Oviposition Rates of Instrumentally Inseminated and Naturally Mated Queen Honey Bees (Hymenoptera: Apidae)

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Ann. Entomol. Soc. Am. 79: 112–115 (1986)

ABSTRACT Egg-laying rates of queen honey bees, *Apis mellifera* L., were measured in colonies containing no brood and ca. 3,000 or 12,000 worker bees. Naturally mated queens were heavier and laid more eggs per day than instrumentally inseminated queens. CO_2 narcosis caused weight loss in queens and may account for at least some of the difference. When nitrogen narcosis replaced carbon dioxide during instrumental insemination, queen weights increased, but still did not equal those of naturally mated queens that received no narcosis. The correlation between egg-laying rate and queen weight was $r = 0.73$ ($n = 112$).

HARBO AND SZABO (1984) found that instrumentally inseminated (II) queen honey bees, *Apis mellifera* L., did not survive as long as naturally mated (NM) queens and that colonies with II queens produced less brood. Therefore, even if genetically superior colonies are produced using II, this advantage can be quickly lost by early supersedure or reduced brood production. To take full advantage of controlled breeding, one must produce II queens that are not handicapped by the insemination process.

The purpose of this study was to describe the differences between II and NM queens in more detail and, thus, begin to find the causes of these differences. I used oviposition rate, brood production, and queen weight (perhaps a measure of egg production status) to evaluate the queens.

The variables, CO_2 narcosis and N_2 narcosis, were included in some experiments because CO_2 narcosis is known to be detrimental to honey bees as well as other insects. CO_2 narcosis slows larva growth in *Heliothis zea* (Boddie) (Edwards 1968), reduces fecundity in *Blattella germanica* (L.) (Brooks 1965) and *Drosophila melanogaster* Meigen (Perron et al. 1972), changes the behavior of worker bees (Ribbands 1950, Skowronek and Jaycox 1974), and reduces the production of the pheromone 9-oxodec-*trans*-2-enoic acid in queen bees (Pain et al. 1967). Skowronek (1979) found that when queens were treated with CO_2 shortly after natural mating, the treated queens laid more eggs per day initially (July), but the controls produced more eggs per day in the following year.

Materials and Methods

Instrumental Insemination Versus Natural Mating. Ten experiments compared queens that were II or NM. Five compared II and NM queens in small colonies containing ca. 3,000 bees (Table 1), and five compared II and NM queens in colonies that started with ca. 12,000 bees (Table 2). None of the colonies started with brood. The queens in each experiment were sisters of the same age that were reared in the same colony.

The following treatment was given to all queens except those in experiment 4. Each queen was free in a small colony (ca. 3,000 bees) at the time of the insemination. Narcotization was performed only during insemination while the queen was in the plastic holder. A flow rate of ca. 35 ml/min for CO_2 or ca. 45 ml/min for N_2 was maintained for 1–2 min. During this time the queens were given 2 μl of semen [for II(CO_2) and II(N_2) groups] or 0.3 μl of saline (for CO_2–NM groups). (Abbreviations are explained in footnote a of Table 1.) The saline consisted of 0.85% NaCl and 0.25% dihydrostreptomycin sulfate. Queens in experiment 3 were inseminated only once, whereas all others were inseminated three times on consecutive days.

The treatment of queens in experiment 4 differed from the above in the following ways. The queens were stored together in a colony that had no laying queen. Each queen was kept in a separate cage and was given a single insemination of 2 μl of semen when 7 days old. On the next 2 days, they were treated for 90 s with CO_2 or N_2. The queens were put into nuclei when 12 days old and released 3 days later. The N_2–NM and CO_2–NM queens were given one 90-s narcosis when 8 days old and were not inseminated with saline.

All queens were evaluated after they had laid eggs. Queens in experiments 1–5 were weighed 3 days after initial oviposition (when larvae began to hatch). For weighing, each queen was removed from her colony for 5–10 min. Egg counts were made after the queens were returned (caged) to the colony. Eggs were counted in experiments 1 and 3, but in experiments 2, 4, and 5, the numbers of eggs were estimated using a wire grid to mea-

Table 1. Effect of insemination and narcosis on the egg-laying rate and weight of queens laying in a colony with 2,000–4,000 workers (data are mean ± SD)

Experiment	Treatment	No. of queens	Eggs per day in first 3 days	Queen wt (mg)	
				Day 3	Day 9
1 (Apr. 1982)	NM	7	681 ± 165	253 ± 10	244 ± 11
	II(CO_2)	7	542 ± 131	229 ± 7	231 ± 11
2 (May 1982)	NM	5	809 ± 89	280 ± 16	261 ± 20
	CO_2–NM[a]	6	—	237 ± 9	238 ± 11
	II(CO_2)	6	498 ± 130	244 ± 7	234 ± 13
3 (June 1982)	NM	11	670 ± 98	262 ± 10	—
	CO_2–NM[a]	6	—	235 ± 15	—
	II(CO_2)	6	320 ± 187	232 ± 21	—
4 (July 1982)	NM	7	—	262 ± 14	—
	N_2–NM	5	—	253 ± 17	—
	II(N_2)	6	617 ± 75	251 ± 12	—
	CO_2–NM	3	—	241 ± 28	—
	II(CO_2)	6	459 ± 133	233 ± 14	—
5 (Mar. 1983)	NM	6	360 ± 78	260 ± 16	—
	II(N_2)	6	336 ± 67	243 ± 5	—
	II(CO_2)	6	311 ± 78	237 ± 12	—

[a] Of 12 CO_2–NM queens in experiments 2 and 3, only 3 of the queens mated. The remaining nine laid unfertilized eggs.

sure the area of comb that contained eggs (assuming 1 egg per cell and 3.9 cells per cm^2) (Moeller 1961).

Egg laying in the larger colonies was estimated by measuring the areas of capped brood (experiments 6–10). Because eggs hatch ca. 72 h after oviposition, the colonies were checked for larvae 3–4 days after the queens were released to establish when egg laying began. When larvae were first observed, they were counted, and the time of counting was designated as age 72 h for the brood. Thus, time zero was slightly after the first egg was laid. Exactly 11 days after time zero, the area of capped brood was measured with the wire grid mentioned above. Worker brood is capped ca. 7.7 days after the egg is laid, so the amount of capped brood at 11 days corresponds to the number of eggs laid during the first 3.3 days. To estimate the number of eggs laid in the first 3.3 days, I multiplied cm^2 of capped brood by 3.9 and subtracted the number of larvae counted on day 3. I calculated the number of eggs laid on days 4–6 by measuring capped brood on day 14 and subtracting the 11-day measurement.

The number of spermatozoa in the spermathecae was counted only in the queens from experiments 9 and 10. Counts were made spectrophotometrically. The technique was similar to that described by Harbo (1975) except the contents of each spermatheca were diluted to 10 rather than to 5 ml of diluent (0.5 *M* NaCl). The conversion formula was $y = 15.74x - 0.41$ (x = absorbence at 230 nm; y = millions of spermatozoa per 10 ml).

Oviposition rates and queen weights of the various treatments were compared using analysis of variance. Experiments 6–10 were treated as five blocks in a single split-plot analysis. Differences at the 0.05 level were accepted as significant.

Egg-laying Rate and Body Weight. Linear regression was used to measure the correlation between oviposition rate and body weight. I measured this relationship in a variety of queens to broaden the sampling base. The intent was to use body weight only to predict the egg-laying status of a queen. Queens were removed from their colonies and weighed to the nearest milligram. Then the number of eggs in the colony was estimated by counting eggs or measuring comb area. There were 75 observations of II(CO_2) and NM queens from experiments 1–5 and 37 from NM queens in miscellaneous colonies having populations as high as 25,000 bees. All queens were laying fertilized eggs and sampling dates ranged from March to October.

CO_2 Narcosis After Natural Mating. A change in queen weight was used to test the effect of CO_2 on queens after natural mating. Sister queens were allowed to fly from small colonies to mate naturally. The queens were weighed 4 days after initial egg laying. When all the queens had been weighed, they were caged and brought into the laboratory. Half were chosen randomly for CO_2 treatment and the others served as controls. The queens were 20 (experiment 11) and 16 (experiment 12) days old at this point. The treated group was put into a plastic bag that was rapidly inflated with CO_2 gas until the queens were immobilized. The bag was then closed, and the queens were left in the CO_2 atmosphere for 2 min. All the queens were returned to their respective colonies. After 3 days (experiment 11) or 7 days (experiment 12), the brood combs were replaced with empty combs and the queens were released. The queens were reweighed 4 days later.

The change in weight from the first to the second weighing was recorded for each queen. With these values, the test and control groups were compared (t test).

Table 2. A comparison of brood produced in colonies with naturally mated or instrumentally inseminated queens; all colonies started with no brood and ca. 12,000 bees (data are mean ± SD)

Experiment	Treatment[a]	No. of queens	Eggs laid per day[b]		
			Days 0–3	Days 4–6	Days 0–19[c]
6[d] (Apr. 1982)	NM	5	918 ± 162	1,148 ± 75	948 ± 68
	II(CO_2)	5	738 ± 147	939 ± 66	771 ± 49
7 (June 1982)	NM	4	952 ± 106	968 ± 128	875 ± 62
	II(CO_2)	4	784 ± 66	848 ± 40	886 ± 55
8 (Sept. 1982)	NM	6	586 ± 103	604 ± 100	597 ± 99[e]
	II(CO_2)	5	502 ± 126	578 ± 200	593 ± 59[e]
9 (Apr. 1983)	NM	6	678 ± 59	1,118 ± 87	1,086 ± 92
	II(CO_2)	5	673 ± 171	1,016 ± 234	873 ± 239
10 (Apr. 1983)	NM	4	907 ± 65	1,278 ± 203	1,172 ± 67
	II(CO_2)	4	698 ± 132	1,122 ± 222	1,117 ± 115

[a] Each II queen received three inseminations of 2 μl of semen and ca. 2 min of CO_2 narcosis during each insemination.
[b] Estimates were based on measures of capped brood. Since egg and larval mortality account for some loss, the estimates may be ca. 10% low.
[c] Workers began to emerge 19 days after initial egg laying, so 19 days represents one complete brood cycle.
[d] Queens in experiments 6 and 9 were 1 month old, those in experiment 7 were 2 months old, and those in experiments 8 and 10 were 6 months old during the test period. All were mated at 1–2 weeks of age.
[e] Each lost two observations, so means represent four and three queens.

Results

Instrumental Insemination Versus Natural Mating. When laying in small colonies having ca. 3,000 bees, NM queens consistently laid more eggs per day than II(CO_2) queens during the first 3 days of oviposition (Table 1) ($P < 0.01$). Moreover, body weights of NM queens were significantly heavier than the weights of II(CO_2) queens 3, 6, and 9 days after initial egg laying (6 and 9 day data were from experiments 1 and 2 only) ($P < 0.05$).

When N_2 narcosis replaced CO_2 narcosis, the egg-laying rate and body weight increased. II(N_2) queens had higher oviposition rates and greater body weights than II(CO_2) queens, but their body weights were still less than that of NM queens (experiments 4 and 5) ($P < 0.05$).

Queens given CO_2 or N_2 narcosis and then allowed to naturally mate (CO_2–NM and N_2–NM queens in experiments 2, 3, and 4) were very similar in weight to their respective II(CO_2) and II(N_2) queens. Although many did not mate naturally after receiving narcosis, those that did mate did not weigh as much as normal NM queens. Nine of 12 CO_2–NM queens in experiments 2 and 3 did not mate, and 3 of 6 CO_2–NM and 2 of 7 N_2–NM queens in experiment 4 did not mate. The weights of all queens were reported in experiments 2 and 3 (the weight of the one mated queen in experiment 2 was 4 mg below the group mean and weights of the two mated queens in experiment 3 averaged 8 mg above the mean). Only the mated queens are reported in experiment 4.

II(CO_2) queens laying in colonies of 12,000 bees laid fewer eggs per day than NM queens (Table 2). Significant differences were detected at all intervals tested (0–3 days, 4–6 days, and total), but the differences were greatest at the onset of egg laying (df = 1,38; $F = 13.8$, $P = 0.0007$ for 0–3 days; $F = 9.2$, $P = 0.004$ for 4–6 days; and $F = 6.8$, $P = 0.014$ for 0–19 days).

There was no significant correlation between the number of spermatozoa in the spermatheca and the number of eggs laid per day ($r = -0.18$ in experiment 9 and $r = 0.31$ in experiment 10). After experiments 9 and 10, the II queens had mean sperm counts of 5.15 and 3.87 million and the NM queens had means of 4.27 and 5.17 million, respectively.

Egg-laying Rate and Body Weight. The correlation between egg-laying rate and body weight was $r = 0.73$ ($n = 112$). The formula was $y = 15.9x - 3{,}433$ (y = number of eggs laid per day; x = mg body weight). Observations included eggs per day from 100–1,550 and queen weights from 200–315 mg.

CO_2 Narcosis After Natural Mating. CO_2 narcosis after NM caused queens to lose weight (Table 3). In June 1982, the queens treated with CO_2 lost 9.2 ± 5.6 ($\bar{x}$ ± SD) mg, whereas controls gained 2.6 ± 4.9 mg ($P < 0.01$). In August 1984, treated queens each lost 1.3 ± 7.1 mg; controls gained 6.8 ± 9.9 mg ($P < 0.05$).

Table 3. Weight change of queens that were treated with 2 min of CO_2 narcosis after they had naturally mated and begun to lay eggs

Experiment	Treatment	No. of queens	Mean wt before treatment (mg)	Net wt change ($\bar{x}$ ± SD)[a]
11 (June 1982)	Control	5	259	2.6 ± 4.9 mg
	CO_2	6	264	−9.2 ± 5.6 mg
12 (Aug. 1984)	Control	10	260	6.8 ± 9.9 mg
	CO_2	10	262	−1.3 ± 7.1 mg

[a] In both experiments, the queens treated with CO_2 lost significantly more weight than the control ($P < 0.01$ in experiment 11; $P < 0.05$ in experiment 12).

Discussion

Instrumentally inseminated queens that received CO_2 narcosis laid fewer eggs per day than NM queens. This response was not absolute, but was proportional to the worker population in the queen's colony. For example, during the first 3 days of egg laying, II(CO_2) and NM queens laid ca. 500 and 700 eggs per day, respectively, when laying in colonies with ca. 3,000 bees (Table 1) and ca. 700 and 900, respectively, when laying in colonies with ca. 12,000 bees (Table 2). Thus, both II(CO_2) and NM queens responded to larger populations by producing more eggs. The difference was that the NM queens responded to both populations with more eggs per day than did the II(CO_2) queens. This lower response may be associated with the early supersedure that was reported for II(CO_2) queens (Harbo and Szabo 1984).

Experiments 4 and 5 were designed to test N_2 as a substitute for CO_2 narcosis. N_2 did not immobilize the queens as quickly as CO_2 but was adequate and similar to CO_2 in that it stimulated the queens to lay eggs. II(N_2) queens laid more eggs per day than II(CO_2) queens, but further tests are needed before CO_2 is replaced by N_2. Ribbands (1950) reported that N_2 had about the same effect on worker bees as CO_2. The use of N_2 may be similar to the use of lower concentrations of CO_2 recommended by Ebadi and Gary (1980).

Data in experiments 2–4, 11, and 12 point to CO_2 narcosis as a cause of weight loss in queens. This is somewhat paradoxical because CO_2 also stimulates II queens to start egg laying much sooner than if CO_2 is not given (Mackensen 1947). Thus, CO_2 stimulates weight gain and egg laying, but the queens stimulated in this manner are not as heavy and do not produce as many eggs per day as queens stimulated to lay eggs through natural mating. Moreover, CO_2 seems to have a dominating effect because queens treated with both CO_2 and NM are similar to the CO_2 rather than to the NM groups.

The weight loss caused by CO_2 after NM was about 10 mg per queen (Table 3). Based on the above formula, a 10-mg drop in body weight indicates a reduction of 160 eggs per day, which may explain much of the difference between the egg production of NM and II queens (Tables 1 and 2). However, the difference in the body weight of II(CO_2) and NM queens was >10 mg (Table 1), so it is possible that other factors present or absent in II queens may account in part for the lower egg production and body weight.

Acknowledgment

Shirley Painter and Susan Cobey (Bee Breeding and Stock Center Laboratory) assisted in all parts of this project. Michael Brown (USDA-ARS, Stoneville, Miss.) gave advice on statistical procedures. This work was done in cooperation with La. Agric. Exp. Stn.

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Received for publication 1 April 1985; accepted 29 August 1985.