

Occurrence and size of laying worker eggs in *Apis florea* colonies

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SUMMARY

The occurrence of laying workers in *Apis florea* colonies in Thailand was observed. Laying workers were active in 'hopelessly queenless' colonies, and deposited eggs in large numbers even in colonies rearing queens. Laying worker brood disappeared after virgin queens emerged, well before they started to lay eggs. Thirty eggs were collected from each of three colonies with queens and three queenless colonies with laying workers. The average lengths of eggs deposited by queens were 1.540, 1.558 and 1.618 mm, and by workers 1.633, 1.694 and 1.783 mm. The average widths were 0.348, 0.374 and 0.392 mm, and 0.418, 0.438 and 0.452 mm, respectively. Thus, laying worker eggs were larger than those deposited by queens, although the length of the worker abdomen is about half of that of the queen. A high correlation was found between the average length and width of eggs from different colonies. However, the correlation was weak for eggs deposited by particular queens.

Keywords: *Apis florea*, laying worker honey bees, queen honey bees, eggs, morphometrics, Thailand

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INTRODUCTION

Laying workers appear in queenless honey bee (*Apis mellifera*) colonies without brood. Woyke (1993b) showed that laying workers also appeared in Sudanese honey bees (*A. m. yemenitica*) during queen rearing in the presence of queen cells. Woyke (unpublished) observed a similar phenomenon during queen rearing in *A. cerana* colonies. In both cases queen cells were not destroyed. For several years *A. m. capensis* colonies were kept in an apiary with *A. mellifera* colonies at the Bee Division in Warsaw, Poland. During queen rearing, *A. m. capensis* workers entered those colonies, and the queen cells were destroyed. There has been only one report of laying worker eggs in an *A. florea* comb, seen in a market in Thailand (Sakagami & Yoshikawa, 1973).

The size of eggs of *A. mellifera* was measured by Hejtmanek (1961) and Jordan (1961), and of three Asian honey bee species by Woyke (1975). Woyke (1993a) also showed that eggs of honey bees change size during their incubation period. Until now, the size of eggs laid by queens and laying workers has not been compared. This question is especially interesting to study in *A. florea* where the difference in size between workers and queens is very large (Koeniger *et al.*, 1993).

We report the occurrence of laying worker eggs in *A. florea* colonies, and compare their size with eggs deposited by queens.

MATERIALS AND METHODS

The investigations were conducted in the Bee Biology Research Unit of Chulalongkorn University in Bangkok, Thailand, in February and March 1992. *A. florea* colonies were collected in Bangkok or in Samut Songkhram province, and moved to near the Bee Biology Research Unit for observation. *A. florea* combs with brood and honey were also bought from the Bangkok weekend market. The combs were placed in an incubator and emerging workers were added to the colonies. Combs with honey and brood were also used to replace empty combs occupied by bees. Honey and brood combs were also hung next to empty combs on which bees were sitting.

Thirty eggs were collected from each of three colonies with queens and from three queenless colonies with laying workers. The length and width of all eggs were measured with the micrometer of a stereo microscope using a 30 x magnification. Altogether 360 measurements were made. The length of some living queens and workers was also measured using a ruler.

Analysis of variance and a Newman-Keuls multiple comparison test were used to determine significant differences among means. The *t*-test was used to compare specific pairs of means.

RESULTS

Occurrence of laying workers

Three queenless colonies with laying workers were observed: these contained eggs in drone cells as well as in worker cells. Many eggs per cell were observed. Two further combs, having only drone brood in worker cells, were bought from the weekend market. Most of these two combs were occupied by sealed brood, and larvae and eggs were present on comb edges. No queen cells or younger female larvae were present in any of the five combs. Thus laying workers of these colonies were active in 'hopelessly queenless' colonies, as happens in *A. mellifera* colonies.

Laying workers were found in three other colonies which contained queen cells and large amounts of sealed worker brood. Queen cells were on the edges of the combs and it was difficult to determine whether they were swarm or emergency cells. The eggs were deposited in drone cells, up to 20 eggs per cell. The laying workers must have appeared in the period between the loss of the old queen and emergence of the new one. Several virgins emerged, fought, but did not kill each other. On several occasions two or three queens were found in a colony. After the virgins emerged, but before they started to lay eggs, the eggs deposited earlier by laying workers disappeared. No larvae were found in drone cells where previously the laying worker eggs were present.

Length of queens and workers

Six virgin queens and 10 workers were measured. As we measured live unnarcotized bees, it was difficult to conduct precise measurements. The average length of all virgin queens was estimated at 15 mm, and of their abdomens 9 mm. Average length of the workers was estimated at 9 mm and of their abdomens 5 mm. Thus the total length of a worker was about equal to the length of a queen's abdomen. The length of a worker's abdomen was only a little longer than half of a queen's abdomen.

Size of eggs deposited by queens and laying workers

The length of eggs laid by the three queens ranged from 1.450 to 1.650 mm. The length of all eggs deposited by laying workers in the three queenless colonies ranged from 1.550 to 1.900 mm (table 1). Thus the longest eggs produced by queens were similar to the shortest eggs produced by laying workers. The longest average length of eggs produced by a queen was shorter than the shortest average length of eggs deposited by a laying worker (table 1). ANOVA (table 2) showed that differences

among the lengths of eggs from all six colonies (d.f. = 5) were highly significant ($P < 0.001$).

Eggs produced by laying workers were significantly longer (1.703 mm) than eggs deposited by queens (1.572 mm) (d.f. = 178, t -value = 12.802, $P < 0.001$).

The Newman-Keuls multiple comparison showed that eggs of one queen were significantly longer than those of eggs laid by two other queens. Differences between all means of the length of eggs produced by laying workers were statistically significant. Variation in the length of laying worker eggs (table 1) was about three times higher (s.d. range 0.060–0.069), than in eggs laid by queens (s.d. range 0.020–0.030).

The width of eggs laid by three queens ranged from 0.325 to 0.400 mm and that of eggs deposited by laying workers from 0.375 to 0.500 mm (table 1). Thus the widest eggs produced by queens were only slightly wider than the thinnest ones deposited by laying workers. The maximal range of width of eggs laid by three queens was approximately equal to the minimal width range of eggs produced by laying workers (table 1). Mean width of all eggs produced by three queens was $\bar{x} = 0.371$ mm ($n = 90$, s.d. = 0.022), and that of eggs deposited by laying workers in three colonies was $\bar{x} = 0.436$ mm ($n = 90$, s.d. = 0.026). Statistical analysis showed that variation between those two groups was highly significant (table 2). The difference between those two means was highly significant (d.f. = 178, $t = 17.871$, $P < 0.001$). Thus, eggs produced by laying workers were significantly thicker than those deposited by queens. Variation in egg widths among all six colonies (d.f. = 5) was highly significant (table 2). Newman-Keuls multiple comparison showed that differences between all width means were significant (table 1) with eggs laid by laying workers always wider than eggs deposited by queens. Variation of width was greater in laying worker eggs than in eggs deposited by three queens (table 1).

Consequently, laying worker eggs were larger in both length and width than eggs deposited by queens.

TABLE 1. Size (mm) of *Apis florea* eggs deposited by queens and laying workers. Thirty eggs were measured from each colony.

Females	Range	Mean \pm s.d.
Length		
1. Queen	1.500–1.575	1.540 \pm 0.020a ¹
2. Queen	1.450–1.600	1.558 \pm 0.029a
3. Queen	1.550–1.650	1.618 \pm 0.030b
4. Laying workers	1.550–1.750	1.633 \pm 0.069b
5. Laying workers	1.600–1.800	1.694 \pm 0.060c
6. Laying workers	1.650–1.900	1.783 \pm 0.062d
Width		
1. Queen	0.325–0.375	0.348 \pm 0.010a
2. Queen	0.350–0.400	0.374 \pm 0.012b
3. Queen	0.350–0.400	0.392 \pm 0.017c
4. Laying workers	0.375–0.450	0.418 \pm 0.024d
5. Laying workers	0.400–0.450	0.438 \pm 0.018e
6. Laying workers	0.400–0.500	0.452 \pm 0.025f

¹Different letters indicate significant differences among means ($P < 0.05$).

Relationship between length and width of eggs

Eggs which were on average longer, were also wider (table 1). The correlation coefficient for the six pairs of averages for length and width was $r = 0.82$ (d.f. = 4, $P = 0.045$), indicating a significant positive correlation. This trend is confirmed by length and width measurements of all individual eggs ($r = 0.699$, $n = 180$, d.f. = 178, $P < 0.001$). A similar correlation was

TABLE 2. Results of analysis of variance of size of eggs deposited by *Apis florea* queens and laying workers.

Variation	Length			Width	
	d.f.	F-ratio	P	F-ratio	P
Total	179				
Six colonies	5	103.519	0.000	140.292	0.000
Egg type (queen vs. laying worker)	1	163.897	0.000	319.385	0.000
Colony within type of eggs	4	46.523	0.000	34.838	0.000
Error	174				

found using data from eggs of queens ($r = 0.627$, $n = 90$, d.f. = 88, $P < 0.001$). A significant, although smaller, correlation was found using data from individual eggs from laying workers ($r = 0.234$, $n = 90$, d.f. = 88, $P = 0.027$).

Correlation coefficients were also calculated separately for each queen. These coefficients for queens 1, 2 and 3 were $r = 0.084$, $P = 0.658$; $r = 0.080$, $P = 0.676$; and $r = 0.144$, $P = 0.448$, respectively. Thus some females produced larger eggs which were both longer and wider. However, among eggs produced by particular females, this correlation was not found.

DISCUSSION

A. florea laying worker eggs have been reported from only one comb in the market in Bangkok (Sakagami & Yoshikawa, 1973). However, a cross-section of comb presented by those authors suggests that it may have been an *A. andreniformis* comb, thus it is not clear whether the eggs were produced by *A. florea* or by *A. andreniformis* laying workers. *A. florea* workers laying eggs in the presence of queen cells are apparently more active than laying workers of the Sudanese honey bee *A. m. yemenitica* ecotype *nubica* (Woyke, 1993b).

It is difficult to generalize about the frequency of laying workers in *A. florea*. However, the presence of large numbers of laying worker eggs in all three colonies with queen cells suggests that laying workers are common in the period between the loss of a queen and emergence of the new one. Whether or not all brood produced by laying workers disappears after the emergence of the new virgin requires verification. If the queen cells were destroyed, or the queens did not emerge, drones from the laying workers would supply genes of those colonies.

Eggs laid by laying workers were larger than those laid by the queens. This was unexpected since the length of the worker abdomen is about the half the length of a queen's abdomen. Woyke (1993a) showed that older eggs are smaller than younger ones. In this study the age of eggs was not known. However, it can be assumed that eggs of varying ages were collected from each colony. The large difference between the size of eggs laid by laying workers and queens is unlikely to be caused by random variation.

A. florea queen eggs measured by Woyke (1975) in India (1.675 x 0.414 mm) were a little larger than those from Bangkok. They were rather similar to laying workers' eggs from Bangkok.

It is interesting that a strong correlation existed between the length and width of eggs collected from different colonies, but the same correlation was weak between eggs deposited by particular queens.

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