respects.

The number of cells with eggs and the egg positioning of each queen are summarized in Table 1. During the 3-day test period, queens inseminated with live semen produced from 3.0–9.6 times more cells with eggs than the other queens in their respective test group. Each uninseminated queen and queen inseminated with frozen semen produced at least 45% of their cells with eggs lying sideways on the base or attached to the side of the cell; daily observations ranged from 33–94%. In contrast, queens inseminated with live semen produced only 0.1–3.6% of their cells with eggs positioned nonuniformly; daily observations ranged from 0–7%. Fig. 1 shows the positioning of eggs by an uninseminated queen and by a queen inseminated with live semen.

Spectrophotometric counts of sperm (Harbo 1975) revealed that only the 5 queens inseminated with live sperm had spermatozoa in their spermathecae. Queen 12 had 4.1×10^6 spermatozoa, queen 7 had 3.8×10^6, queen 1 had 3.7×10^6, queen 10 had 3.4×10^6, and queen 4 had 2.1×10^6.

In the supplementary test where I gave queens a choice of laying in worker or drone cells, mated queens oviposited in 926 drone cells and 3937 worker cells (ten 24-h observations); the unmated queens laid in 776 drone cells and 472 worker cells (seven 24-h observations). The egg positioning in drone cells was similar; 23% and 24% nonuniformly positioned by the mated and unmated queens respectively. But the egg positioning in worker cells was significantly different ($P < 0.01$ with analysis of variance): 6% and 35% improperly positioned by mated and unmated queens respectively.

DISCUSSION

I believe that the daily rotation of queens from one colony to another minimized the effect of workers on my results. The 3 queens in each test group had equal contact with the same groups of workers. Changes in daily egg production of each colony corresponded to the queen rotations; thus egg production reflected the activity of the queens rather than that of the resident worker population. Then since the unfertilized queens showed a consistent tendency to lay fewer eggs and to position those eggs nonuniformly, one may look to the queen for an explanation.

Significantly, reduced and nonuniform egg laying

Table 1.—Effect of insemination on egg laying rate and positioning by queen honey bees. Groups A–C consisted of 3 queens and 3 colonies; groups D and E each had 2 queens and 2 colonies. Each queen spent 24 h in each colony in its group.

Group no., queen no. & insemination	Average daily count of cells with eggs ($\bar{x}\pm s_{\bar{x}}$)	Cumulative % of cells with nonuniform egg positioning
Group A		
1 live semen	588± 69	0.2
2 not inseminated	192± 28	69
3 frozen semen	160± 69	84
Group B		
4 live semen	538±193	4.7
5 not inseminated	59± 14	82
6 frozen semen	80± 35	82
Group C		
7 live semen	764± 77	0.7
8 not inseminated	258± 18	47
9 frozen semen	115± 26	53
Group D		
10 live semen	575±255	0.1
11 not inseminated	60± 4	62
Group E		
12 live semen	860± 9	0.9
13 not inseminated	96± 5	69

occurred only when a nonmated queen laid an egg in a worker-sized cell, a cell that normally receives a fertilized egg. When I counted only drone-sized cells, cells that normally receive an unfertilized egg, the laying behavior of mated and nonmated queens did not differ. Perhaps reduced and nonuniform egg laying were the result of egg laying disruptions caused by queens having no sperm trying to fertilize eggs.

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