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Research article

Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality

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Summary. The requeening process was investigated under emergency conditions in honey bee colonies (*Apis mellifera* L.). The progression of queen cell construction was closely monitored after removal of the mother queen, and the newly-emerged queens were measured for several physical traits to quantify their reproductive potential (= quality). The results suggest that workers regulate the queen rearing process by differentially constructing cells. Workers built different numbers of queens cells from different ages of brood and non-randomly destroyed over half (53%) of the initiated cells before their emergence. For those queens whose cells were not torn down, the variation in reproductive quality was limited, varying only slightly among age groups for queen size. Several hypotheses are discussed which might explain the adaptive benefit of worker regulation during queen rearing.

Key words: Queen rearing, social behavior, *Apis mellifera*, reproduction, polygyny.

Introduction

Honey bee colonies are monogynous for the vast majority of their life histories. However, colonies raise multiple queens to create a state of temporary polygyny during short-lived phases of their life cycle which ultimately result in queen replacement. Temporary polygyny almost always occurs during supersedure and emergency queen rearing, and frequently occurs at the conclusion of swarming. Nonetheless, the requeening process encompasses two discrete stages: (1) queen rearing, to create temporary polygyny, and (2) polygyny reduction, to re-establish monogyny through queen competition.

The requeening process is a critical event in the life cycle of a colony because its future fitness is dictated by the reproductive potential of the final replacement queen. Therefore, it is likely that selection has acted upon the two stages of queen replacement to regulate the outcome of such events. Little is known about polygyny reduction, although some evidence suggests that it is non-random (Tarpy and Fletcher, 1998). On the other hand, queen rearing has been of great concern in experiments on nepotism (see Visscher, 1998 for review) and descriptive studies of the three reproductive situations (Butler, 1957; Fletcher and Tribe, 1977a, b; Winston, 1979; Punnett and Winston, 1983; Fell and Morse, 1984). Apart from what is known about queen rearing, relatively little is understood concerning its potential regulation during phases of queen replacement.

Previous studies have demonstrated significant variation in the number of queens among colonies and among the three reproductive events during queen rearing (Fletcher and Tribe, 1977b; Fell and Morse, 1984; Pettis et al., 1994). There is also marked variation among queens with respect to their quality and other potential fitness criteria (Eckert, 1934; Clarke, 1989; Fischer and Maul, 1991), particularly as a function of the age of brood at which queen rearing is initiated (Weaver, 1957; Woyke, 1971). Given significant variation among queens, it is possible that selection has acted upon the queen rearing process to select higher quality queens from those available. However, no study has measured quality among queens as it occurs during emergency queen rearing. Therefore the purpose of this study was to quantify the magnitude of the variation in quality among queens reared naturally by the workers after the loss of their queen, as well as to describe in detail the regulation of queen rearing by the workers.

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Materials and methods

The honey bees were maintained in two research apiaries located approximately 14 km apart. All colonies were kept in two Langstroth brood chambers both of which contained nine standard-sized frames. A total of eight populous colonies were used, each with an estimated minimum of 30,000 bees to ensure that they would be capable of rearing an adequate number of queens for the study.

During the period of July through August, 1994, each colony was dequeened to prepare it for queen rearing. All frames were first examined for queen cells 24 h after dequeening. Thereafter, the colonies were examined once per day at the same time each day to minimize disruption. The frames in both brood chambers were numbered consecutively and the two sides of each comb were individually labeled to facilitate recording the positions of the queen cells constructed by each colony. Each side was further sub-divided into six equal areas ($A = 140\ cm^2$) by means of placing a plexiglass grid over the comb upon removal. The location of each queen cell was then recorded according to the number, side, and area on the frame.

The age of the egg/larvae from which each cell was initially constructed was approximated by calculating backwards from the day the queen cell was sealed. Although development time may vary as a function of several factors (Jay, 1963), the duration of this stage was assumed to be five days for all queens. Since genotype and temperature are the two most influential factors of development time, this assumption is most likely to be valid because all colonies were presumably of European origin and were standardized for their worker populations to control for brood nest temperature. A wire cage was positioned around the cell six days after it was capped to capture the queen when she emerged. Once the first queen emerged from a particular colony, each cell was checked twice a day to prevent workers from releasing queens by chewing away the comb next to the cages. Newly emerged queens were removed from the colony, killed by placing them in a freezer at –20 °C, and labeled according to their colony and position.

Sampled queens were immediately measured for the following morphological characteristics: (1) wet weight – the queens were weighed to the nearest 0.001 gram as soon as they were dead; (2) wing lengths – both wings were removed from each queen and measured from the point of articulation to the tip of the wing; (3) wing widths – each wing was measured at the widest point for all queens; (4) thorax width – the width of the thoraces were measured using an eyepiece micrometer in a dissecting microscope; (5) thorax length – the thorax length for each queen was similarly determined; (6) poison sac volume – the poison sac length (from the end of the sac to the beginning of the poison duct) and width (at the widest part of the poison sac) were measured for all queens using the dissecting microscope, and the volume was calculated using the formula for an ellipsoid: $2[\pi \int^a b^2[1-(x^2/a^2)]dx]$; (7) hind gut mass – measured for each queen by removing the hind gut and weighing it; (8) spermatheca volume – the length and the width of the spermathecae were measured using the eyepiece micrometer. The average radius of each spermatheca was then used to calculate the spherical volume: $(4/3)(\pi)(r^3)$; (9) ovariole number – both the left and the right ovaries were removed from each queen and preserved in Bouin's fluid. Following standard histological procedures, all ovaries were embedded in wax, sectioned transversely using a microtome into sections 10 μm thick, mounted on slides, and stained using Ehrlich's formula. The number of ovarioles in each ovary was then counted using a microscope.

Characters (1)–(5) were measured to quantify queen size. Numbers (6) and (7) were examined because they could be relevant during polygyny reduction (Tarpy, 1995). Finally, spermatheca volume and ovariole number were measured in attempts to quantify the potential fecundity of the emerged queens.

Results

After removal of the mother queens, the majority of cell construction was initiated within 24 h in all eight colonies. Additional queen cells were constructed for up to two days after dequeening, but no further queen cells were started on or after the third day. The colonies differed significantly both in the number of queen cells they built ($\chi^2 = 155$, df = 7, $p < 0.001$) and in the number of queens that emerged ($\chi^2 = 45$, df = 14, $p < 0.001$; Table 1). The number of cells capped per colony ranged from 6 to 56 (27.1 ± 14.17, mean ± s.d.), and the number of queens that emerged in each colony ranged from 3 to 20 (11.4 ± 5.83, mean ± s.d.). Since the colonies represented various scenarios that could occur in the natural situation, the data were combined. From the eight colonies, a total of 217 queens cells were capped, but only 91 queens (41.9%) emerged. The remainder of the cells were either torn down by worker bees (53.0%) or the queens did not emerge from them (5.1%).

There was a highly significant effect of position on queen rearing ($\chi^2 = 244$, df = 24, $p < 0.0001$; Fig. 1). No significant difference was found between the upper and lower brood chambers with respect to the number of capped queen cells ($\chi^2 = 2.16$, df = 2, $p > 0.25$), nor was there an effect of comb area on cell construction ($\chi^2 = 3.67$, df = 2, $p > 0.15$). However, there was a significant difference of frame position on where queen cells were constructed ($\chi^2 = 44.9$, df = 3, $p < 0.0001$; Table 2). Most of the queen cells (46.1%) were found on frames located in the central three frames of both brood chambers. Similarly, the majority of queens emerged from cells on frames that occupied the center of the nest ($\chi^2 = 138$, df = 24, $p < 0.0001$). However, these centralized results are intuitive because the brood nest occupies the center of the hive. Therefore, the expected distribution is not uniform, and thereby inflates the significance of the result.

Table 1. Variation among colonies in the number of constructed queen cells. Although colony size was roughly equivalent for all replicates, there were significant differences among colonies for all outcomes of queen rearing

Outcome	Colony number								Total
	1	2	3	4	5	6	7	8	
Emerged	8	7	15	3	9	11	18	20	91
Torn down	43	18	8	3	13	13	4	13	115
Unemerged	5	1	0	0	1	2	0	2	11
Total	56	26	23	6	23	26	22	35	217

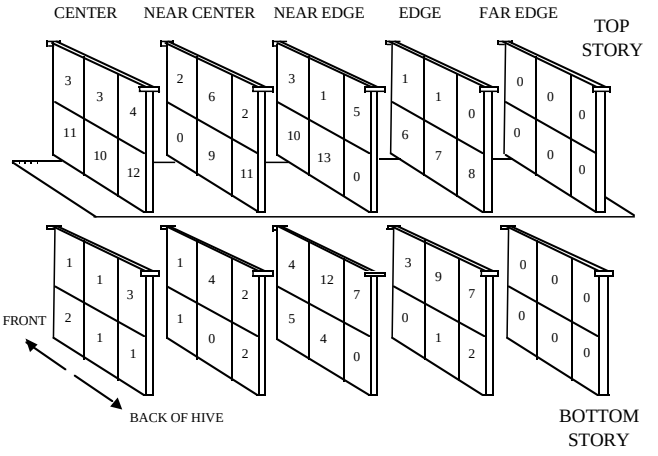

Figure 1. A three-dimensional distribution of queen cell construction. The data reported are the total number of cells constructed in all eight replicates. The center frames for each story are represented by only one frame (5 and 14), whereas the other positions are sums of two frames each (see Table 2). More queen cells are constructed towards the center of the brood nest

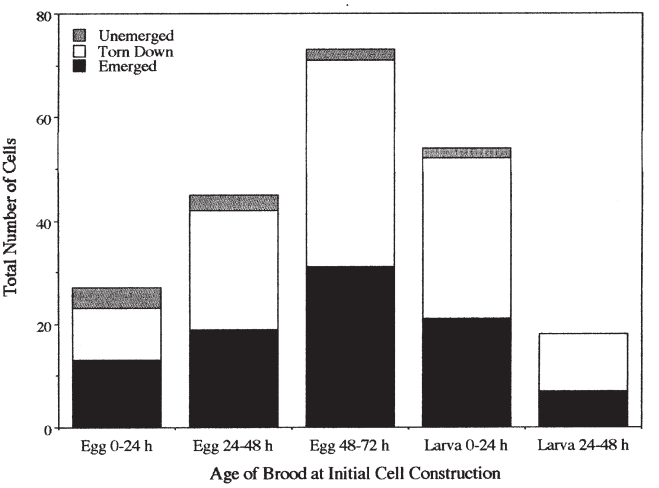

Figure 2. The non-random construction of queen cells. Workers preferentially rear queens from older worker eggs and tend to tear down a greater proportion of cells built around old larvae. The results are summed among colonies

On the other hand, the further a queen cell was located from the center of the nest, the more likely it was to be torn down ($\chi^2 = 17.0$, df = 3, $p < 0.001$; Table 2). Only 41.0% of the capped cells on frames in the 'center' and 'near center' frames were torn down compared to 71.1% of the cells on the more peripheral frames. Therefore, position within the colony is certainly significant in cell construction, but more importantly it influences the probability of a queen being reared to emergence.

Queens were reared from all ages of eggs and from worker larvae that were up to two days old on the day of dequeening (Fig. 2). It should be noted that the age categories are not necessarily discrete because there is an inherent 24-hour measurement error. However, a significant difference was found in the ages of eggs/larvae from which queens were reared ($\chi^2 = 17.9$, df = 4, $p < 0.005$). The majority of queens were reared from individuals that were eggs (69.2%) at the time of dequeening, and about half of these (34.1%) were eggs 48-72 hours old. Additionally, the age of the worker brood from which their occupants were reared had an effect on the percentage of cells destroyed before development was complete ($\chi^2 = 29.0$, df = 4, $p < 0.001$; Fig. 2); only 37.0% of the cells constructed around eggs 0–24 h old were torn down, while more than half (61.1%) of the cells started around larvae 24–48 h old were torn down before the queens could emerge.

Two of the 91 queens that emerged escaped capture and were lost, therefore only 89 were available for measurement. Data on each separate criterion were found to be normally distributed based on standard diagnostic techniques. Unlike the differences in cell number and likelihood of deconstruction, there was no significant effect of position on any of the morphological characters of the queens. There were, however, some significant differences found among the five age groups for two of the criteria, namely queen weight ($F = 3.17$, df = 4, $p < 0.05$) and thorax length ($F = 3.13$, df = 4, $p < 0.05$; Table 3). In these two cases, individual comparisons were made between the means of the five age groups using Fisher PLSD tests. Queens reared from eggs 48–72 h old were significantly heavier ($p < 0.05$) than queens reared from either larvae 0–24 h old or larvae 24–48 h old. Also, queens reared from eggs 48–72 h old had significantly longer thoraces ($p < 0.05$) than queens reared from eggs 24–48 h old or from larvae 0–24 h old.

Table 2. Lateral position effect of queen rearing. More cells were built near the center of the colony. There was also a higher percentage of emerged queens toward the middle frames. However, the probability of cell demolition increased with distance from the center of the brood nest

Position	Frame numbers	Total No. cells	Average No. cells per frame per colony	% emerged	% unemerged	% torn down
Center	5, 14	52	3.25	48.1	3.8	48.1
Near center	4, 6, 13, 15	48	1.50	62.5	4.2	33.3
Near edge	3, 7, 12, 16	72	2.25	36.1	5.6	58.3
Edge	2, 8, 11, 17	45	1.40	22.2	6.7	71.1
Far edge	1, 9, 10, 18	0	0	–	–	–

Table 3. External and internal measurements for naturally reared queens. There were few differences among age cohorts for measures of queen quality. There was only a significant size difference among certain groups of queens built from different-aged brood. Data are given as means ± SEM

Age group	n	Weight (mg)	Thorax width (mm)	Thorax length (mm)	Wing length (mm)	Wing width (mm)
Egg 0–24 h	13	162 ± 13	4.73 ± 0.21	4.50 ± 0.46	9.47 ±0.38	3.17 ± 0.20
Egg 24–48 h	18	166 ± 25	4.66 ± 0.25	4.37 ± 0.32	9.37 ± 0.46	3.13 ± 0.16
Egg 48–72 h	30	173 ± 22	4.72 ± 0.18	4.65 ± 0.34	9.38 ± 0.34	3.14 ± 0.16
Larva 0–24 h	21	156 ± 23	4.59 ± 0.18	4.34 ± 0.29	9.33 ± 0.35	3.13 ± 0.13
Larva 24–48 h	7	148 ± 22	4.77 ± 0.19	4.43 ± 0.34	9.57 ± 0.40	3.31 ± 0.09
F Value		3.174*	2.023	3.129*	0.675	2.009

Age group	n	Ovariole No.	Spermatheca volume (mm^3)	Poison sac volume (mm^3)	Hind gut (mg)
Egg 0–24 h	13	316 ± 94	1.39 ± 0.24	9.27 ± 3.90	8.85 ± 4.78
Egg 24–48 h	18	352 ± 109	1.36 ± 0.24	7.02 ± 1.79	9.94 ± 6.43
Egg 48–72 h	30	305 ± 85	1.34 ± 0.24	8.62 ± 3.47	10.47 ± 4.88
Larva 0–24 h	21	325 ± 127	1.21 ± 0.43	7.71 ± 3.62	10.14 ± 4.48
Larva 24–48 h	7	342 ± 85	1.34 ± 0.16	9.98 ± 3.21	6.71 ± 4.50
F Value		0.685	1.066	0.158	0.907

* = $p < 0.05$.

Table 4. Correlation coefficients for the nine measurements of queen quality. There were no strong relationships between any of the characters measured in the 89 experimental subjects

	Weight	Thorax width	Thorax length	Wing length	Wing width	Ovariole no.	Spermatheca vol	Poison sac vol.	Hind gut
Weight	1								
Thorax width	0.523	1							
Thorax length	0.391	0.451	1						
Wing length	0.572	0.558	0.415	1					
Wing width	0.391	0.542	0.310	0.710	1				
Ovariole No.	0.049	0.185	0.138	0.196	0.247	1			
Spermatheca vol.	0.534	0.407	0.218	0.551	0.431	0.218	1		
Poison sac vol.	0.324	0.386	0.262	0.463	0.435	0.102	0.297	1	
Hind gut	0.241	0.057	0.061	0.032	−0.189	−0.016	−0.053	0.147	1

Finally, there were no significant correlations among the morphological characters (Table 4). Most notably, there was no relationship between queen weight and ovariole number, irrespective of egg or larval age group.

Discussion

Our results suggest that colonies produce some notable differences in the quality of queens during the requeening process, although the magnitude of such differences is not profound. The only criteria that proved to be significant among the emerged queens were wet weight and thorax length, such that queens reared from older eggs were larger than other age cohorts. These findings differ from Woyke (1971), who found a negative relationship between grafting age and several characters, including ovariole number and spermatheca volume. However, Woyke (1971) did not note the ages of grafted eggs and used larvae up to and including four-days-old. Interpretation of the present finding is unclear. It is possible that bigger queens have an advantage during polygyny reduction, although no study has ever directly addressed this point (but see Butz and Dietz, 1994). It is also possible that heavier queens have higher levels of polyandry (D. Tarpy, unpublished data), which could provide a fitness benefit to the colony by a number of different potential mechanisms (see Ratnieks and Boomsma, 1995 for review). On the other hand, queen size may have little to do with reproductive potential. No significant correlation was found between queen size and ovariole number, a result that supports previous studies when age is held constant (Weaver, 1957; Woyke, 1971; Eckert, 1934; but see Fischer and Maul, 1991). Ovariole number may therefore not be an accurate measure of fecundity, and/or queen size may be correlated with a more meaningful determinant of fitness. Regardless, there appears to be limited variation in the reproductive potential of replacement queens reared by workers during the requeening process.

Despite the resultant quality of queens during the requeening process, the data demonstrate that workers regulate queen rearing to some degree. The most telling evidence of worker involvement derives from the non-random destruction of queen cells. Peripheral cells are preferentially torn down over those towards the center of the brood nest. Differences in temperature (Fukuda and Sakagami, 1968) or nurse bee encounters (Visscher, 1986) may account for this phenomenon, and most likely results in the overall position effect of cell acceptance (Visscher, 1986; Tarpy, 1995). Also, workers tear down a higher proportion of cells built around older larvae than from younger eggs. These abiotic and age differences may influence queen quality beyond our detection, since it was impossible to sample queens from torn down cells. In addition to cell demolition, the construction of queen cells also appears to be regulated by the workers. Disproportionally more queens are reared from older eggs, the same age cohort that tends to be larger than its counterparts. This non-random choice of brood used for queen rearing may be significant to the overall requeening process, although the mechanism remains unknown. It should be noted that this finding is in contrast to Fell and Morse (1984), who found almost no cell construction from eggs. Finally, worker regulation is implied by the requeening rate. Construction of queen cells began within 24 h after removal of the mother queen in all eight replicates, indicating that bees detect the loss of their queen and prepare for her replacement within this short time period.

Given that the major stimuli of queen rearing are most likely shared among the three reproductive processes, the regulation of queen rearing during emergency queen replacement may have broader significance. It is unclear precisely how worker control over queen rearing may be adaptively significant, if at all. If only one queen is needed for the perpetuation of the colony, then why do the workers construct more than one cell and regulate the outcome of the requeening process? Here we briefly entertain four hypotheses why colonies may regulate cell construction. (1) Colonies raise multiple queens to guarantee the requeening process. As long as the costs of queen rearing are low (which they presumably are), there is little selection against multiple cell construction. On the other hand, the failure to requeen ensures the death of the colony. If only one queen cell is raised, it has a decreased probability of emergence (by 5.1% in the present study) since deleterious alleles, homozygosity at the sex locus, and colony disturbance are all potential sources of queen mortality during development. The smaller costs of multiple cell construction are outweighed by the extreme cost of requeening failure, so colonies raise multiple queens as an "insurance policy." (2) If the conditions are right, raising multiple queens may provide a reproductive opportunity. Certainly in the case of after-swarming, multiple queens must be reared. Furthermore, sometimes in cases of emergency queen replacement following queen loss, colonies may take advantage of fortuitous favorable environmental conditions by producing swarms (Fletcher and Tribe, 1977a; Winston, 1979). Multiple cell construction may therefore be driven by potential reproductive events. (3) The number of constructed queen cells is merely an incidental result of age demographics in the brood and/or workers. Differences among colonies with respect to the number of queen cells they produce may be a result of the relative abundance of workers performing cell construction (see Pankiw, 1997). Similarly, the variance in brood cohorts during queen rearing most likely has an effect on the number of cells built since we demonstrate a non-random bias of cell construction from different ages of eggs and larvae. The large variation in cell number among colonies in this and other studies may be simply a reflection of these factors. (4) Workers rear multiple queen cells because it affords them an opportunity to select among the variants. The observed worker control suggests that such selection may be taking place. Workers are preferentially rearing queens of different age groups that result in different-sized queens, as well as non-randomly tearing down cells. It is possible that workers are recognizing lower quality queens and selectively destroying them before emergence. The remaining queens would therefore be of similar quality, any of which would be sufficient to take over the nest after polygyny reduction. However, without knowing the quality of the occupants of the destroyed cells, there is no way to verify the extent of worker selection during queen rearing.

By no means are these hypotheses mutually exclusive. However, the regulation of queen rearing is apparent, and the outcome of such control seems to have a stabilizing effect on the quality of emerged queens, i. e. all fully-developed queens are very similar in several reproductive characteristics. At this point, it is unclear which hypothesis has the greatest influence during queen rearing and therefore the greatest impact on future colony fitness. Further studies which directly test these effects are needed to address these questions.

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