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RESEARCH ARTICLE

Honey bee colonies regulate queen reproductive traits by controlling which queens survive to adulthood

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Abstract The production of new queens in honey bee colonies is one of the most important determinants of reproductive success, and it involves cooperative behavior among hundreds or thousands of workers. Colony members are generally expected to benefit by optimizing the reproductive traits of prospective replacement queens, but potential conflicts of interest among colony members could result in suboptimal queens. We studied the degree to which colonies regulate adult queen traits by controlling access to developing queens that survived from pupation to adulthood. We also searched for evidence of strong conflict among patrilines by comparing the contribution of patrilines to new queens and new workers, although we found no evidence for the existence of significantly queen-biased patrilines or for any association between patriline contribution to new queens and queen traits. However, adult queens emerging from cells accessible to workers were larger in terms of compared to adult queens emerging from cells that were not accessible to workers. These results suggest that colonies regulate queen quality traits by curtailing low-quality queens from fully developing, which is further evidence that cooperation predominates over potential conflict within honey bee colonies.

Keywords Social physiology · Colony-level selection · Royal cheats · Queen reproductive potential

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Introduction

The number and quality of new queens and males produced by a honey bee colony define colony reproductive success and represent the culmination of colony-level activities including foraging, brood rearing, and nest defense. Colony-level selection is expected to strongly shape colony-level processes and all the traits of colony members that affect the colony output of new queens and males. At the same time, the caste fate of individual female larvae strongly affects individual reproductive success; workers are facultatively sterile, so within-colony selection may also shape individual-level traits affecting caste fate. Thus, while honey bee colonies are often considered to be exemplar cooperative units that maximize colony productivity, honey bee life history traits may also be strongly shaped by individual conflicts of interest.

The production of new queens is an emergent, colony-level process involving the coordinated activities of hundreds or thousands of adult workers through a series of sequential stages. Queen rearing is initiated when the current queen dies or needs to be replaced (supercedure) or as the colony prepares for swarming (colony division). In cases of queen replacement or supercedure, workers first build special queen cells from a small fraction of available worker cells with young larvae. The precise factors determining which larvae are reared as queens are unknown, but workers preferentially build queen cells from worker cells that

contain brood of particular ages (Fletcher 1978; Fell and Morse 1984; Hatch et al. 1999; Tofilski and Czekonska 2004). Most queens that are raised, at least under "emergency" circumstances, originate from the oldest eggs or from the youngest larvae present at the initiation of queen rearing.

Over the course of larval development, nurse-aged workers provide queen and worker cells with qualitatively and quantitatively different nutrition (colloquially known as "royal jelly" vs. "worker jelly"; Haydak 1970; Brouwers et al. 1987), which induces divergent queen versus worker developmental trajectories. This social control of the larval nutritional environment is critically important, as demonstrated by the fact that queen-worker dimorphism disappears when social control is eliminated and larvae are reared in vitro (Linksvayer et al. 2011).

Finally, workers determine which queen-destined brood is actually allowed to survive to adulthood. Workers frequently tear down queen cells after they have been constructed (Allen 1956; Gary and Morse 1962; Winston and Taylor 1980; Melathopoulos et al. 1996), and queen cells that are started with older brood are destroyed more often than cells initiated with eggs (Hatch et al. 1999). Given that queen reproductive potential (i.e., body size, predicted mating success, fecundity, and longevity; Tarpy et al. 2011; Rangel et al. 2013) is negatively correlated with the age at which an egg or larva is initiated as a queen (Woyke 1971; Fischer and Maul 1991; Dedej et al. 1998; Gilley et al. 2003), this bias in cell destruction might suggest that workers affect the outcome of the queen-rearing process by decreasing the variation in queen traits in general, and specifically queen reproductive potential (Winston 1987). Indeed, Hatch et al. (1999) found that the surviving, worker-selected queens that were allowed to emerge did not differ significantly with respect to several measures of fecundity regardless of larval age when queen rearing was initiated. Most notably, there were no significant differences in ovariole number between the queens produced from the different age cohorts. This suggests that there is a final step in the social control of queen traits, whereby workers may be "weeding out" lesser-quality queens, which is the focus of the current study.

If workers are selectively destroying low-quality queens prior to emergence, one would expect that surviving queens have higher average reproductive potential than queens that were destroyed. The best way to test this hypothesis would be to compare queens from undestroyed and destroyed cells, but it is difficult or impossible to measure queen traits from queen cells that are being destroyed or have been destroyed. To circumvent this problem, we experimentally prevented workers from destroying a subset of queen cells and compared the traits of adult queens emerging from these worker-excluded queen cells to the traits of adult queens emerging from the remaining queen cells that were accessible to workers.

While colony-level selection is expected to act to optimize queen reproductive quality traits, since these traits closely linked to colony fitness (e.g., Rangel et al. 2013), within-colony conflicts over which individual larvae are reared as new queens could mitigate queen quality traits. A range of studies in honey bees and ants find evidence suggestive of patriline and genotype biases (i.e., "royal cheats") in queen development (Tilley and Oldroyd 1997; Osborne and Oldroyd 1999; Châline et al. 2003; Hughes and Boomsma 2008; Dobata et al. 2009). As a secondary goal, we searched for evidence of strong biases of patriline contributions to new queens versus new workers, which would be consistent with the presence of caste-biasing alleles segregating in the population.

Materials and methods

We removed the mother queen from each of six two-story colonies to initiate emergency queen rearing. Once a day, we inspected the brood frames within each colony for the presence of newly constructed queen cells. We numbered each new queen cell and recorded its position within the nest. We monitored each cell daily as the queen larva grew and the cell was elongated by the workers, at which point we randomly placed it into a "worker-excluded" or a "worker-accessible" group. We constructed the cages from wire mesh (3.25 mm^2) and they were sufficiently large to press into the comb to the mid-rib to deter the workers from accessing the cells. We fully caged one-third of the cells (the "worker-excluded" group), chosen at random by flipping a weighted coin, while the remaining two-thirds were caged with queen excluder to permit worker access yet control for the caging manipulation (the "worker-accessible" group). Given that workers destroy approximately half of the capped queen cells that they construct (Hatch et al. 1999), this procedure would ideally yield an equal number of adult queens in the two treatments at the end of the rearing period.

Upon emergence, we estimated the reproductive potential (=quality) of each queen using non-destructive means. First, we recorded the wet weight of each queen by measuring her to the nearest 0.1 mg on a digital Mettler microbalance. Second, we measured the thorax width of each queen using digital calipers. For consistency, we measured the width of the thorax between the two wing junctions of each queen. Other morphometric measures (see Tarpy et al. 2011; Rangel et al. 2013) were not analyzed because we did not place each queen into their own mating nucleus colonies to mate and begin oviposition. We then froze each queen in separate microcentrifuge tubes at −20 °C for subsequent genetic analysis.

Three weeks after dequeening, we sampled ~100 workers from each colony by emerging a frame of capped

brood in an incubator set at brood nest conditions (34 °C and ~50 % RH) to remove the possibility of drifting workers from another colony. These workers were, therefore, of the approximate cohort of brood from which the emergency queen cells were constructed. We then subjected all workers and queens to microsatellite genotyping following standard techniques (see Delaney et al. 2011; Evans et al. 2013). Briefly, we extracted genomic DNA using Chelex® resin, obtained PCR products for each individual at eight microsatellite loci (A24, A28, A79, A88, A107, Ap43, Ap66, and Ap81), and determined the paternal marker set for each using GeneMapper® 4.0 and COLONY® 1.2. We were, therefore, able to assign paternity to each worker or queen, which enabled us to distinguish their subfamily or patriline. In doing so, we calculated the effective paternity frequency for each queen following Nielsen et al. (2003).

Results

One of the colonies was unexpectedly already rearing queens at the time of queen removal, so we excluded it from all further analyses because we could not properly track the development of the queen cells. Of the remaining five colonies, 61 queen cells were reared to the point of capping, ranging from 4 to 22 per colony. 19 (31 %) of the cells were assigned to the "worker-excluded" treatment and the remaining 42 cells (69 %) were assigned to the "worker-accessible" treatment. Four of the "worker-excluded" cells were unexpectedly torn down by the workers, because they successfully circumvented the cages by chewing through the comb. Twenty-four of the "worker-accessible" cells were successfully torn down by the workers, so that overall 57 % of the queen cells were torn down after capping.

Of the 33 total adult queens fully reared during the study, 15 were from the "worker-excluded" group and 18 from the "worker-accessible" group. Using a two-way ANOVA with Colony and Treatment (with their interaction), the wet weights of queens were significantly different among colonies ($F_{4,22} = 5.40$, $p < 0.005$) and were marginally different between the two treatment groups ($F_{1,22} = 3.43$, $p = 0.08$; Fig. 1) with no interaction with colony, such that those from the worker-accessible group were numerically but not statistically heavier than those from the worker-excluded group. The same effect was statistically significant for thorax width, with queens from the worker-accessible

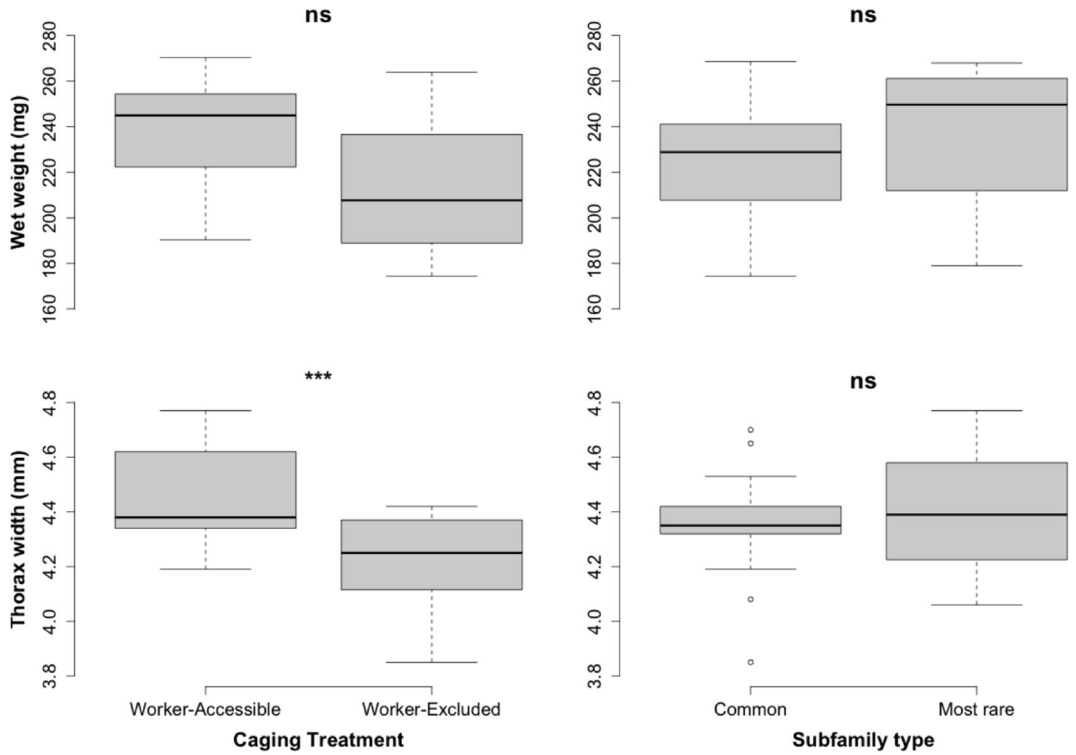

Fig. 1 Effect of queen treatment on two different measures of queen reproductive potential. Shown for each group are standard box plots describing the respective distributions, with minimum value (*bottom bar*), 25th percentile (*lower box bound*), median (*center box line*), 75th percentile (*upper box bound*), and maximum value (*top bar*). Individual data points are also shown. *** $p < 0.005$. *Left* Queens that derived from worker-accessible cells were significantly larger (*bottom*) than those derived from worker-excluded cells, with a non-significant trend for weight (*top*). *Right* Queens from unique or the most rare subfamilies did not differ in either measure of queen quality, suggesting that the rare "royal subfamilies" are not likely selectively favored at the individual queen level

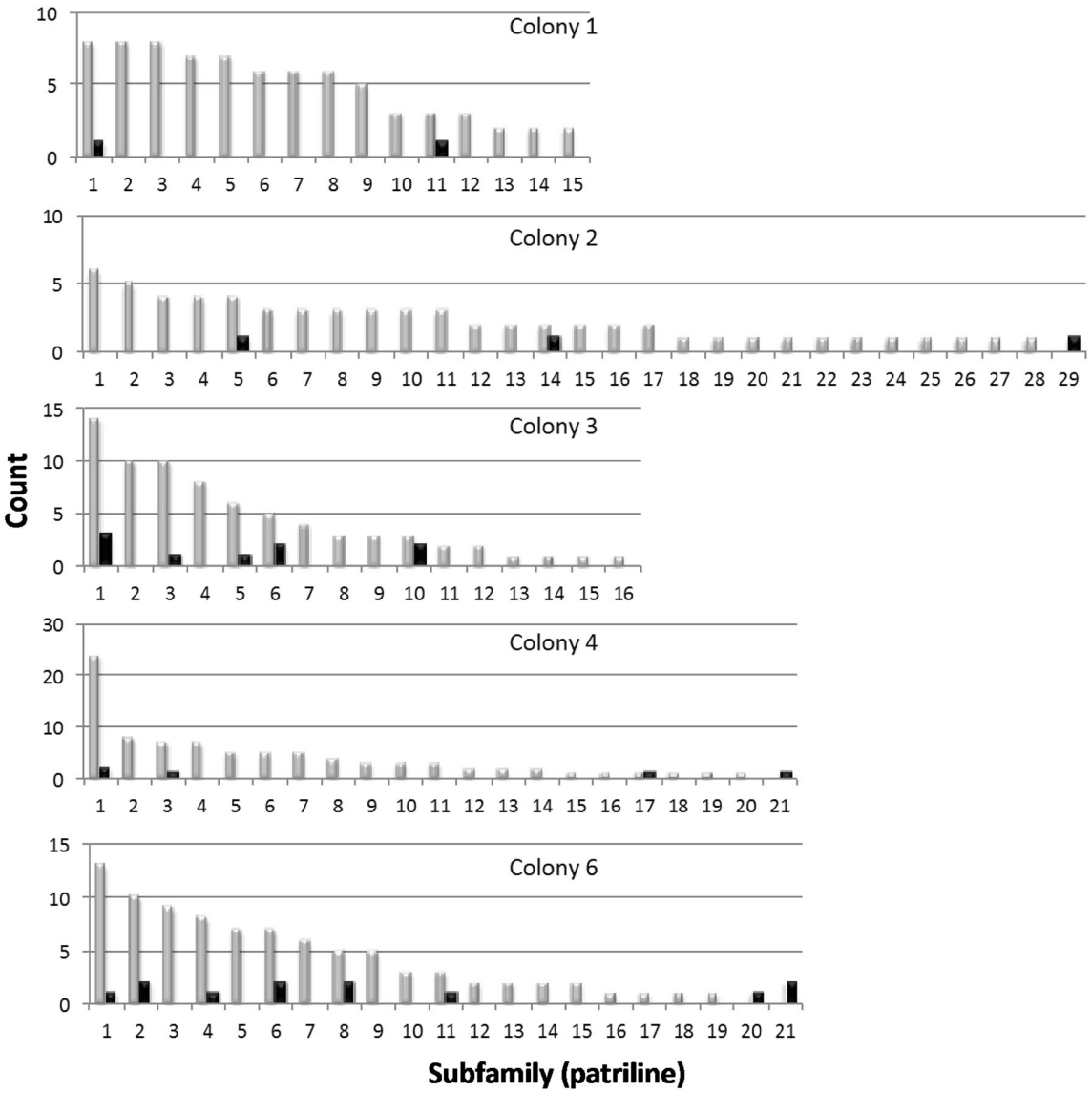

Fig. 2 Subfamily distributions of each colony. *Gray bars* are the counts, in decreasing order, of the workers within each colony. *Black bars* are the number of queens within each same subfamily. We detected 15–29 subfamilies across the five colonies, with some queens deriving from patrilines that were rare or even absent in the workers, although we did not find evidence for a significant patriline bias in queen production across all colonies

group being larger than those from the worker-excluded group ($F_{1,23} = 5.81$, $p < 0.05$; Fig. 1). The variance between the two groups was not significantly different for either measure (weight $p = 0.50$; thorax width $p = 0.90$).

We estimated that the maternal queens of the workers and daughter queens mated with between 15 and 29 drones (Fig. 2). Because of skews in paternity (unequal representation of drone fathers among the worker offspring), the effective paternity frequency (m_e) of the queens ranged from 9.1 to 29.7.

A few patrilines were only detected among adult queens and not among adult workers (Fig. 2), consistent with the phenomenon previously referred to as "royal subfamilies." However, this apparent bias in queen production among patrilines was not significant for any colony (Fisher's Exact Test, all $p > 0.15$), and similarly we did not find evidence for a significant patriline bias in queen production across all colonies (Chi-squared test, $\chi^2 = 121.1$, $df = 101$, $p = 0.084$). Four of the 33 queens analyzed did not generate sufficient microsatellite data for successful genotyping, and so were not included in the analyses. When pooled among colonies and categorized into the most common, common (above median representation), rare (below median representation), and rarest subfamily, the queens were significantly more likely to be reared from subfamilies that were most rarely represented (if at all) among the workers

(Pearson Chi Square, $\chi^2 = 15.66$, $df = 3$, $p < 0.005$). When comparing the proxies of queen quality (wet weight and thorax width) between queens from the most rare versus common subfamilies, there are no significant differences between the two groups (Fig. 1). We also observed no relationship between the proportional investment of patrilines in new queens and queen mass or thorax size (glm with binomial residuals; mass $z = 0.90$, $df = 26$, $p = 0.37$; thorax size $z = 1.08$, $df = 27$, $p = 0.28$).

Discussion

Social insect reproductive caste is considered to be an exemplar polyphenism, whereby alternate developmental trajectories depend on environmental conditions (Evans and Wheeler 1999). However, unlike polyphenisms found in other insects (Nijhout 2003), social insect queen-worker dimorphism is largely socially controlled (Linksvayer et al. 2011). In honey bees, this social control involves presumed worker control over exactly which larvae are chosen to be reared as queens, as well as social control over the nutritional environment provided to larvae, which determines whether the larvae develop as queens or workers (Haydak 1970; Brouwers et al. 1987; Linksvayer et al. 2011). Besides this worker control during larval development, here we demonstrate another level of social control. We find that queen cells accessible to workers produce larger adult queens (as measured by thorax width) than from queen cells from which workers are experimentally excluded. These results indicate that adult workers may be "weeding out" lower quality queens.

Although the precise mechanism by which queen quality is assessed by workers is not known, the end result of this additional social filter on colony investment in new queens may be expected to maximize colony-level fitness. Previous work has found that higher quality queens tend to be larger (i.e., higher wet mass, larger thorax and spermatheca), resulting in an increased likelihood to win fights with other queens, mate with an increased number of males (Tarpy et al. 2011), and head colonies that are more productive (Rangel et al. 2013). Thus, preferentially rearing larger queens to adulthood selects for queens of higher reproductive potential. The lack of a reduction in variance among the worker-accessible group, however, suggests that other mechanisms may also be responsible here, most notably the opportunity for incubation of developing queen cells (DeGrandi-Hoffman et al. 1993; Schneider and DeGrandi-Hoffman 2002). Thus, there may be multiple factors that influence the collective decisions of queen rearing during these episodes.

We also looked for evidence of patriline biases in the contribution to new queens versus new workers, which would be consistent with the presence of "royal cheats", queen-biasing alleles (Tilley and Oldroyd 1997; Osborne and Oldroyd 1999; Châline et al. 2003; Hughes and Boomsma 2008; Dobata et al. 2009; Van Dyken et al. 2011). Such caste-biasing genotypes may also be expected to be associated with different, perhaps lower quality queens. We found no evidence for such patriline biases or associations between queen traits and the proportional representation of patrilines in new queens and workers, although we ended up with little power to detect such biases because of the overall low number of queens produced across our replicate colonies. Future studies of potential caste-biasing should also consider influences on queen traits.

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