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Report

Promiscuous Honey Bee Queens Increase Colony Productivity by Suppressing Worker Selfishness

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Summary

Queen monogamy is ancestral among bees, ants, and wasps (Order Hymenoptera), and the close relatedness that it generates within colonies is considered key for the evolution of eusociality in these lineages [1]. Paradoxically, queens of several eusocial species are extremely promiscuous [2], a derived behavior that decreases relatedness among workers and fitness gained from rearing siblings but benefits queens by enhancing colony productivity [3–9] and inducing workers to rear queens' sons instead of less related worker-derived males [10–13]. Selection for promiscuity would be especially strong if productivity in a singly inseminated queen's colony declined because selfish workers invested in personal reproduction at the expense of performing tasks that contribute to colony productivity. We show in honey bees that workers' ovaries are more developed when queens are singly rather than multiply inseminated and that increasing ovary activation is coupled with reductions in task performance by workers and colony-wide rates of foraging and waggle-dance recruitment. Increased investment in reproductive physiology by selfish workers might result from greater incentive for them to favor worker-derived males or because low mating frequency signals a queen's diminished quality or future fecundity. Either possibility fosters selection for queen promiscuity, revealing a novel benefit of it for eusocial insects.

Results and Discussion

In three studies, we tested the hypothesis that honey bee (*A. mellifera*) workers selfishly redirect resources toward their own reproductive physiology when queens are monandrous (inseminated by a single male), and that this costly investment is linked to a reduction in the performance of tasks that are critical to colony function. Our first study determined whether the degree of workers' ovary activation was influenced by the mating frequency of queens. Most bee, ant, and wasp queens are monandrous; however, extreme polyandry (insemination by multiple males) has evolved in several hymenopteran lineages, including all species of honey bees [1, 2, 14]. Although untested in honey bees, interspecific comparisons show that workers' reproductive activity increases as mating frequency drops and nestmates become more closely related [15–18]. In 2008, ovary activation was compared for workers in honey bee colonies where queens were inseminated by either one male per queen or multiple males per queen. Given the scarcity of workers with fully developed ovaries in our colonies (see Figure S1 available online), we distinguished between workers that had resting ovaries and workers that had some degree of ovary activation. We first assessed whether the fraction of workers with activated ovaries was affected by the technique of instrumental insemination, independently of queen mating frequency. A bootstrap analysis (20,000 runs) found no difference between colonies with naturally mated versus multiply inseminated queens in the mean fraction of workers with activated ovaries (Figure 1; bootstrapped 95% confidence interval [CI] for mean difference [−0.117, +0.107]; $p < 0.05$; differences are statistically significant when 95% CI does not overlap zero). Given that the latter distributions were so close to each other, we pooled these treatments into a single group for comparison to workers from colonies with singly inseminated queens. A significantly higher mean fraction of workers showed some degree of ovary activation when queens were singly rather than multiply inseminated (Figure 1; Figure S1; bootstrapped 95% CI [0.03, 0.281]; $p < 0.05$).

It is unlikely that workers with singly inseminated queens laid eggs more often than workers with multiply inseminated queens. In both types of colonies, workers with fully activated ovaries comprised <1% of those sampled (Figure S1), a frequency that is only marginally higher than that reported previously for *A. mellifera* [11, 12, 19]. Workers with partially developed ovaries occur frequently in queenright colonies [20–22], which reflects our findings (Figure 1; Figure S1). However, colonies with singly inseminated queens had the highest levels of partial ovary development, suggesting that some workers modulated investment in reproductive physiology in response to either the low mating frequency of queens or the presence in colonies of a single patriline (family fathered by a single male).

With the discovery that the proportion of workers with activated ovaries increased when a colony's queen was singly inseminated, our next study determined whether this response was associated with a decrease in the rate at which workers executed critical tasks. This hypothesis is supported by observations of reduced task execution under atypical conditions where worker reproduction is usually high for honey bees [20, 23–27]. Specifically, we examined tasks related to acquiring food from the environment because reductions in the rate at which workers forage are associated with a loss over time of colony productivity and other fitness proxies [7, 28]. In 2009, we correlated ovary activation among workers with colony-wide foraging and recruitment activity in a new set of colonies, each headed by a singly inseminated queen. We found that the higher measures of mean ovary activation were among workers, the lower were the rates at which foragers visited a food source (Figure 2A; Spearman correlation; $\rho = -0.67$; $p = 0.007$) and the less time they spent advertising it with waggle dances (Figure 2B; $\rho = -0.55$; $p = 0.034$).

Workers with fully developed ovaries were not observed in the colonies that were studied in 2009 (Figure S1). However, a large fraction of workers had partly developed ovaries and at a frequency that was comparable to the other colonies with singly inseminated queens that were examined in 2008 (Figure 1; Figure S1). Also similar to the previous year, the

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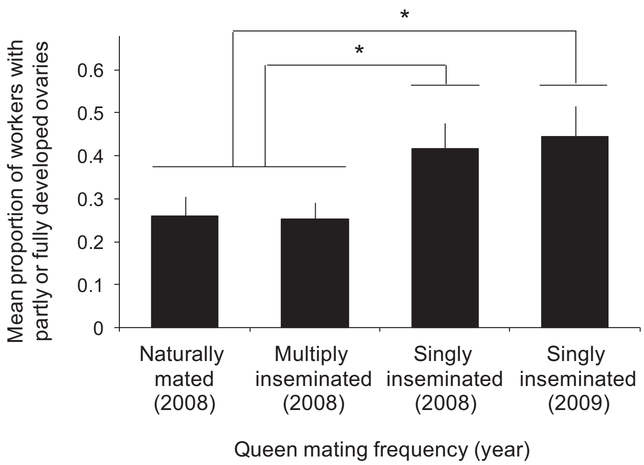

Figure 1. Queen Monandry Induced Ovary Development among Workers

Ovary activation among workers was compared over 2 years between colonies that had queens that were naturally mated (n = 11 colonies in 2008), instrumentally inseminated by multiple males (n = 12 colonies in 2008), or instrumentally inseminated by a single male per queen (n = 14 colonies in 2008; n = 15 colonies in 2009). Multiply inseminated queens received semen from different groups of 15 males either in small volumes (equivalent to a single mating; n = 9 queens) or in large volumes (equivalent to the capacity of a queen's spermatheca; n = 3 queens). Data were pooled across high-volume and low-volume treatments because of a small sample size in the first treatment and a similarity between groups in the fraction of workers with at least partly developed ovaries (mean ± SEM: 0.20 ± 0.04 and 0.27 ± 0.04, respectively). The proportion of workers with partly or fully developed ovaries was determined for each colony and compared across groups (mean + SEM are presented); groups that separate at $p < 0.05$ are indicated by asterisks (two-tailed bootstrap test). See Experimental Procedures and Supplemental Experimental Procedures for further details; see also Figure S1.

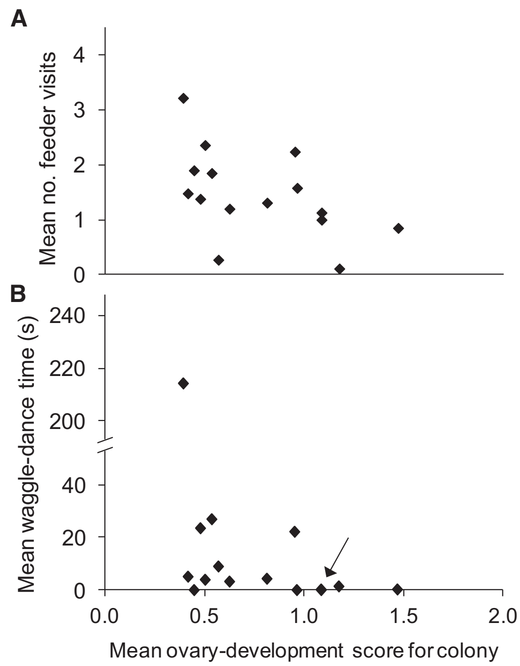

Figure 2. Foraging and Waggle-Dancing Rates Decreased in Colonies as Ovary Development Increased among Workers

Mean ovary-activation scores for workers in colonies were correlated against mean per capita rates of (A) feeder visitation and (B) waggle-dance recruitment for focal foragers as they visited a food source. Rates of visitation and recruitment were determined for each colony by transferring ~2,000 workers and their queen into a two-frame observation hive in a greenhouse, where foraging conditions were standardized across replicates. Foragers were trained to visit a sugar-water feeder (1.0 M sucrose solution; mean 27 workers per colony; range 11–40 workers per colony). After training was complete, the feeder was removed for at least 1 hr, then it was restocked (2.0 M sucrose solution), and activity rates of focal individuals were determined over the subsequent hr. The arrow indicates two overlapping data points. See Experimental Procedures and Supplemental Experimental Procedures for more details about assessment of workers' ovaries and foraging behavior.

fraction of workers with partly activated ovaries was significantly higher in the 2009 colonies with singly inseminated queens than it was in the 2008 colonies with multiply inseminated queens (Figure 1; bootstrapped 95% CI [0.047, 0.337]; $p < 0.05$). Our observation that foraging and recruitment productivity waned as ovary activation increased within singly mated queens' workforces is remarkable, especially given that none of the sampled workers were fully reproductive and differences among colonies in mean ovarian activation were incrementally small, ranging from resting to only minimal development, but were associated with substantial changes in colony-wide productivity (Figure 2). Our findings suggest that small adjustments to ovary development by workers in response to the mating frequency of queens (or genetic architecture of their offspring) are concomitant with large effects on colony productivity.

Our final study explored the causative nature of the relationship between ovary activation and worker productivity by comparing task performance among individuals who varied in the degree to which their ovaries were activated. To overcome the challenge of getting adequate numbers of workers with developed ovaries in queenright colonies, we divided into halves the worker population and comb contents of three different colonies (all headed by polyandrous, naturally mated queens). One half of each colony remained queenright and the other half was made queenless, with the expectation that many workers in the second half would undergo ovary development and some would commence egg laying [29]. Once eggs were found frequently in queenless halves of colonies, marked workers from both halves of the same colony were reunited in a single observation hive (with their original queen), the extent to which they performed colony tasks was evaluated, and their ovarian development was subsequently determined. Ovary activation among focal workers did not differ based on source colony, so data were pooled across colonies for analysis (colony-source effect in a colony half × source two-way ANOVA: $F_{2,350} = 2.6$, $p = 0.08$; colony-half effect: $F_{1,350} = 179.5$, $p < 0.0001$; effects interaction: $F_{2,350} = 1.3$, $p = 0.27$).

In total, ovary-activation scores and behavioral data were obtained for 356 workers, with mean 4.0 ± 0.1 (SEM) observations per worker. A "performance index" showed that the activity level of workers was affected by the extent to which their ovaries were developed (Figure 3; one-way ANOVA: $F_{2,353} = 3.8$, $p = 0.02$). Workers with resting ovaries were significantly more likely to be actively engaged in a task than doing nothing compared to workers with fully developed ovaries, who were conversely more likely to be observed motionless than at work (Figure 3). Workers with partly developed ovaries fell between the two extremes (Figure 3). It is possible that colony conditions prior to merger (i.e., presence or absence of a queen) skewed worker behavior once colony halves

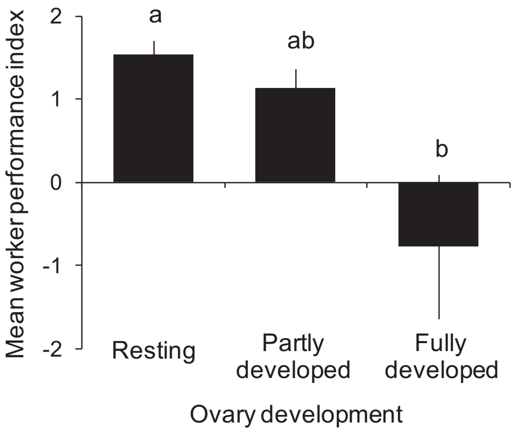

Figure 3. Workers with Resting Ovaries Were Engaged in Colony Tasks More Often than Workers with Fully Developed Ovaries

Task performance and ovary development of focal workers was evaluated in three colonies with naturally mated queens; data were pooled across colonies (see Results and Discussion). A performance index was calculated for each worker by classifying their behavior (observed during repeated scans of observation hives) as either inactive (i.e., stationary) or active (i.e., walking, grooming, feeding other adults or brood, maintaining the nest, guarding, entering/exiting, or dancing) and then subtracting the number of observations of inactive behavior from the number of observations of active behavior. A positive index meant a worker was observed actively engaged in a task more often than she was motionless; a negative score meant that she was motionless more often than active. Ovary-activation scores were determined after all behavioral data were collected. Mean indices (+ SEM) were compared among workers with resting (n = 289 workers), partly (n = 58 workers), and fully developed ovaries (n = 9 workers).

were reunited, especially considering that the majority of workers with resting ovaries came from queenright halves of colonies (92%) and all workers with fully developed ovaries came from queenless halves (workers with partly developed ovaries came evenly from both halves: 56% and 44%, respectively). However, we presume this potential skew to be minimal, given that, once colony halves were merged, average performance indices were similar between workers from queenright and queenless colony halves (for all workers: colony-half effect in a colony half × source two-way ANOVA, $F_{1,350}$ = 2.1, p = 0.15; colony-source effect: $F_{2,350}$ = 0.4, p = 0.69; effects interaction: $F_{2,350}$ = 0.1, p = 0.95; for workers with partly developed ovaries only: t test, t_{56} = 1.3, p = 0.20). In other words, activity levels of workers were affected only by their reproductive physiology on the day that they were observed, not by whether a queen was present in the half of the colony that they lived in before they were observed. This final study shows that workers who live in queenright colonies perform less work if their ovaries are fully activated compared to workers who remain infertile, with partial ovary development affecting worker performance to an intermediate degree.

Our evidence identifies a novel cost of monandry for honey bee queens (i.e., increased ovary activation among workers is coupled with lower colony productivity) and shows how changes in worker behavior can generate these costs, thus providing a strong additional selective benefit for polyandry in the eusocial Hymenoptera, one that is distinct from, and a new alternative to, the efficiency benefits of genetic diversity [30] and suppressed reproduction by workers [10]. A substantial proportion of honey bees (41%–45% of workers; Figure 1; Figure S1) responded to colony conditions created by monandrous queens with a subtle shift to heightened ovary development, negatively affecting work rates in a way that worsened as workers' ovaries became more activated. We propose two (not mutually exclusive) hypotheses to explain this shift: (1) workers may increase their investment in personal reproduction in response to a greater likelihood that worker policing will be weakened at some point in the future, given that the incentive for policing against such selfishness is reduced as mating frequencies of queens decrease, and (2) singly inseminated queens may inadvertently signal to workers that they are in poor condition (low expected fecundity or survival), to which workers respond by increasing investment in possible future selfish reproduction, as they would when a colony becomes queenless. It is possible that workers, assessing that they have enhanced future reproductive opportunities for either reason, partially activate their ovaries and reduce their work levels to increase their likelihood of successfully capitalizing on those opportunities, but do not fully activate their ovaries and lay eggs without additional evidence that those opportunities can be realized (e.g., queen loss or weakened policing levels). Workers with partially developed ovaries may invest in reproductive "readiness" to capitalize quickly on reproductive opportunities when they finally arrive, even though this readiness may occur at some cost to colony output.

These findings are exciting because they unify two hypothesized explanations for the evolution of extreme polyandry in the eusocial Hymenoptera by causally linking increases in worker fertility to decreases in their productivity, which can explain colony-wide loss of productivity as more workers activate their ovaries (Figure 2). Importantly, this link provides an alternate explanation for why single-patriline colonies are repeatedly less productive than multiple-patriline colonies [3–9], one that does not exclude (but is possibly synergistic with) the roles that patriline membership and task participation by workers play in generating efficient division of labor in colonies with polyandrous queens [9, 30]. Patriline differences in task performance seem most consistent with genetic diversity hypotheses for the benefits of queen promiscuity, but such differences might also arise from the reproductive conflict revealed by our results if differences in worker selfishness exist among patrilines and are maintained by frequency-dependent selection.

How do workers assess colony conditions to modulate their fertility? Workers are capable of detecting changes in queen pheromone that are caused by insemination volume and reflect mate number [31]. However, such differences among queens were controlled for here. It is possible that genetic diversity within a colony yields an increase in the complexity of colony odor, which workers may evaluate, given that patrilines vary in the chemical recognition cues that their workers produce [32–34]. Without understanding the mechanisms that generate it, the ability of selfish workers to make subtle shifts in their fertility in response to mate number is surprising, given that *Apis* queens are universally polyandrous [14] and there is extremely weak or no selective pressure in the natural world for workers to invoke this response. Yet, workers may have evolved a facultative response to the natural range of variation in the mating frequency of queens, with such facultative assessment most clearly revealed under the unusual case of monandry. Honey bee workers have shown a similar ability to adjust their fertility in response to shifting relatedness asymmetries after colony fission [20]. In other situations where conflict between queens and workers changes depending on the mating frequency of hymenopteran queens, such as sex allocation, workers show sensitivity to mating frequency and correspondingly alter their behavior in ways that maximize their fitness interests [35, 36].

At the colony level, selection for worker reproduction when queens are monandrous may be counterbalanced by selection against it if this switch confers large productivity costs to colonies. These costs are confirmed here and they are not trivial, but they are likely curbed by the scarcity of fully reproductive workers. Tension between selfish reproduction by workers and its cost to colonies is further supported by observations that honey bees recognize and act aggressively toward reproductively active colony members [29], causing these workers to give up nutrients to nestmates and hide to avoid attack [37]. Workers have two avenues for minimizing costly reproduction when queens are singly inseminated: self-restraint, which we found here to be only partially relaxed, and worker policing. Indeed, there are species for which production of males by workers is predicted because of queen monandry, yet worker-laid eggs are consistently policed [17], presumably because of costs to colonies of worker reproduction. Reproduction by honey bee workers may be checked if social sanctions such as policing or aggression are upheld when queens are monandrous. Conversely, workers may facultatively suspend policing despite costs to productivity, a phenomenon that is not been clearly documented within a single hymenopteran species [38, 39]. Partial self-restraint by workers in colonies where queens are monandrous, either voluntary or enforced in the face of social sanctions [18], suggests that flagging work rates impart sizable enough costs to colonies to counterselect against selfish-worker reproduction without additional information that such reproductive opportunities have arisen. It will be important to confirm whether policing of workers or their eggs continues when honey bee queens are monandrous to understand how selfish investments by individuals are received in light of colony interests.

In summary, we found that the mating frequency of honey bee queens induces changes in the reproductive physiology of workers, which in turn alters activity levels of individuals and the pace at which vital work is performed within colonies. This relationship between worker reproduction and productivity has profound consequences for our understanding of the interplay of factors that have selected for the evolution of extreme polyandry in the Hymenoptera.

Experimental Procedures

Within each year, instrumentally inseminated queens were maximally related to one another (r = 0.75) and inseminating drones were selected randomly from a pool of 1,000 drones that were sourced from 20 unrelated colonies. Whenever experimental queens were introduced into a host colony, data were not collected until the new queens had totally replaced the host colonies' worker populations (at least 8 weeks later). All ovary development and behavioral data were collected in the blind to avoid observer bias.

In all 3 years, ovary activation was scored using a conventional scale that ranged from resting (score = 0) to completely developed (score = 4) [40]. Each worker's ovaries were scored separately and a mean score was determined per individual; nestmates' scores were used to determine colony means in 2008 and 2009. Activation categories were defined as resting (score < 1), partly developed (score 1–3), or fully developed (score > 3). Workers were collected from drone comb in 2008 (n = 20 workers per colony) and 2009 (n = 90 workers per colony); their ages were not known, but we chose younger workers who had likely not yet initiated foraging (i.e., minimal hair loss, no wing wear, no pollen on body) and, thus, had the greatest probability of having activated ovaries [41].

Expanded details of colony management, worker sampling, ovary assessment, behavioral assays, and data analyses can be found in Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes one figure and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.08.021.

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