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Original article

First symptoms of queen loss in a honeybee colony (*Apis mellifera*)

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Abstract – Approximately 50 min after removing the queen, the bees remained indifferent to the traces of the nestmate queen pheromone on the wall of the experimental cage. The worker's responsiveness to the cages then increased and peaked after approximately 165 min. The cage was licked (30.3%) and antennaed (69.7%) by workers. Three days after the removal of the queen the electroantennogram responses of workers from the queenless colonies were 2.7 times greater than those from the queenright colonies. The first symptom that reflects the loss of the queen in a bee colony is increased sensitivity of workers to the queen pheromone, which occurs approximately 50 min following the removal of the queen.

***Apis mellifera* / queen pheromone / sensitivity of bee / chemoreceptor / queen rearing / electroantennogram**

1. INTRODUCTION

Queen loss is one of the most serious events that may occur to a colony of honeybees (Laidlaw and Eckert, 1950; Ruttner, 1981; Winston, 1979, 1987, 1998). Investigations of the events that follow queen loss have provided insights into colony functioning and responses to stress (Winston, 1987) and promoted the development of the methods of queen rearing (Ruttner, 1981), etc.

The process by which workers recognize that a queen has been lost is unclear. Butler (1958) and Ruttner (1981) reported that if the queen was removed from a honeybee colony, the workers started to run around the entrance of the hive as if looking for the lost queen. Gubin (1969) questioned whether this behaviour of workers reflects the loss of the queen. Juška and Skirkevičius (1975) observed this behaviour of workers in only 1 of 22 cases and only within 6 h after dequeening. Thus, removing the queen from a colony does not always cause the workers to run around the entrance of the hive as if searching for their lost queen.

The mechanism most commonly associated with worker recognition of queen loss is the timing of the construction of queen cells. Most queen cells are constructed during the first 12–48 h after queen loss (Ruttner, 1981; Punnett and Winston, 1983). However, queen cell construction may begin within 3–4 h (Butler, 1958), 10 h (Seeley, 1979), 2–4 days (Gubin, 1969), or even 9 days (Winston, 1987) after queen loss. This variability in the onset of queen rearing suggests that the timing of queen cell construction may not be a very precise indicator of when a honey bee colony begins its transition to the state of queenlessness. Indeed, transition to the queenless state may occur within only a few hours following queen loss. Colonies readily accept worker larvae for rearing new queens within 3–6 hours after queen removal (Laidlaw and Eckert, 1950; Hůsing and Nitschman, 1987; Bilash et al., 1999). Also, Juška and

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Skirkevičius (1975) found that on average three hours after the removal of the queen from the colony workers began to behave differently around the worker larvae from which queens would be reared than around the other worker larvae. Nevertheless, workers must recognize the queenless condition before queen rearing can begin, and this is likely to involve signals emanating from the queen herself.

Honey bee queens produce a variety of pheromones from the mandibular glands (Free, 1987; Kaminski et al., 1990; Neumann et al., 1991; Melathopoulos et al., 1996; Winston and Slessor 1998; Wossler, 2002) that influence many aspects of worker behavior, including the rearing of new queens. Queen loss could alter worker sensitivity to these compounds. Thus, an examination of worker responses to queen pheromones during the first few hours after queen removal could provide a valuable mechanism for assessing when the transition to the queenless state begins. Skirkevičius (1976) found that after the removal of the queen the behavioral reaction of worker bees to traces of the queen pheromones intensifies. Because this response is closely associated with the worker olfactory system (de Jong and Pham-Delègue, 1991; Skirkevičienė and Skirkevičius, 1994; Vetter and Visscher 1997), behavioral changes may reflect changes in the responsiveness of chemoreceptors to queen pheromones. However, it is unknown when worker sensitivity to queen pheromones increases or how queen loss may affect worker chemoreceptors.

The objective of the present study was to identify the changes occurring in the reactions of worker bees to queen pheromones after the removal of the queen from a honeybee (*Apis mellifica* L.) colony as soon as workers begin to select larvae to rear the new queens. We examined a combination of behavioural responses and electrophysiological responses of the olfactory system. This may aid in more precise detection of the first symptoms that reflect the state of queenlessness in a bee colony and to understand the reasons for their origin.

2. MATERIALS AND METHODS

We examined worker response to queen loss in honey bee colonies hived in 16 frame (435 × 300 mm) standard hives. All colonies contained brood in all stages, sufficient workers to adequately cover the brood nest, a 2-year-old mated egg-laying queen, honey and pollen.

Workers were exposed to two sources of queen pheromones: traces of the queen pheromone of the experimental colonies and queen extract from other colonies. The reaction of worker bees to queen pheromone was investigated behaviourally and at the level of the olfactory system (electroantennogram responses). This study was conducted in June 1998–1999.

2.1. Monitoring behavioral responses to queen pheromones

Worker responses to the loss of a queen were examined in two ways. First, four colonies were dequeened and the behavioural responses of workers to traces of queen pheromone were recorded throughout the following 3 h period. In approximately 3 h after the removal of the queen from the colony, workers select larvae for rearing a new queen (Juška and Skirkevičius, 1975). In each colony one trial was conducted. Behavioural responses of workers were estimated by counting the number of worker surrounding and interacting with a source of pheromone traces from their own queen (Skirkevičius, 1976). Such sources of pheromones were used to preclude the possibility of any side effect of queen pheromone from a foreign bee colony.

To obtain queen-pheromone traces, the queen was removed from an experimental colony and confined in a small cage constructed so that her body was in contact with the cage walls (Fig. 1). Because queen mandibular pheromones are distributed over the body surface of a queen (Butler et al., 1974), this ensured that pheromones were transferred to the walls of the cage. For each experiment, each queen was kept in a cage for 10 min (Skirkevičius, 1986), because previous work has demonstrated that the pheromone traces deposited during this time attract workers (Skirkevičius, 1976, 1980). The queen was then removed and the cage was presented on the entrance board of the experimental colony. Every 10 min we changed the cage and counted the number of bees attracted to the cage and the number that licked and antennated the cage within a period of 5 min. These responses are typical of those normally exhibited when workers form a retinue around a live queen. The counts were repeated 5 times. The interval between the counts was 1 min. The first observation was made 15 min following the removal of the queen.

At the same time that the trace-pheromone cage was presented to an experimental colony, we also presented a control cage that contained no traces of

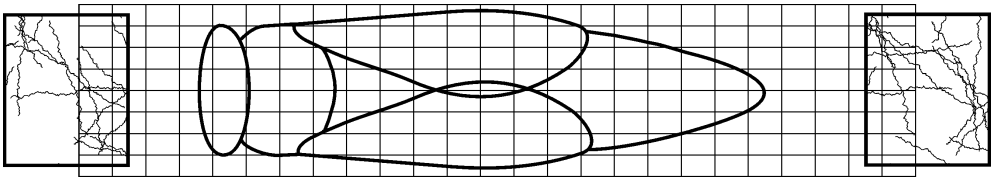

Figure 1. The cage in which the queen was kept 10 min before the test. The queen could move only backwards and forwards. In this position, the queen must deposit the pheromone necessary for the experiment on the wall of the cage.

queen pheromones. The distance between the control and the experimental cages was 10 cm. All cage presentations were conducted on the entrance boards of the hives, to minimize disturbance to the experimental colonies. If we had presented the cages inside the colonies, this would have required that the brood nest would have been open for observation for extended periods (179 min, excluding of queen removal time) and during this period 17 experimental and 17 control cages should be replaced.

For our second method for examining worker behavioral responses, we determined if the trace-pheromone cages influenced worker behavior in queenright colonies in a manner similar to that observed in the queenless colonies. A trace-pheromone cage was first presented to a queenless colony, then to two queenright colonies, and finally to the same queenless colony again. A control cage was presented simultaneously with each trace-pheromone cage, as described above. In this manner, we were able to determine if the presence of a queen influenced worker reactions to the cages and to assess if the pheromone traces in the cages diminished over time. Four queenless and eight queenright colonies were tested using the methods described above. Worker responses were monitored as previously described. The queenless colonies were dequeened 2.5–3 h before examination.

2.2. Generating electroantennogram of workers

The purpose of these experiments was to examine how queen loss influenced the responsiveness of worker pheromone receptors. An increase of electroantennogram (EAG) responses to the same stimulus was considered to be a sign of an increase in the sensitivity of pheromone receptors and their decrease was regarded as indicative of a decrease in their sensitivity (Gershuni, 1971; Skirkevičius, 1986; de Jong and Pham-Delègue, 1991; Vetter and Visscher, 1997).

Ethanol extracts of queens were used as a stimulus. Mated egg-laying queens were placed in a flask and soaked in ethanol. The material collected was kept in a refrigerator at a temperature of 4 °C (Apšegaitė and Skirkevičius, 1995). The extract was calibrated according to the amount of *E*-9-oxo-2-decenoic acid (9-ODA). The queen's extract containing 100–150 μg of 9-ODA (Slessor et al., 1988; Apšegaitė and Skirkevičius, 1995) was equated to one bee queen equivalent (Qeq).

EAG responses were recorded from an isolated antenna. An antenna flagellum with the pedicel and scape was removed from the head of a bee with forceps. All bees were taken from the entrance board of the hive and tested directly. Workers from 3 colonies were investigated before the removal of the queen (21 individuals from each colony) and 3 days following the removal of the queen (12 individuals from each colony). Workers from 3 queenright colonies were also measured, to serve as controls. The workers from the control colonies were tested two times. The first time (13 individuals from each colony), they were tested at the same time as the experimental colonies (before the removal of the queen) and the second time (15 individuals from each colony), simultaneously with the removal of the queen. We think that such bees should better reflect changes occurring in the colony as a functional unit.

The electrodes were strips of filter paper (Whatman No. 1), which were put into tubes of organic glass filled with physiological solution (0.9% NaCl). The strips of filter paper were moistened with physiological solution, too. One of these glass tubes served as a recording electrode, whereas the other one was indifferent. The distal part (distal flagellar segment) of the antenna was placed on the filter paper connected to the recording electrode, whereas its proximal part (scape) was placed on the filter paper connected to the indifferent electrode. The positions of electrodes were adjusted using micromanipulators and controlled with an optical microscope.

The tip of the distal end of the antenna was cut off to ensure its better contact with the physiological solution. The tubes were connected to a DC preamplifier (input impedance 100 MΩ amplification 100 ×) through the Ag-AgCl electrodes. Signals were amplified using a DC amplifier (Fig. 2).

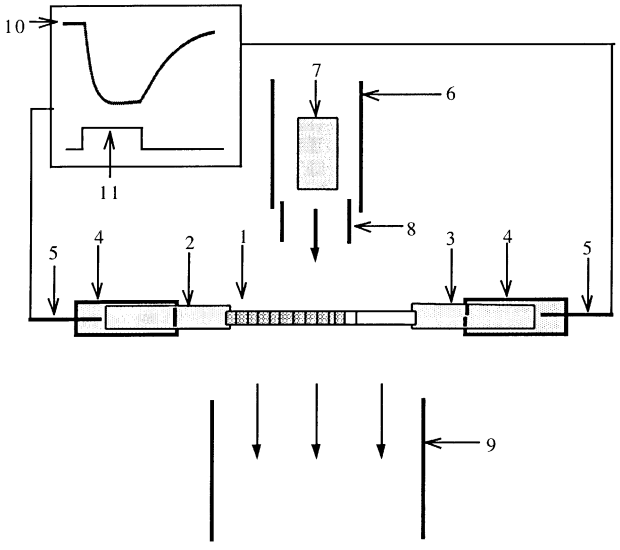

Figure 2. The arrangement of items for the recording of the electroantennogram (EAG).
1 - antenna of a worker bee; 2 - strips of filter paper serving as a recording electrode; 3 - strips of filter paper serving as an indifferent electrode; 4 - glass tubes filled with physiological solution; 5 - Ag/AgCl-wires; 6 - glass tube for odour stimulation; 7 - filter paper on which the extract was pipetted; 8 - silicon tube; 9 - metal tube for odour deflation; 10 - EAG; 11 - duration of the stimulation.

An oscilloscope and a pen-recorder were used to display the EAG signal. A peak-amplitude was used for evaluation of EAG response. For control of the overall amplification of the equipment, a stabilised 0.5 mV DC voltage was applied to the input of EAG preamplifier before each experiment.

To stimulate the antennae, an air stream was diverted through a glass tube (3 mm in diameter) containing a piece of filter paper (7×15 mm), which was loaded with 0.01 ml of the ethanol extract. Such a stimulus contains 1.57 µg 9-ODA (1×10^{-2} Qeq.). The content of 9-ODA in the ethanol extract was determined with a gas chromatograph (Dr. V. Apšegaite, the Chemoreception Laboratory of the Institute of Ecology, Lithuania). Filter paper loaded with 0.01 ml of the solvent was used for control. Filter papers were placed in a glass tube after the solvent had evaporated. The charcoal-filtered and humidified airflow containing a stimulating substance was blown (0.08 m/s) onto the antenna through a 2-mm silicon tube located at a distance of 3 mm from the antenna. The duration of stimulation was 0.5 s. Each antenna was stimulated only one time. The speed of recording of the EAG was 2.5 mm/s.

The electrical response of the antennae to the stimulation with the control paper was subtracted from the electrical response to the stimulation with the paper to which the queen extract had been applied. Thus, the influence of responses of mechanoreceptors on the EAG amplitude was eliminated.

2.3. Data analyses

One-way ANOVA was used to compare the behavioural responses of workers from queenless colonies exposed to experimental and control cages 3 h after the removal of the queen. An ANOVA followed by a post-hoc (Tukey's HSD) procedure was used to identify significant differences in the behavioural responses of workers from queenless colonies exposed to experimental cages every 10 min. One-way ANOVA and Tukey's tests were also used to compare the behavioural response of workers in queenless and queenright colonies to experimental and control cages. Two-way ANOVA and Tukey's tests were also used to test for a significant difference between the means of the EAG responses for two colony types (with or without a queen) and for two variables: before the removal of the queen and 3 days following the removal of the queen. Student's t-tests were used to compare the number for workers that licked and antennated the experimental and control cages. All means are presented as ± one standard error. All statistical tests were performed with Statistica (vers. 6.0) and SPSS (vers. 11.5) software.

3. RESULTS

3.1. Worker behavioral responses to queen pheromone traces

The removal of the queen altered the behaviour of worker bees ($F = 371.6$, $df = 16,323$, $P < 0.001$ for number attracted at the different time periods, Fig. 3, Experimental cages). However, this change in behaviour occurred only after a certain period of time. Approximately 50 min after the removal of the queen,

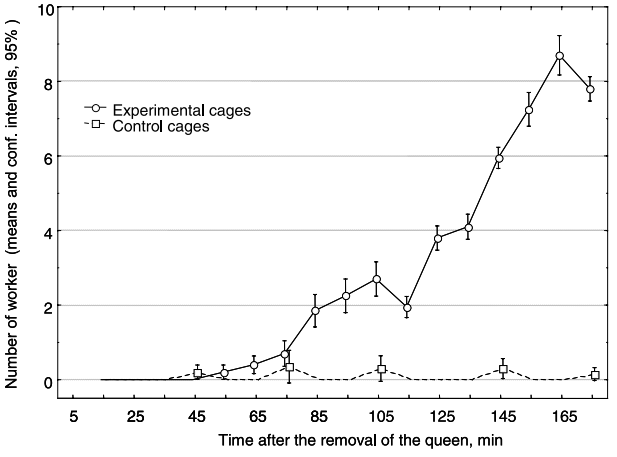

Figure 3. Average number of worker bees gathered around the experimental and control cages (in 5 min) after the removal of the queen. Four queenless colonies were investigated. The first observation of the bees attracted by the cage took place 15 min after the removal of the queen.

the bees remained indifferent to the traces of the queen pheromone on the wall of the experimental cage. The worker's responsiveness to the cages then increased and peaked after approximately 165 min. There were two main increases in responsiveness): a first, minor increase between 85–115 min following queen loss, and the second, major increase between 155–175 min. Mean responsiveness levels during the major peak was significantly greater than that observed during he minor peak, although the levels observed within each peak did not differ from one another (Tukey HSD, homogeneous subset for alpha = 0.01, Fig. 3, Experimental cages). In contrast, the control cages attracted very few workers throughout the 180 min observation periods (F = 1.5, df = 16,323, $P = 0.071$ for number attracted at the different time periods, Fig. 3).

After 155–175 min following the removal of the queen, we identified the behaviour of workers around the cages. We found workers of two kinds: those that antennaed the cage and those licking the cage. In a queenless colony, the cage with queen pheromone traces was licked on the average by 30.3% and antennaed by 69.7% of workers (Tab. I). Workers of the queenless colony rarely or never antennated or licked the control cages.

The attractiveness of the experimental and control cages to workers was established not only for queenless but also for queenright colonies. In a queenless colony, a cage with queen pheromones traces attracted on the average 7.3 ± 0.46 worker bees per 5 min within 150–180 min following dequeening. In contrast, in the first queenright colony it attracted only 0.7 ± 0.15 worker bees (Tukey HSD, $P < 0.001$) and in the second queenright colony the same cage attracted only 0.3 ± 0.13 workers (Tukey HSD, $P < 0.001$). Attraction to the cage in the second queenright colony was less that in the first queenright colony (Tukey HSD, $P = 0.05$; Fig. 4), which suggests that the traces of the queen pheromones had evaporated during the experiment. However, when the same cage was returned to a queenless colony, it still attracted 3.7 ± 0.41 bees,

Table I. 150–180 min after the removal of the queen from a colony the experimental (with traces of its own queen pheromone) and control cages attracted workers within a period of 5 min.

	Experimental cages	Control cages
Number of colonies	4	4
Number of tests	25	25
Mean ± *SE* number of workers	7.9 ± 0.59[a]	0.3 ± 0.12[a]
Number of workers licking:		
a. Mean ± *SE*	2.4 ± 0.21[b]	0.0 ± 0.00[b]
b. Percent	30.3	0.0
Mean ± *SE* number of workers palpating	5.4 ± 0.59[c]	0.3 ± 0.12[c]

SE – standard error. Row means followed by the same letter are different at the $P < 0.001$ level (*t*-test).

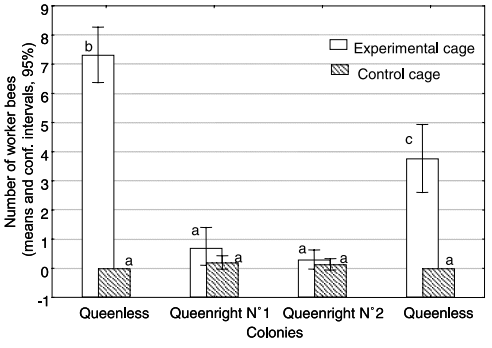

Figure 4. Average number of worker bees gathered around the trace of the queen pheromone (experimental cage) in queenright and queenless colonies in 5 min. The cage that contained the queen for 10 min was first investigated in the queenless colony, then in two queenright colonies and finally in the same queenless colony again. Four queenless and eight queenright colonies were investigated. Bars with different letters are significantly different between EAG responses of bees (Tukey HSD; $P < 0.001$).

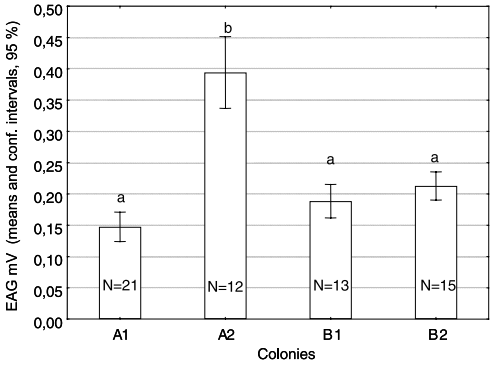

Figure 5. Electroantennogram (EAG) responses of worker to the doses of the queen extract equal to 1×10^{-2} Qeq. before and after the removal of the queen from the colonies. Three colonies were investigated before the removal of the queen (A1) and 3 days following the removal of the queen (A2). Three queenright colonies were investigated as control at the same time as the experimental colonies (B1 and B2). N: number of investigated antennae from each colony. Bars with different letters are significantly different (Tukey HSD; $P < 0.001$).

although this was significantly fewer bees than were attracted during the first presentation to the queenless colony (Tukey HSD, $P < 0.001$). Thus, though the attraction of bees to the cage diminished twice as much during the 20-min period of the experiment, in the queenless colony it still remained 5 to 10 times higher than in the queenright colonies.

In the queenright colonies, the control cages attracted 0.29 ± 0.11 bees in 5 min and the trace-pheromone cages attracted 0.5 ± 0.21 bees (Tukey HSD, $P > 0.05$). Thus, the few bees attracted to the cages in the queenright colonies were probably responding to incidental stimuli and not the traces of pheromones.

3.2. EAG-responses from worker bee antennae

The response of the chemoreceptors of workers (EAG) taken from the experimental colonies when they were queenright colonies was 0.14 ± 0.026 – 0.18 ± 0.025 mV (Fig. 5) and did not differ among bees (Tukey HSD, $P > 0.05$). In contrast, three days following the removal of the queen from these colonies, the EAG responses of workers were 0.39 ± 0.039 mV, or 2.7 times greater (Tukey HSD, $P < 0.001$). All the antennae responded to the stimulus (1×10^{-2} Qeq). The EAG responses of worker bee antennae from the queenright control colonies were 0.18 ± 0.025 mV during the queenright period of the experimental colonies and 0.21 ± 0.032 mV during the queenless periods of the experimental colonies (Tukey HSD, $P > 0.05$).

Thus, the results of our study suggested that the increase of the EAG responses to the queen extract (1×10^{-2} Qeq) in the queenless colonies is related to queen loss. The change of the EAG responses to the same stimulus shows that the sensitivity of the worker bee pheromone receptors changes too. It seems reasonable to conclude that the dequeening of a colony increases the sensitivity of the worker bee antenna pheromone receptors.

4. DISCUSSION

In this study, we have described new symptoms following queen loss in honeybee (*Apis mellifera* L.) colonies and showed the possible reasons for their origin. Our results suggest that worker respond to the queenless condition within 50 min following queen loss (Fig. 3).

Roberts (see Laidlaw and Eckert, 1950), Tresko (1955) Woyke (1956) established that the queen leaves the hive for mating for a period of 3 to 45 min. Thus, the maximum time of the possible absence of the queen from the colony (45 min) and the maximum delay time of the worker bees' reaction to the removal of the queen from the colony (50 min) are similar. The queen abandons the colony for a certain period of time more than once. Tresko (1955), Ruttner (1956), Woyke (1956) and others found that most queens take 1 or 2 short orientation flights and 1 to 5 mating flights, all over a 2-to-4-day period and 2 or 3 flights per day (Winston, 1987). Thus, it is quite possible that in the process of evolution mechanisms protecting the honeybee colony from false alarms about the loss of the queen have arisen.

One such mechanism may be a prolongation of the queen pheromone effect (or its latent action) on the worker for about 50 min. Later its action begins to lessen and breaks down. This process may result in an increase of the workers' sensitivity to queen pheromone and this is manifested in two ways. First, after about 50 min following queen loss, workers began to respond to the pheromone traces, which until then had not elicited a reaction (Fig. 3). Within 50 to 155 min after dequeening a colony the number of such workers increased from 0 to 7. The behaviour of workers around the experimental cages was similar to that in a queen retinue. We observed 30.3% of workers licking cages and 69.7% of workers antennating them (Tab. I). In a queen retinue, 29.4% of workers lick the queen and 70.6% of workers antennate her (Skirkevičius, 1968). Around a strip of paper moistened with ethanol extract of queens, 19.6% of workers lick the paper and 80.4% antennate it (Skirkevičius, 1965). In contrast, the control cages attracted very few workers in the queenless colonies (Figs. 3 and 4), and both pheromone-trace and control cages attracted few workers in the queenright colonies (Fig. 4), and there were no licking at all under these circumstances. Second, the dequeening of a colony increased the sensitivity of pheromone receptors, because after dequeening EAG responses to the same stimulus were 2.7 times greater (Fig. 5). Thus, the dequeening of a colony after a lapse of time elicited changes not only in the behavioural responses of workers to the same doses of queen pheromones but in chemoreceptor responses as well.

Three hours after removal of the queen from a colony (Fig. 3) the sensitivity of workers to queen pheromone traces was highest (the cage was most attractive to workers). Furthermore, within a three-hour period following queen loss workers change their behaviour around the bee larva from which they begin rear new queens (Juška and Skirkevičius, 1975). Thus, in this period of time workers must gain an awareness that the colony is in the queenless state. The period of time approximately 1–3 h after the dequeening of the colony can be called the stage of the transition of a bee colony to the state of queenlessness, because only in this period of time the workers' responses to queen pheromone showed the first changes as a result of their increased sensitivity to queen pheromone. Thus, the increased sensitivity of workers to the queen pheromone may be the first symptom reflecting the loss of the queen in a bee colony.

We have described a hypothetical scheme of the transition of a bee colony to the state of queenlessness. The duration of separate events that follow queen loss can fluctuate due to various circumstances, because workers start constructing queen cells in very different spans of time after queen loss: most often within 12 to 48 h (Ruttner, 1981; Punnett and Winston, 1983; Winston, 1987). However, it remains unknown what the mechanisms of their control are. To elucidate these problems further studies are necessary.

ACKNOWLEDGEMENTS

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Résumé – Premiers symptômes de la perte de la reine dans une colonie d'abeilles domestiques (*Apis mellifera*). Nous avons étudié les réponses comportementales et les électroantennogrammes (EAG) des ouvrières d'abeilles en réaction aux phéromones de la reine quand celle-ci est retirée de la colonie. Dans un premier dispositif, quatre colonies

ont été orphelinées et les réponses comportementales des ouvrières aux traces de leur propre reine sur la paroi de la cage (Fig. 1) ont été enregistrées toutes les 10 min durant les 3 h qu'a duré l'expérience. La cage était présentée sur la planche d'entrée de la colonie testée. Dans le deuxième dispositif nous avons déterminé si les cages avec des traces de phéromone influaient sur le comportement des ouvrières de la même façon dans des colonies avec reine et dans des colonies sans reine. Le but des expériences avec les EAG était d'examiner comment la perte de la reine agissait sur la réponse des récepteurs phéromonaux des ouvrières (Fig. 2).

Environ 50 min après le retrait de la reine, les abeilles sont restées indifférentes aux traces de phéromone de leur propre reine présentes sur les parois de la cage (Fig. 3). Il est tout à fait possible que durant ce laps de temps l'action de la phéromone royale soit prolongée pour protéger la colonie de fausses alarmes lorsque la reine quitte la ruche pour s'accoupler. Ensuite l'action de la phéromone a diminué et disparu. Le résultat de ce processus peut être un accroissement de la sensibilité des ouvrières à la phéromone royale. A ce moment là les ouvrières se sont mises à reconnaître les traces de phéromones, qu'elles avaient jusqu'alors ignorées (Fig. 3). Le comportement des ouvrières autour des cages expérimentales a ressemblé au comportement de cour : 30,3 % d'entre elles léchaient les cages et 69,7 % établissaient des contacts antennaires (Tab. I). Par contre les cages témoins ont attiré très peu d'ouvrières dans les colonies, qu'elles aient une reine ou pas (Fig. 4), exactement de la même façon que la cage expérimentale dans les colonies avec reine (Fig. 4), et aucun léchage n'a été observé. La suppression de la reine d'une colonie a augmenté la sensibilité des récepteurs phéromonaux de 2,7 fois (Fig. 5). Ainsi l'orphelinage de la colonie a provoqué après un certain laps de temps non seulement des changements dans les réponses comportementales des ouvrières aux mêmes doses de phéromone royale, mais aussi dans les réponses de chimiorécepteurs. Cette augmentation de la sensibilité des ouvrières à la phéromone royale pourrait bien représenter le premier symptôme qui reflète la perte de la reine dans la colonie.

***Apis mellifera* / phéromone royale / sensibilité / chimirécepteur / électroantennogramme**

Zusammenfassung – Erste Symptome der Weisellosigkeit im Bienenvolk *(Apis mellifera).* Diese Studie untersuchte die Antwort im Verhalten und Elektroantennogramm (EAG) von weisellosen Arbeiterinnen auf Königinnenpheromone. Die Antwort der Arbeiterinnen auf den Verlust der Königin wurde auf zwei Arten untersucht. Im ersten Ansatz wurden aus vier Völkern die Königinnen entnommen, und danach wurden die Verhaltensantworten von Arbeiterinnen auf Spuren des Pheromons ihrer eigenen Königin an der Wand eines Versuchskäfigs (Abb. 1) über einen Zeitraum von 3 Stunden in Intervallen von jeweils 10 Minuten registriert. Der Käfig wurden am Eingang des jeweiligen Testvolks angeboten. Im zweiten Ansatz untersuchten wir, ob Käfige mit Pheromonspuren das Verhalten von Arbeiterinnen in weiselrichtigen Völkern in ähnlicher Weise beeinflussen, wie zuvor in weisellosen Völkern beobachtet. Mit den EAG-Experimenten verfolgten wir die Absicht, die Antwort der Pheromonrezeptoren bei Arbeiterinnen auf den Verlust ihrer Königin zu untersuchen (Abb. 2).

Bis etwa 50 min nach der Entnahme der Königin erwiesen sich die Arbeiterinnen als indifferent gegenüber den Pheromonspuren ihrer Königin auf der Wand des Versuchskäfigs (Abb. 3). Es ist möglich, dass in diesem Zeitraum der Effekt des Königinnenpheromons noch persistiert und somit kein eventueller Fehlalarm beim Ausflug der Königin zur Paarung ausgelöst wird. Danach klingt dieser Pheromoneffekt ab und bricht zusammen. Das Ergebnis dieses Prozesses könnte in einer Zunahme in der Sensitivität der Arbeiterinnen auf das Königinnenpheromon liegen. Ab diesem Zeitpunkt beginnen die Arbeiterinnen auf das im Versuchskäfig angebotene Pheromon zu reagieren, das zuvor noch keine Beachtung gefunden hatte (Abb. 3). Das Verhalten der Arbeiterinnen am Versuchskäfig entsprach dem eines Hofstaats: dabei leckten 30,3 % der Arbeiterinnen an den Käfigen und 69,7 % betasteten sie mit den Antennen (Tab. I). Kontrollkäfige ohne Pheromonspuren waren für weisellose Arbeiterinnen (Abb. 3 und 4) genauso wenig attraktiv, wie es die Versuchskäfige mit Pheromon für die weiselrichtigen Arbeiterinnen waren (Abb. 4). Die Entnahme der Königin erhöhte die Sensitivität der Pheromonrezeptoren um den Faktor 2,7 (Abb. 5). Der Verlust der Königin löste somit nach einem bestimmten Zeitraum nicht nur die Verhaltens- sondern auch die Chemorezeptorantwort der Arbeiterinnen auf die gleiche Dosis an Königinnenpheromon aus. Der Anstieg in der Sensitivität auf das Königinnenpheromon könnte das erste Symptom darstellen, das den Verlust der Königin im Bienenvolk widerspiegelt.

***Apis mellifera* / Königinnenpheromon / Sensitivität der Bienen / Chemorezeptor / EAG**

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