EFFECT OF QUEENLESSNESS ON WORKER SURVIVAL, HONEY GAIN AND DEFENCE BEHAVIOUR IN HONEYBEES

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Summary

The effects of queenlessness on worker honeybees (*Apis mellifera*) were tested with 50 colonies in groups of 10 (five treatments and two replicates) in August, October and December, 1984 and February and April, 1985 in Baton Rouge, Louisiana. The 10 colonies in each group were all from a single heterogeneous mixture of bees, and each colony began with about 6000 workers and no brood. The five treatments each lasted for 28 days and consisted of (1) caged queen for 28 days, queenless for 0 days; (2) caged queen for 21 days, queenless for 7 days; (3) caged queen for 14 days, queenless for 14 days; (4) caged queen for 7 days, queenless for 21 days and (5) caged queen for 9 days, laying queen for 19 days (control). With prolonged queenlessness worker survival, colony weight gain and defence behaviour (number of stings) decreased. Queenlessness did not induce drifting.

Introduction

A queen honeybee affects a colony in more ways than by just laying fertilized eggs. She inhibits ovary development in workers (Voogd, 1955; Butler, 1956, 1957; Butler & Fairey, 1963), and if she is absent certain workers lay eggs (Perepelova, 1926; Ruttner & Hesse, 1981) and show mandibular gland development (Costa-Leonardo, 1985). The absence of a queen lowers survival in groups of up to 100 bees (Roger & Pain, 1966; Filipović-Moskovljević, 1972); in colonies with about 10 000 bees (Jaycox, 1970a); and in caged diseased bees (Kulinčević et al., 1973). Queen pheromone, 9–oxodec–*trans*–2–enoic acid, or the presence of a queen, stimulates nectar foraging (Jaycox, 1970a). Workers leaving the presence of a queen transfer food more often than do workers observed at random (Ferguson & Free, 1980).

Few studies have used a variety of queenless conditions. Accordingly in this study we used different queenless periods and compared their effects on (1) worker survival, (2) colony weight gain per bee per day , (3) worker drift (bees changing their residence to another colony), (4) colony temperature and (5) colony defence behaviour.

Materials and Methods
General
Fifty colonies were tested in a randomized block design. Each block (trial) consisted of 10 colonies (five treatments and two replicates). Each treatment began with caged queens which were released or removed at different times: caged queen for 28 days and queenless for 0 days (QL-0); caged queen for 21 days and then queenless for 7 days (QL-7); caged queen for 14 days and then queenless for 14 days (QL-14); caged queen for 7 days and then queenless for 21 days (QL-21); and caged queen for 9 days and then free-laying queen for 19 days (control, QR). Queen cages were made of hardware cloth (8 mesh per 2·5 cm) rolled into a rectangle and closed at each end with a wooden plug. Trials were run in August, October, December, 1984, February and April, 1985 in Baton Rouge, Louisiana.

For each trial about 12 kg of bees were collected from five to seven normal colonies into a common cage. After 1–2 days, bees from the cage were divided into 10 smaller packages of about 6000 bees each.

The bees were put into similar hives. Ten small, broodless hives were set up at the beginning of each trial, each with six pre-weighed combs (worker cells) containing enough honey to feed the bees during the trial. Large-mesh screening was placed across the front of the hives to keep skunks from eating bees. Hives were placed 20–50 metres apart facing SE. Treatments were randomly assigned to the hives in each trial.

The bees were put into the hives in the late afternoon of day 0. A caged queen (all queens were sisters that had mated naturally) was placed inside each hive between the two centre combs, then a package of bees was placed on top of each open hive and secured with tape.

Excluder material across the package openings kept drones and dead workers in the packages for later removal. Colony entrances were kept closed until the next morning when the packages were removed.

Worker drift was checked to confirm that moving bees did not alter the data for survival and honey gain. A paint colour was assigned to each colony on day 5, and 300 bees from each colony were marked on the thorax (colony treatments were not yet begun and each colony had a caged queen at this point). During the final examinations, bees of non-resident colour were noted.

During the trial, we periodically removed queens. Queens in the QL-21 group were removed on day 7 and queens in the remaining treatment groups were removed at weekly intervals. Control queens were not released until day 9 to prevent emergence of young bees during the test period. Data for survival, wt gain, and drift were collected on day 28.

Survival
Estimates of the original population for each colony were made by weighing bees and counting samples. About 750 g of bees were poured into each of the 10 packages which were pre-weighed. The exact weight of bees was recorded for each package. During this process, three samples of about 100 bees were taken from the 12-kg cage, weighed, and counted in order to obtain average wt of workers. After the bees had entered the hives the packages were removed and the numbers of drones and dead workers in each were counted. Weight of drones (0·2 g per drone) was subtracted from the total bee wt for each package, and the difference (worker wt) divided by mean worker wt to estimate the number of workers in the hive. Finally, the number of dead workers in the package was subtracted from this value to estimate the original population of each colony.

To determine final population sizes we closed colony entrances on the morning of day 28 when there was no flight activity. Each hive with bees was weighed to the nearest gram. The bees were then brushed from the combs and the hive was re-weighed to find the final wt of bees. About 100 bees were sampled, weighed, and counted and the final population was estimated. Percent survival and mean population size were calculated from the initial and final populations.

Weight gain
Weight gain or loss was found by weighing the combs (Harbo, 1983) and estimating the weight of bee foreguts. All frames of comb were weighed to the nearest gram. Foregut weights were calculated for each colony with the following regression equation (Harbo, unpublished): $Y = 0·76X - 70·4$ ($r = 0·83$, $n = 115$) where $X =$ wt per bee (mg) and $Y =$ foregut wt per bee (mg). We multiplied the foregut wt by the population size to estimate the wt of honey stored in the foreguts. Net wt gain was estimated by subtracting comb and foregut wts for the original population from the corresponding values for the final population.

Brood wt was estimated for colonies with brood. Total brood area (cm^2), excluding eggs, was measured then multiplied by 3·9 (number of worker cells per cm^2) to give the total number of brood cells. Brood wt per cell was estimated by weighing brood at various stages and using the larval wt estimates of Nelson et al. (1924). In colonies without laying queens mean brood wt varied with age of brood. The means were found by estimating the age of the oldest brood and then using the wt estimates. Mean brood wt per cell multiplied by the number of brood cells gave total wt of brood.

Net weight gain of a colony was then adjusted for the wt of honey and pollen that had been used to rear the brood. Rosov (1944) estimated that the weight ratio of food to brood was 2:1. Weight adjusted gain included the net gain (or loss) of food that had been stored plus that which had been fed to larvae.

To find mg gained or lost per bee, we assumed a linear change in colony populations and determined the mean (original + final population for each colony/2). Colony gain was then divided by mean population to give wt gain per bee. Dividing this value by the number of days in the experiment (28) gave average daily gain (or loss) per bee.

Colony temperature
To find mean colony temperature, an 18-cm wooden rod with thermocouples attached to it was

placed next to the caged queen between the two centre combs of each colony, 20 cm from the front and 30 cm from the back of the hive. Thermocouples on the rod were (from top to bottom) at the 4-cm, 9-cm and 15-cm marks. Thermocouple wires remained outside the colony and had numbered plugs for a digital thermometer. Readings were taken at least twice during the last week of the experiment. A temperature for each colony observation was found from the mean of the three thermocouple readings. The overall mean was used as the colony temperature. Data for colonies without brood were analysed separately to compare the effect of queenlessness and presence of brood on temperature.

Defence behaviour

The test colonies were compared for stinging behaviour by counting the number of stings on red suede–leather patches that were dragged lightly across the tops of the exposed frames. A long rod with a clip at one end was used to hold the patches, each of which was 5 cm square. At time 0 the hive lid was removed without using smoke. At 10 s an unused patch was dragged front to back over the frames, then back to front, then twice side-to-side. The time for each 'pass' was 5 s and the 20-s sequence was repeated three times to give the patch a 60-s exposure to the bees.

This test was used only for trials 3, 4 and 5. For each trial the test was done at least twice during the last week of the experiment. The total count of stings for each colony was used in the analysis of defence behaviour.

Statistical analysis

Analysis of variance (randomized-block design) with separation of means by LSD was used to show treatment differences in all variables. Sources of variation were treatment, trial, and treatment-trial interaction (error–term). Trials, or month-effects, were blocked because the experiment covered only one year and months were not replicated. Orthogonal polynomial contrasts (Steel & Torrie, 1980), excluding QR colonies, were used to show slope trends in the treatment means. The arcsin transformation was used on percentage data. Significance at $\propto$ = 0·05 was accepted as indicating a difference.

Results and Discussion

Survival

There were differences among treatments (P = 0·01, Table 1). In the four QL treatments worker survival showed a negative linear slope with prolonged queenlessness (P = 0·0005).

TABLE 1. Analysis of variance of five dependent variables for newly established honeybee colonies with queens removed for different periods.
Tm = treatment; Tr = trial. Tm × Tr interaction was used as the error–term.

Variable	Source of variation	df	F	P
Survival	Tm	4	4·71	0·0105
	Tr	4	12·76	0·0001
	Tm × Tr	16	1·81	0·0893
Total wt gain	Tm	4	10·72	0·0002
	Tr	4	87·33	0·0001
	Tm × Tr	16	1·22	0·3238
Adjusted wt gain	Tm	4	14·41	0·0001
	Tr	4	81·66	0·0001
	Tm × Tr	16	1·19	0·3380
Temperature	Tm	4	9·35	0·0004
	Tr	4	24·14	0·0001
	Tm × Tr	16	1·69	0·1173
Defence	Tm	4	3·98	0·0459
	Tr	2	0·94	0·4284
	Tm × Tr	8	0·57	0·7873

TABLE 2. Effects on newly established honeybee colonies of removing the queen for different periods. Each colony began with no brood and a caged queen. The laying queen was released after 9 days and no bees were added during the 28-day test. Means are from combined data of five trials. Means and LSD mean separation followed by different letters are different at $\propto$ = 0·05. The mean square error is given in parentheses for each variable.

Treatment[1]	Survival (%) (1·2)	Total wt gain[2] (mg) (6·3)	Adjusted wt gain[3] (mg) (7·6)	No. of brood cells[4] (21043)	Colony temp. (°C) (1·8)	No of stings (238)
QR	65·0bc	−0·7a	4·3a	5310a	33·2a	26·2a
QL-0	73·7a	0·3a	0·3b	0c	29·4c	16·8ab
QL-7	69·8ab	−3·4b	−3·4c	0c	29·5c	11·8ab
QL-14	62·3cd	−4·4b	−4·1cd	300c	30·2c	3·0b
QL-21	55·2d	−7·1c	−6·1d	1175b	32·0b	4·5b

[1] Colonies queenright (QR) or number of days queenless (QL).
[2] Colony wt gained per bee per day.
[3] Colony wt gained per bee per day adjusted for brood reared.
[4] Cells with eggs not included.

Survival was highest in broodless colonies with caged queens. Survival in the QL-0 treatment (caged queen, no brood) was higher than in the QR colonies with brood of all ages (P < 0·05, Table 2). It seems that brood rearing, or the activity required for it (such as increased pollen foraging), was associated with reduced worker survival as shown by Maurizio (1961).

However, queenlessness alone reduced survival. Among the four QL treatments the linear slope of treatment means showed that there were no sudden changes in survival after brood rearing began (with the QL-14 and QL-21 treatments). This supports earlier conclusions that queenlessness may cause enough stress to kill certain workers (Milne, 1982), and that the level of queen pheromone in the colony affects worker survival (Jaycox, 1970a).

Weight gain
There were differences among treatments for total colony wt gain (P = 0·0002, Table 1). In the four QL treatments wt gain showed a negative linear slope with prolonged queenlessness (P = 0·0001).

There were also differences among treatments for colony wt gain adjusted for brood (P = 0·0001, Table 1). In the four QL treatments wt gain showed a negative linear slope with prolonged queenlessness (P = 0·0003). Adjusted wt gain was highest in colonies with brood and a laying queen, and the number of brood cells differed among the treatments (P < 0·0001). Brood affected adjusted wt gain in the QR and QL-0 treatments. More food was collected (P < 0·05, Table 2) in the QR colonies with brood than in the QL-0 treatment (caged queen, no brood). Jaycox (1970b) showed that extracts of larvae increased foraging in colonies with caged queens. Similarly, brood may have stimulated foraging in our study and caused the higher adjusted wt gain in the QR treatment. The small amount of drone brood in queenless colonies probably did not increase foraging. It seems that stress from queenlessness overcame any stimulus to forage that may have come from the drone brood.

Queenlessness alone reduced wt gain. Neither the QL-0 nor the QL-7 treatment had brood, but wt gain was lower in the QL-7 group (P < 0·05, Table 2). The linear slope of treatment means showed no abrupt changes in wt loss with prolonged queenlessness. The slope for wt gain among treatment means was negative and linear for each trial even when all values were negative because of nectar dearth. The lack of treatment–trial interaction (P = 0·3, Table 1) showed that nectar availability did not alter treatment effects. The treatment group with the smallest loss in times of nectar dearth also gained the most during nectar flow.

Colony temperature
There were differences among treatments (P = 0·0004, Table 1, Table 2). In the QL treatments temperature showed a positive linear slope with prolonged queenlessness (P = 0·003).

Queenlessness alone did not seem to increase temperature. With broodless colonies there were no differences among treatments (P = 0·7). This suggests that the changes in temperature with prolonged queenlessness were affected by brood produced by workers. Brood alone probably produced little colony heat (Himmer, 1927) but stimulated workers to produce heat (Ritter & Koeniger, 1977; Kronenberg, 1979).

Defence behaviour

Queenlessness alone reduced stinging. Although the variance (MSE) was high for number of stings (Table 2), the queenright treatment produced more stings than queenlessness for 14 or 21 days (P < 0·05, Table 2). The slope for the QL treatment means was not significant (P = 0·06); however, the order of the means (Table 2) suggest that colonies became more gentle with prolonged queenlessnes.

Drift

Few workers (36/15 000 marked bees) changed their residence to other colonies. The QL-0 colonies lost the most (24) bees and the QL-7 colonies received the most (15) bees. Most cases of drifting (24/36) were between adjacent colonies.

Queenless colonies in our experiment neither attracted drifting workers nor encouraged workers to drift to another colony. Free and Spencer–Booth (1961) report that drifting bees may enter queenless colonies at a slightly greater frequency than they enter queenright colonies. Worker drift was found by Free (1958) to be greatest on the third and fourth days of life, which was probably their age at the time of their orientation flights. In our experiment drift was minimal, probably because (1) none of the bees were very young ($\geqslant$ 6 days old at the beginning and $\geqslant$ 36 days old at the end of the test period), (2) all bees had four days for orientation flights before the experiment began, and (3) all colonies were > 9 m apart (Jay, 1966, found little drifting between colonies > 9 m apart).

The low frequency of drift in our study confirmed that the differences in worker survival (Table 2) truly reflected a higher mortality rate among workers that had been in queenless colonies for 14 days or more.

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L94

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