Does the Queen Mark Pheromone in the Wall of Cell?

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Abstract

In order to test the hypothesis whether the queen can mark pheromone on the wall of the cells where the queen laid eggs, three experiments were designed and conducted. The first experiment is to transfer three kinds of eggs—queen laid diploid eggs (QD), queen laid haploid eggs (QH) and worker laid haploid eggs (WH), which came from the same colony into the cells where the queen has laid eggs and hasn't laid eggs respectively, then observing and counting the number of eggs remained. The second experiment is similar to the first one, but three kinds of eggs came from the different colonies. The third experiment use only QD to investigate. The results of these experiments do not support the hypothesis that the queen can mark pheromone in the wall of the cell, but support the viewpoints of worker policing and recognizing in the honeybee.

Keywords: honeybee, queen, pheromone, cell, eggs.

Introduction

Honeybee as the typical social insect consists of three casts: the queen (diploid), the worker bees (diploid) and the drone (haploid), which are reared from different cells, such as queen cells, worker cells and drone cells[1-3], respectively. In the normal honeybee colony, the task of reproduction is conducted only by the queen honeybee, whereas worker bees usually do not reproduce and instead help in rearing the offspring. By measuring the diameter of different cell types with their forelegs[4], queen honeybees can precisely lay diploid eggs (QD) in worker cells and haploid eggs (QH) in drone cells[5]. This behavior improves the colony's efficiency of reproduce and feed.

When the colony loses its queen and is unable to raise a replacement, the ovaries of some worker bees will develop. These worker bees with developed ovaries become laying workers, and lay haploid eggs (WH) [6]. Sometimes worker bees also can lay WH eggs in the normal honeybee colony. In this case, the WH are usually either removed or eaten by other bees called policing workers[7-12]. How can policing workers distinguish different kind of eggs? A popular hypothesis is that the queen can mark their eggs with pheromone, indicating that their eggs are queen-laid[13-20], but these results are not consistent with egg marking pheromone. Based on the conflicts about egg marking pheromone, we hypothesize that, instead of marking the surface of the eggs, the queen might mark the wall of the cells where she laid eggs, and designed this study to test our hypothesis.

Material and Methods

Experimental colonies and eggs preparation: Experiments were conducted during the summers of 2006 at laboratory of honeybee biology, INRA, Avignon, France. The colonies were maintained according to standard techniques and supplied with adequate food. Three strong source colonies A (*Apis mellifera mellifera*), C (*Apis mellifera ligustica*) and D (*Apis mellifera mellifera*) with high quality queens were prepared.

To stimulate the worker honeybees building the drone cells rapidly, two frames of comb foundation for drone cells were put into colony A. After the two frames of drone cells were built up and removed for later use.

Three combs with honeybee and brood from colony A were examined one by one to make sure there is no queen in the comb and then removed to buildup a queenless colony B (*Apis mellifera mellifera*). To destroy the queen cells, the colony B was checked every day until no new queen cells appeared. After about 20 days, the worker began to lay WH eggs.

When worker bees in the colony B began to lay eggs, put two frames of drone cells into the colony A. the queen was restricted in the two combs of drone cells, to lay haploid eggs (QH).We utilized "Cupularve" (an artificial box-like brood containing removable worker cells) (Figure 1) to collect queen laid diploid eggs (QD). The "Cupularve" with some honey was put in the hive. After worker bees cleaned up the cells, each of "Cupularve" was divided into two parts by one small plastic. The queen was restricted to lay eggs in one of the two sections.

Material and Methods

In a dusk right after the QH and WH eggs were prepared, put the queen of colony A in one "Cupularve" which was fixed in the centre of comb (the comb had cut the same size hole in the centre as "Cupularve"). Put the comb containing the "Cupularve" back to colony A. On the next morning, take out the "Cupularve" from colony A, the position of the cells where the queen had laid egg was recorded in a special form which had same shape and number cells as "Cupularve". Take out all cells from "Cupularve", put cells with queen laid eggs together and back to "Cupularve". About half of the empty cells were transferred with QH eggs from colony A, the other empty cells were transferred with WH eggs from

colony B as Taber described[21]. Sequentially put back the cells containing the three kinds of eggs to "Cupularve". Then put the "Cupularve" in colony A. Observe and record the cells that still had eggs at 3h, 6h, 9h, 24h and 48h interval, respectively.

In the same way, put the queen of colony C in one "Cupularve" which was fixed in a comb. Put the comb containing the "Cupularve" back to colony C. In the next morning, take out the "Cupularve" from colony C, the position of the cells where the queen had laid egg was recorded in a special form which had same shape and number cells as "Cupularve". Take out all cells from "Cupularve", put cells with queen laid eggs together and back to "Cupularve". About half of the empty cells were transferred with QH eggs from colony A, the other empty cells were transferred with WH eggs from colony B. Sequentially put back the cells containing the three kinds of eggs to "Cupularve". Then put the "Cupularve" in colony C. Observe and record the cells that still had eggs at 3h, 6h, 9h, 24h and 48h interval, respectively,.

Put the queen of colony D in one "Cupularve" which was fixed in a comb. Put the comb containing the "Cupularve" back to colony D. On the next morning, take out the "Cupularve" from colony D, the position of the cells where the queen had laid egg was recorded in a special form which had same shape and number cells as "Cupularve". Reorganize the cells in "Cupularve" by putting the cells with queen laid eggs together. Insert the "Cupularve" back to colony D. Observe and record the cells that still had eggs at 6h, 24h, 48h and 72h interval, respectively.

Statistical analyses: All analyses were performed using the general linear models imbedded in StatView 5 package (SAS Institute Inc.). The number of replicates of each experiment is 5 times. Differences in the percentage of eggs remaining in the different modalities were analyzed as ANOVA. Data did not belong to a particular treatment. Means (without transformation) and standard errors (SE) are used in the figures and throughout the text.

Results and Discussion

As shown in Figure 2, the percentage of eggs remaining between the cells which queen had laid eggs and the cells which queen had not laid eggs were not significant at the same time intervals for all the studied three colonies ($P > 0.05$ in all trials). In other words the percentage of eggs remaining was not correlated with the cells whether the queen had laid egg or not.

Figure 3 shows that QD＞QH＞WH in both two colonies A and C. At 3h stage, there is no significant difference in the eggs remaining between QD and QH in colony A ($P > 0.05$), but has significant difference in the percentage of eggs remaining between QD and QH in colony C ($P < 0.05$). Comparison of the percentage of eggs remaining of QH and WH between colony A and colony C, the QH and WH were eliminated faster and more in colony C than that of colony A.

The results of these experiments do not support the hypothesis that the queen can mark pheromone in the wall of cell, but its support the viewpoints of worker policing and recognizing in the honeybee.

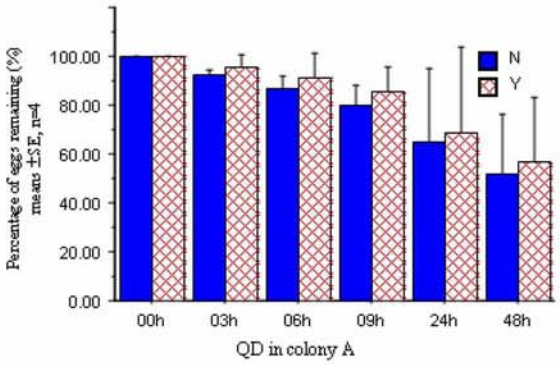
We inferred that the queen did not secrete pheromone from its gland of abdomen to mark the wall of cell when the queen laid eggs. Because if the queen marked the wall of cell, the worker honey bees would know which eggs that the queen laid, and would eliminate more eggs in the cells where the queen had not laid eggs, but it was not the fact.

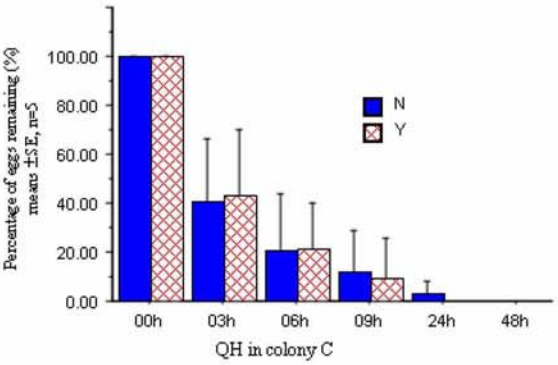
The worker honey bees have eliminated more WH than QH, this result was consistent with previous studies [7, 13, 19]. This indicated that worker policing did happen in experimental colonies.

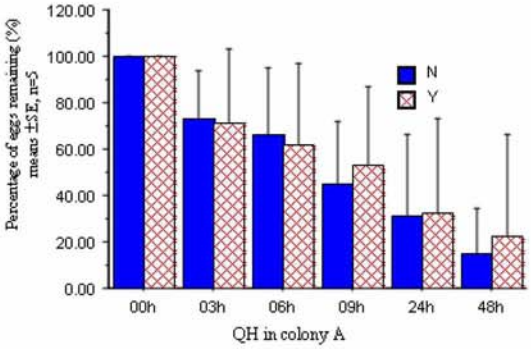
The comparison of the percentage of eggs remaining of QH and WH between colony A and colony C, showed the QH and WH were eliminated faster and more in colony C than that of colony A, because three kinds of eggs came from the same colony and have same ancestry in colony A, while three kinds of eggs came from different colonies in colony C. it indicates that the worker honey bees could discriminate and recognize the eggs by relative relation.

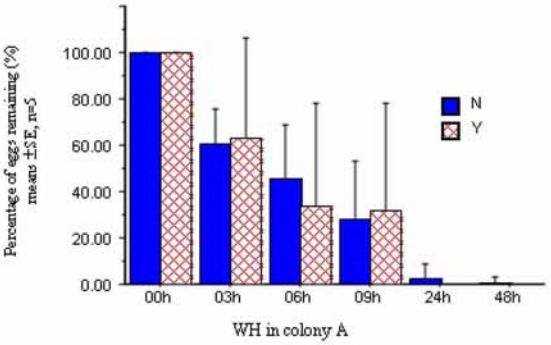
Our methods of investigation are different from the previous studies in worker policing[7- 9, 11-12], Ratnieks et al. transferred QH and WH into the comb of empty drone cells, we transferred QH and WH into "Cupularve" comb of empty worker cells, but we got some similar results as previous studies, for example, the WH was almost eliminated in 24-48h; the speed of elimination of WH was faster than QH. However, there were some different results, for example, Our results show that the percentage of eggs remaining of QH are lower than those in the previous studies, which was likely to indicate that the worker honey bees could decide to eliminate eggs or not, according to synthesis information from the type of cells and the eggs pheromone, we do not know which the first work that the worker honey want to do, examining the type of cells or getting the eggs pheromone? While many studies had been conducted on the queen laid-eggs[4-5], it was not very clear the mechanism of the queen laid-eggs.

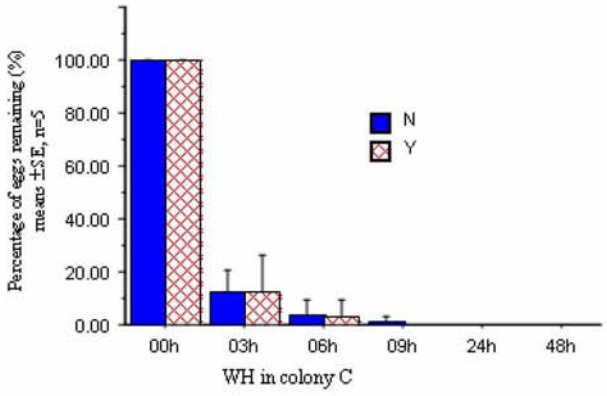
Figure 1. Cupularve in comb

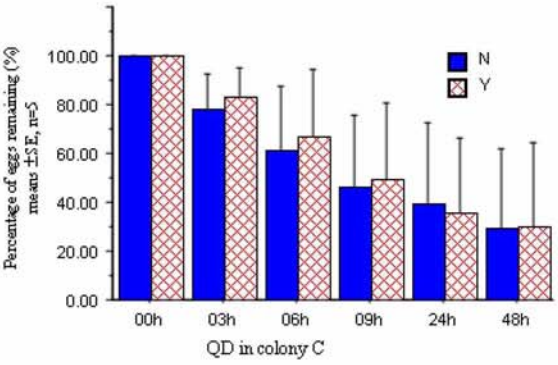












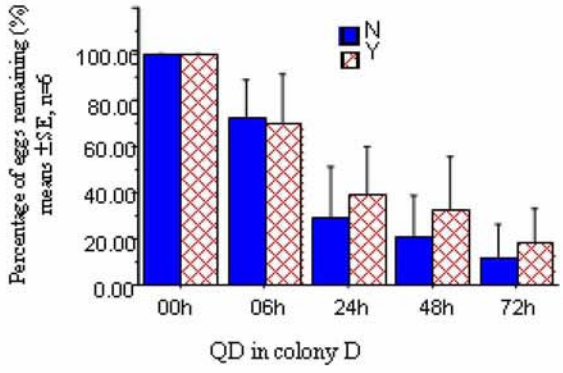


Fig. 2: Comparison of the percentage of eggs remaining between "N" and "Y"

"N" means the cells which queen had not laid eggs; "Y" means the cells which queen had laid eggs. It showed no significant difference in the percentage of eggs remaining between "N" and "Y" in the same time ($P > 0.05$ in all trials).

Conclusion

The percentage of eggs remaining did not show a consistent pattern in responding to that the queen can mark pheromone in the wall of cells leading to worker policing. The future experiments must studies on the chemical and behavioral biology of queen laid-eggs and search the relation between chemical and behavioral biology of queen laid-eggs.

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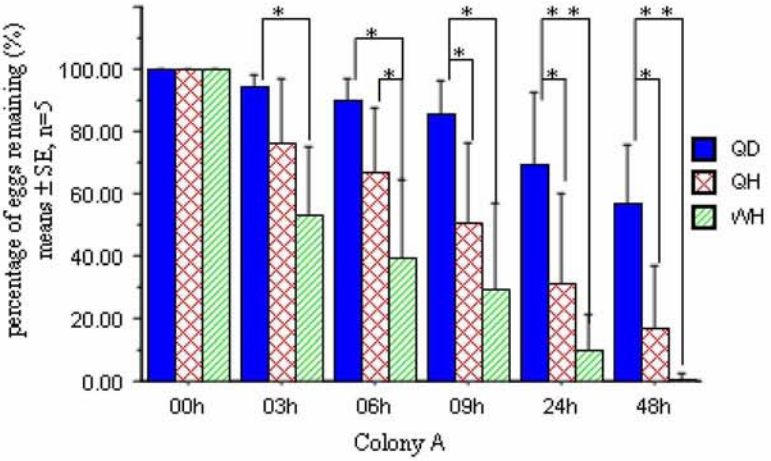

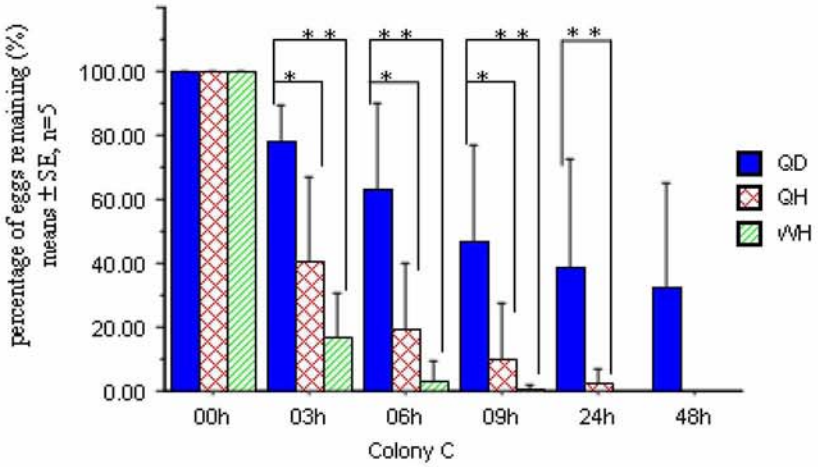

Fig. 3: Comparison of the percentage of eggs remaining with "QD","QH" and "WH" in the same time
Note: * indicates significant difference (P<0.05); * *indicates very significant difference (P<0.01); lack * or * * indicates no significant difference (p>0.05).

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