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The honeybee queen influences the regulation of colony drone production

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Social insect colonies invest in reproduction and growth, but how colonies achieve an adaptive allocation to these life-history characters remains an open question in social insect biology. Attempts to understand how a colony's investment in reproduction is shaped by the queen and the workers have proved complicated because of the potential for queen–worker conflict over the colony's investment in males versus females. Honeybees, in which this conflict is expected to be minimal or absent, provide an opportunity to more clearly study how the actions and interactions of individuals influence the colony's production and regulation of males (drones). We examined whether honeybee queens can influence drone regulation by either allowing or preventing them from laying drone eggs for a period of time and then examining their subsequent tendency to lay drone and worker eggs. Queens who initially laid drone eggs subsequently laid fewer drone eggs than the queens who were initially prevented from producing drone eggs. This indicates that a colony's regulation of drones may be achieved not only by the workers, who build wax cells for drones and feed the larvae, but also by the queen, who can modify her production of drone eggs. In order to better understand how the queen and workers contribute to social insect colony decisions, future work should attempt to distinguish between actions that reflect conflict over sex allocation and those that reflect cooperation and shared control over the colony's investment in reproduction. *Key words:* cooperation, drone production, honeybee, queen–worker conflict, sequential decision making. *[Behav Ecol 18:1092–1099 (2007)]*

Social insect colonies, like many individual organisms, must allocate resources appropriately between growth and reproduction. How colonies manage to do this is not well understood because little is known about how colony life-history decisions are governed by the actions and interactions of colony members (Bourke and Franks 1995). Recent work has begun to focus on how one colony-level reproductive decision, the investment in males, is influenced by the queen and the workers (Herbers et al. 2001; Beekman and Ratnieks 2003; Beekman et al. 2003; Mehdiabadi et al. 2003; Pen and Taylor 2005; Ratnieks et al. 2006). However, the attempt to understand how the queen and workers cooperate when allocating colony resources to reproduction is complicated by the fact that their actions may instead spring from conflict.

A colony produces males through the actions of the queen and the workers, who are potentially in conflict over their colony's sex allocation, or investment in males versus females (Ratnieks et al. 2006). Although there are other types of queen–worker conflict (see Ratnieks et al. 2006), we consider only conflict over sex allocation for this paper. The potential for this conflict is due to the haplodiploid system of sex determination, whereby the queen is equally related to her sons and daughters whereas workers are on average more closely related to their sisters than to their brothers (Hamilton 1964). When colonies have one queen that is singly mated, workers are 3 times more related to their sisters than to their brothers, leading to the prediction that the queen and workers are in conflict over their colony's sex allocation and that each party should attempt to bias sex allocation in its favor (Trivers and Hare 1976). One way in which workers might do so is by selectively eliminating male larvae, and workers of several species of ants apparently do this (Aron et al. 1994, 1995; Keller et al. 1996; Sundstrom et al. 1996). Given that workers in some species appear to control the colony's investment in males, whether queens can influence male investment by modulating their egg sex ratio has recently gained interest (Aron et al. 1995; Cremer and Heinze 2002; Beekman and Ratnieks 2003; Mehdiabadi et al. 2003; de Menten, Cremer, et al. 2005; de Menten, Fournier, et al. 2005; Koedam et al. 2005).

Although the queen and workers might experience conflict, their inclusive fitness is determined largely by colony success, so they share an interest in adaptively regulating the colony's investment in reproduction. So, although the ability of the queen or the workers to influence their colony's investment in males is consistent with the hypothesis that they are in conflict, an alternative explanation is that queens and workers are assessing local information about the value of rearing males and are cooperatively but sequentially shaping the colony's allocation to reproduction. For example, the queen might modulate her egg sex ratio to ensure that the colony is well supplied in males, and workers might eliminate males later if conditions become unfavorable for raising sexuals. We term this hypothesis queen–worker cooperation. Because queens and workers might agree over their colony's allocation to reproduction but also experience conflict over sex allocation (Herbers et al. 2001), queen–worker conflict and queen–worker cooperation are not mutually exclusive scenarios. Any ability of queens or workers to influence their colony's male investment may therefore reflect a strategy of biasing sex allocation in their favor (conflict), a strategy of obtaining the colony's adaptive allocation to reproduction (cooperation), or some mixture of both. For this reason, it is difficult to interpret the functional significance of queen and worker sex-biasing abilities when there is a high potential for queen–worker conflict.

Although cooperation between queens and workers is likely to be entangled with conflict in many species, highly

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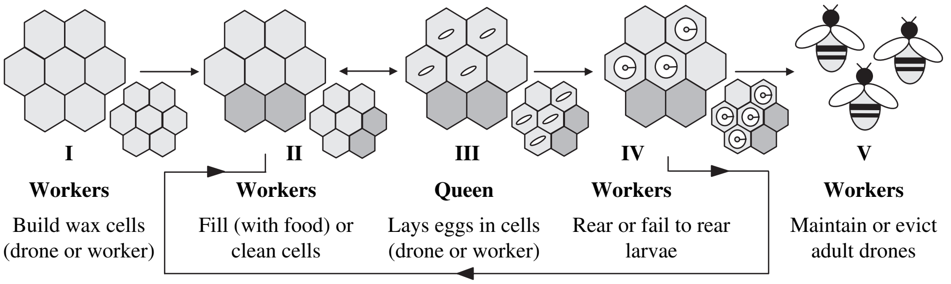

Figure 1
Drone production and maintenance in a honeybee colony: a sequence of actions taken by the workers and the queen. At each step, the bees have an opportunity to influence the colony's investment in drones. Arrows indicate typical sequences of events. Drone cells are larger than worker cells, and drones are normally reared in these larger cells (I). The worker and drone cells are used either for food storage (nectar and pollen; darker cells in diagram) or are cleaned in preparation for rearing brood (II). The queen typically lays unfertilized (drone) eggs into drone cells and fertilized (female) eggs into worker cells (III). Workers then tend the larvae but do not necessarily raise all the larvae to adulthood (IV). Adult drones remain colony members until they mate with a virgin queen in the population, die, or are evicted by workers (V). In this study, we focus primarily on the modulation of drone production that arises from actions taken by the queen (step III).

polyandrous species should have little potential for conflict over sex allocation (Ratnieks et al. 2006). This is because when the queen mates with many males, as in honeybees (Estoup et al. 1994; Neumann and Mortiz 2000), African army ants (Kronauer et al. 2004), and harvester ants (Rheindt et al. 2004), the average relatedness between workers is low, so workers are almost equally related to their brothers and sisters. As a result, the queen and workers should favor a nearly equal investment in the sexes (Moritz 1985; Ratnieks et al. 2006). Therefore, highly polyandrous species provide an opportunity to study whether queens and workers have evolved the ability to influence the colony's investment in males when conflict over sex allocation is not in the picture. In this paper, we investigate one such species, the honeybee, and ask whether the queen has the ability to influence colony patterns of drone (male) production.

The size of the drone population in a honeybee colony depends on a sequence of actions taken by the queen and workers (Figure 1). With each action, there is an opportunity for the bees to alter the investment in drones. Workers construct the wax combs in which broods are reared and thus control the colony's investment in drone and worker cells (Pratt 2004). Drone cells are larger than worker cells and allow drones to develop to full size; therefore, the number of drone cells provides an upper limit for the number of drones that can be reared at any one time. Additionally, workers use some cells for the storage of pollen and nectar and therefore determine the availability of empty cells that can receive an egg. The queen may then use these empty cells for depositing eggs. She produces a female or male egg depending on whether or not she fertilizes the egg and generally lays female eggs in worker cells and male eggs in drone cells (Ratnieks and Keller 1998). The queen sometimes lays eggs in a drone to worker ratio that deviates from the ratio of available drone to worker cells (Henderson 1991), suggesting that she might be able to control the sex of her offspring by selecting what type of comb to use. Next, the larvae are tended by the worker bees, who can decrease investment in drones or workers by failing to provision larvae or by consuming them (Free and Williams 1975; Schmickl and Crailsheim 2001; Sasaki et al. 2004). Finally, the drones that reach adulthood may be evicted from the colony by the worker bees in the late fall or when foraging conditions are poor (Morse et al. 1967; Free and Williams 1975). Because workers initially construct the brood cells and can later alter any queen investment patterns by decreasing the number of immature or adult drones, it is widely assumed that workers control colony drone production.

The value of drones varies with environmental factors. In order to increase colony efficiency by minimizing the number of drones that will be destroyed by the workers, a queen might be expected to adjust her egg-laying patterns to achieve her colony's current optimum investment in drones. Season and energy budget are important factors that affect colony drone production (Free and Williams 1975; Seeley and Mikheyev 2003), and there is some evidence that the queen alters her drone egg production in relation to these (Sasaki and Obara 2001). In addition, colony drone production is inhibited by the presence of drone brood (Free and Williams 1975), indicating that colonies regulate drone production through a negative feedback process. Because this drone brood normally originates from eggs produced by the queen, queens might use oviposition history or the presence of drone brood as an indicator of how many drone eggs to lay. In accordance with this, Sasaki et al. (1996) provide evidence that suggests that honeybee queens decrease subsequent investment in drones if they recently laid drone eggs. However, because the authors used the same queens in multiple treatment conditions staged over time, their data failed to exclude both season and colony energy budget as explanatory variables for the observed queen egg-laying response. Thus, whether queens can adaptively regulate drone egg production remains an open and relevant question.

In this study, we used a between-subjects design to test whether honeybee queens regulate investment in drones. We manipulated the previous egg-laying experience of queens by either allowing or preventing them from laying drone eggs for a period of time, and we then provided them the opportunity to lay both drone and female eggs. If the queen's production of drone eggs is regulated by negative feedback, we expected that queens who were allowed to lay drone eggs for a period of time would subsequently lay fewer drone eggs compared with queens who were initially prevented from laying drone eggs.

METHODS

Honeybee colonies

We conducted this study in the summers of 2004 and 2005 in the countryside surrounding the Michigan State University campus in East Lansing, MI. Twenty-four colonies of Italian honeybees, *Apis mellifera ligustica*, were used, 12 each summer. Because each colony had been purchased as a package in the late spring of the year, it was used in the study, no colony was used twice, and all were approximately the same size (~13 000 bees). Each colony was housed in a standard Langstroth hive consisting of one deep hive body with 10 frames of worker comb. By initially providing the colonies with only, we ensured that drone production occurred mainly during the experimental periods.

General methods

The general strategy was to confine half of the queens to drone comb and half to worker comb for a period of time and then to compare the egg-laying patterns of the 2 groups of queens later, when both types of comb were available.

We randomly assigned colonies to 1 of 2 treatments, DC (drone comb) or worker comb. The treatment labels refer to the type of comb to which we confined the colony's queen before moving her onto a frame with both types of combs. Therefore, before they were moved, queens in WC colonies (WC queens) could not produce viable drone offspring, whereas queens in DC colonies (DC queens) could. These treatments were applied on June 18 in 2004 and on June 16 in 2005. We label those dates as "day 0" of the experiment.

On day 0, we confined each queen to a frame of the randomly assigned type of treatment comb (drone or worker). The cage used to confine the queens was made from queen excluder material, which has small openings through which worker bees can freely pass but the larger queen cannot. We then placed the cage in the colony and positioned it toward an edge of the broodnest, which is where colonies normally rear both drone brood and worker brood.

On day 4, we verified that both the WC and DC queens had laid many eggs on the treatment frame (average proportion of frame area covered with eggs, WC: 0.58, range 0.21–0.91, DC: 0.48, range 0.12–0.84). We then transferred each queen onto a new frame composed of equal areas of drone comb and worker comb (the choice frame). We placed the choice frame into the queen excluder cage and placed the cage at the other edge of the broodnest. Queens are able to switch between laying drones and workers multiple times while successively laying eggs (Sasaki et al. 1996), and this occurs over just a few minutes or hours (Wharton KE, unpublished data). Following the methods of Sasaki et al. (1996), we allowed the queens to lay eggs on this choice frame for 24 h.

On day 5, after we removed each queen from the cage and placed her back into the rest of the colony, we estimated (see below) the number of drone and worker eggs on the choice frame. We used an estimate rather than an actual count because it was faster and thus reduced the exposure of the eggs to the hot summer weather. After making this estimate, we placed the cage containing the choice frame back in the colony. This prevented the queen from laying additional eggs on this frame but allowed the workers to provision larvae that hatched from these eggs. We removed the queen excluder cage from the colonies on days 7 and 15 of the study in 2004 and 2005, respectively.

To estimate the numbers of drone and worker eggs laid in the choice frame, we covered the frame with a grid made from 1.27 × 1.27 cm hardware cloth and counted the number of grid squares containing at least one cell with an egg in the drone comb portion of the choice frame and the number of grid squares containing at least one cell with an egg in the worker comb portion of the choice frame. This gave us an estimate of the area of drone comb and worker comb covered with eggs; this technique is commonly used to obtain estimates of areas of comb covered with young brood (Jeffree 1958). Because drone and worker cells are different sizes, we multiplied the area of drone comb and worker comb by the number of cells in that area (using the convention of 2.60 drone cells per cm^2 and 4.29 worker cells per cm^2; Page and Metcalf 1984) to obtain an estimate for the number of drone and worker eggs laid by the queen, providing a more intuitive notion of queen behavior. This transformation from area to eggs is likely to give a slight overestimate of the actual number of eggs, but the amount of overestimation should be similar for both treatment groups. Because this transformation is applied equally across treatment groups and is linear, it does not affect our analyses or conclusions.

As we were collecting the data on eggs laid by the queen, we noticed that some of the cells in the choice frame had been filled with nectar, which would have deterred queens from laying eggs in these cells. We used the method just described to estimate the maximum area (in square centimeter) of drone comb and worker comb that was used for nectar storage.

After the eggs on the treatment and choice frames reached the pupal stage (and before these pupae emerged as adults), we removed the frames from each hive and took digital photographs of them. We used these photographs to count the number of drone and worker pupae (indicated by capped cells; worker pupae are indicated by flat cappings and drone pupae are indicated by raised cappings) on both the treatment and choice frames for each colony. We noticed that there were some worker pupae on the drone comb treatment frames and on the drone comb half of the choice frames, indicating that the queen had occasionally laid worker eggs in drone cells (as reported in Page et al. 1993). This occurrence was extremely rare on the choice frames: of the colonies that had pupae on the drone portion of the choice frame, the proportion of these pupae that were worker pupae was less than 0.029 in all but 3 colonies. In 2 of the 3 remaining colonies, the proportions of pupae that were workers were 0.051 and 0.105, and the remaining colony had only one pupa (a worker pupa) on the drone comb portion of the choice frame. Because the occurrence of worker pupae in drone cells was so rare on the choice frames, we are confident that our estimate of the number of drone eggs on the choice frames closely approximates the actual number of drone eggs present. In 2004, we photographed the treatment frames on day 17 and the choice frames on day 20. In 2005, we photographed both the treatment and choice frames on day 15.

Drone departure rates

Although we did not provide our colonies with any drone comb prior to the start of the experiment, colonies often build small patches of drone comb in gaps and spaces within the hive. Our colonies had done so and consequently had reared a small number of adult drones before the start of the experiment. Because the presence of adult drones might influence a colony's future production of drones (Rinderer et al. 1985) and therefore might influence the egg-laying decisions of the queen, we measured whether the DC and WC colonies had similar numbers of adult drones near the beginning of the study. To estimate the prevalence of adult drones, we measured the rate of drone departures from each colony. An observer sat near the entrance of a hive, recorded the number of drones leaving during a short period of time (either 1 or 5 min), and visited each hive in a fixed order until each was visited for 10 min in total. We then converted these counts to the rate of drone departure per minute. We made these observations on sunny afternoons, when adult drones in the hive embark on either practice or real mating flights. In 2004, we performed these counts on days 1 and 7 of the study. In 2005, we performed these counts on days 3 and 7 of the study. Thus, for each colony, we obtained 2 rates of drone departures, which were averaged to obtain the colony's drone departure rate.

Statistical analysis

Every queen laid eggs on both the treatment and choice frames, so we included all the colonies ($n = 24$) in our analysis. Because we collected data in both 2004 and 2005, we first checked for differences in our data between years. For each treatment group, we found no differences in our dependent variables across years, so we pooled our data from 2004 and 2005 for the statistical analyses.

To test whether DC and WC queens differed in egg-laying decisions on the choice frame, we used a multivariate analysis of variance (MANOVA) to test for the effect of treatment on queen decisions on the choice frame (number of worker eggs, number of drone eggs). A queen may alter her production of drone eggs through 2 methods: by skewing the sex ratio of her eggs while holding total eggs constant or by increasing her total production of eggs while holding her egg sex ratio constant. Whereas a test for treatment differences in egg sex ratio can only capture the former method, a MANOVA, which tests for differences in centroid locations among treatment groups, can capture either method. We used post hoc analyses to provide further insights into the decisions made by the queens.

To assess whether the egg patterns we observed on the choice frame may have arisen because of worker decisions to fill areas of comb with nectar, we used Student's t-test to assess the difference between DC and WC colonies with respect to the areas of both worker comb and drone comb that were used for nectar storage.

To test whether DC and WC colonies differed in pupae-rearing patterns on the choice frame, we used a MANOVA to test for the effect of treatment on colony decisions on the choice frame (number of worker pupae, number of drone pupae). We used post hoc tests to provide further insights into the colony decisions.

To assess whether worker honeybees might have altered patterns of queen investment differentially across treatments, we used Student's t-test to determine the difference between DC and WC colonies with respect to both drone mortality and worker mortality on the choice frame. We defined drone mortality and worker mortality as the proportion of drone and worker eggs, respectively, that did not reach the pupal stage. We obtained each hive's measure of drone mortality by taking the difference between the numbers of drone eggs and drone pupae and dividing that by the number of drone eggs; worker mortality was obtained in a similar way with worker eggs and pupae. For this analysis, we used data from 2005 only because in that year we kept the choice frame in the queen excluder cage for long enough to ensure that any pupae we counted on our digital pictures were from eggs that the queen laid while she was confined to that frame.

Finally, we used Student's t-test to determine the statistical significance of the difference between the DC and WC colonies with respect to both drone departure rate and the total investment in pupal males by the colonies (the sum total of drone pupae on the treatment and choice frames).

All analyses were performed using MATLAB 7.0. Significance was set at the 0.05 level, and results are reported as mean ± 1 standard deviation.

RESULTS

Does previous comb type influence the queen's egg-laying patterns?

Once moved to the choice frames, WC queens and DC queens differed in their egg-laying decisions (MANOVA, Wilks' $\Lambda_{1,22} = 0.717$, $P = 0.030$) (Figure 2). Specifically, WC queens laid a greater number of drone eggs than did DC queens (number of drone eggs, WC queens: 925.0 ± 665.4, DC queens: 337.9 ± 390.3; $F_{1,22} = 6.950$, $P = 0.015$). However, WC and DC queens did not differ in the number of worker eggs they produced (number of worker eggs, WC queens: 558.7 ± 534.0, DC queens: 762.3 ± 466.1; $F_{1,22} = 0.995$, $P = 0.329$).

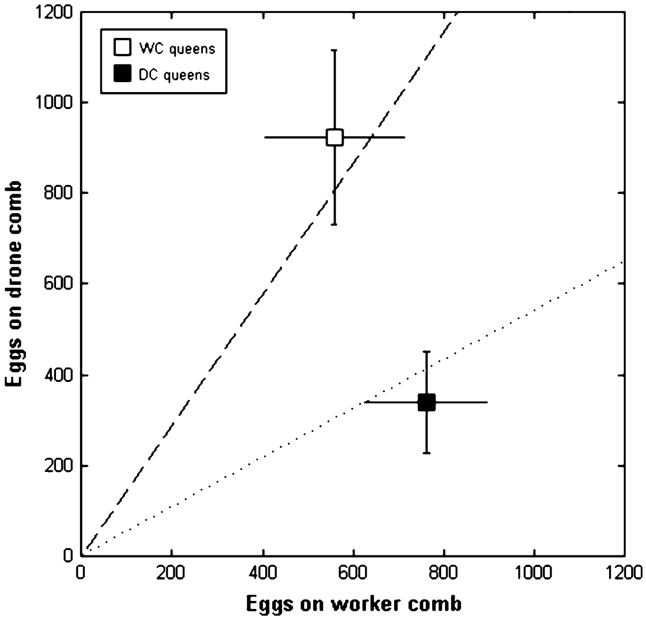

Figure 2
Mean (±standard error [SE]) number of drone eggs plotted against mean (±SE) number of worker eggs on the choice frame for queens previously on worker comb (WC) and queens previously on drone comb (DC). WC queens laid more drone eggs but the same amount of worker eggs as DC queens (see text). The lines represent the egg sex ratio vector on the choice frame; the slope is the average ratio of drone eggs to worker eggs (dashed line for WC queens, dotted line for DC queens). WC queens and DC queens did not produce significantly different egg sex ratios.

To see if the difference in queen behavior was due to queens skewing their egg sex ratio, we compared the egg sex ratio (male eggs divided by total eggs) across treatment groups. WC queens tended to lay a more drone-biased sex ratio (0.59 ± 0.40) than did DC queens (0.35 ± 0.36), although this trend was not significant ($F_{1,22} = 2.340$, $P = 0.140$). To see whether the difference in queen behavior was due to queens altering their total production of eggs, we compared the number of eggs produced across treatment groups. WC queens laid a significantly larger number of total eggs than did DC queens (total eggs, WC queens: 1483.8 ± 337.4, DC queens: 1100.2 ± 439.7; $F_{1,22} = 5.747$, $P = 0.025$). This egg production, which occurred over a period of 24 h, is consistent with reported values of queen egg production, which usually range between 1000 and 2000 eggs per 24 h (Nolan 1925; Seeley 1985).

Do the egg patterns on the choice frame result from the actions of the workers?

The results we reported in the above section could arise from the workers rather than the queen if the egg-laying patterns were simply a by-product of one of the following (refer to Figure 1): worker decisions to selectively clean drone or worker cells, worker decisions to selectively fill drone or worker cells with nectar, or worker decisions to selectively destroy the queen's drone eggs or worker eggs. We address each of these possibilities in this section.

First, the queen's egg-laying pattern might arise from worker decisions to selectively clean drone or worker cells. Workers clean cells by removing debris such as old cocoons, and this cleaning process prepares a cell to receive an egg from the queen (Winston 1987). One way to control for this possibility would be to confine the queen to comb in the absence of workers, but workers tend the queen in many ways, including feeding her. When workers are absent, queens lay very few eggs at all (Wharton KE, unpublished data), but this is a highly unnatural situation and need not have anything to do with the effects of the workers on the cells. In our study, the frames we used had been cleaned by other colonies and stored indoors before they were used for our experiment, so were most likely already prepared to receive eggs from the queens. Even if workers do play a role in preparing cells for egg laying, previous work has demonstrated that workers do not differentially prepare worker or drone cells (Sasaki et al. 1996). In summary, no evidence suggested that workers in our study selectively prepared drone or worker cells for the queen.

Second, the queen's egg-laying pattern might arise from worker decisions to selectively fill drone or worker cells with nectar. For example, if the workers in WC colonies filled fewer drone cells with nectar than did the workers in the DC colonies, the significant treatment difference in drone egg production might arise because WC queens had more cells in which to lay drone eggs than did DC queens. However, the area of drone comb filled with nectar did not differ between the 2 treatment groups (area of drone comb filled with nectar, WC colonies: 17 ± 20 cm^2, DC colonies: 25 ± 34 cm^2; $t = 0.659$, degrees of freedom [df] = 22, $P = 0.516$) (Figure 3). Similarly, the area of worker comb filled with nectar did not differ between the 2 treatment groups (area of worker comb filled with nectar, WC colonies: 325 ± 187 cm^2, DC colonies: 219 ± 126 cm^2; $t = 1.625$, df = 22, $P = 0.118$) (Figure 3). Furthermore, because each choice frame had 825.8 cm^2 of worker comb and 825.8 cm^2 of drone comb, queens in both treatment groups still had plenty of available cells in which they could lay eggs. This trend was particularly notable in the drone comb section of the frame, where on average less than 3% of the total area of drone comb was used for nectar storage, leaving almost all the drone cells available to the queen. Thus, queens were able to make egg-laying decisions independently of worker decisions to fill cells with nectar.

Third, the egg-laying patterns we observed might have resulted from worker decisions to selectively destroy some of the drone eggs. For example, our observation that there were more drone eggs in WC colonies than in DC colonies might be explained by a tendency of the workers in DC colonies to selectively destroy drone eggs. We sampled the eggs when they were 0- to 24-h old, so we cannot exclude the possibility that workers removed drone eggs before we took our estimates. However, in order for the workers to selectively destroy drone eggs, they would need to perceive the sex of each egg. They might distinguish the sex of eggs based on chemical cues, but there is no difference in removal rates for drone and worker eggs when both are transferred into drone cells (Oldroyd and Ratnieks 2000). Furthermore, there is evidence that workers cannot use brood pheromones to distinguish between drone and worker larvae until 7 days after the queen has laid eggs (Sasaki et al. 2004). Alternatively, workers might distinguish the sex of eggs based on cell size alone, but Sasaki and Obara (2001) observed food-starved observation hives and did not witness any cannibalization of eggs in drone cells. Additionally, Woyke (1977) demonstrated that there was no difference in removal rates for eggs in drone and worker cells during the spring or summer (when our study was conducted), and although he found selective removal of immature drones in the spring, a sex-specific difference in removal rate did not occur until the larval stage. For all these reasons, we find it unlikely that workers in our study were selectively removing drone eggs. In general, whether social insect workers can distinguish the sex of eggs remains an open question in social insect biology (Passera and Aron 1996) and is an attractive area for future research. Other social insect studies have measured egg sex ratios from eggs that were 0- to 96-h old and had been exposed to workers (Aron et al. 1994, 1995; Sasaki et al. 1996; Sasaki and Obara 2001), so the methods in our study are consistent with other studies that examine queen egg-laying decisions.

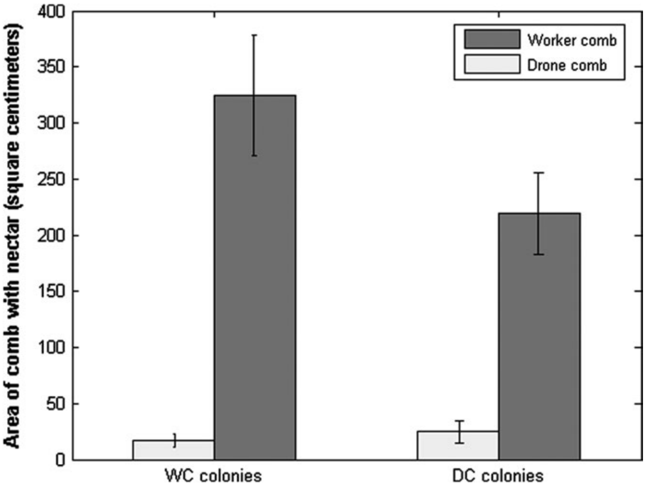

Figure 3
Mean (±standard error) area of drone comb (light bars) and worker comb (dark bars) filled with nectar in the choice frames for WC colonies and DC colonies. The choice frames had 825.8 cm^2 of drone comb and 825.8 cm^2 of worker comb. WC colonies and DC colonies did not differ in the area of DC filled with nectar or in the area of worker comb filled with nectar (see text).

Because it is unlikely that workers in different treatment groups differentially prepared cells, filled cells with nectar, or destroyed eggs, the egg-laying patterns we observed on the choice frames were unlikely due to the actions of the workers but instead were due to the actions of the queen.

Does previous comb type influence pupae-rearing patterns of the colony?

On the choice frames, WC colonies and DC colonies differed in their pupae-rearing patterns (MANOVA, Wilks' $\Lambda_{1,22} = 0.639$, $P = 0.009$) (Figure 4). Specifically, WC colonies raised a greater number of drones than did DC colonies (number of drone pupae; WC: 549.6 ± 351.8, DC: 134.0 ± 210.8; $F_{1,22} = 12.322$, $P = 0.002$) and also produced a more male-biased sex ratio (pupal sex ratio, WC: 0.58 ± 0.36, DC: 0.24 ± 0.26; $F_{1,22} = 5.701$, $P = 0.028$). WC and DC colonies did not differ in the number of worker pupae they produced (number of worker pupae, WC: 458.0 ± 453.6, DC: 475.6 ± 544.7; $F_{1,22} = 0.007$, $P = 0.932$) or in the total number of pupae reared on the choice frame (number of total pupae, WC: 1007.6 ± 564.3, DC: 609.6 ± 628.0; $F_{1,22} = 2.667$, $P = 0.117$).

Thus, just as WC queens had produced more drone eggs on the choice frames than did DC queens, WC colonies produced more drone pupae on the choice frames than did DC colonies. This result indicates that the negative feedback pattern of drone egg production persisted at the colony level.

Does previous comb type influence drone mortality or worker mortality?

These results are for data taken in 2005. We did observe both drone and worker mortality on the choice frame, meaning

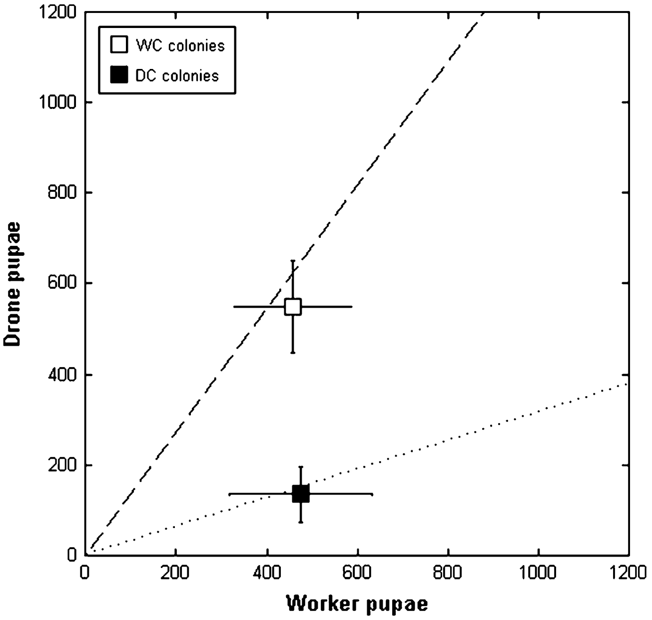

Figure 4
Mean (±standard error [SE]) number of drone pupae plotted against mean (±SE) number of worker pupae on the choice frame for colonies with queens previously on worker comb (WC) and colonies with queens previously on drone comb (DC). WC colonies raised more drone pupae but the same amount of worker pupae as DC colonies (see text). The lines represent the pupal sex ratio vector on the choice frame; the slope is the average ratio of drone eggs to worker eggs (dashed line for WC colonies, dotted line for DC colonies). WC colonies produced a more male-biased sex ratio than did DC queens.

that our estimates of drone and worker eggs were higher than the numbers of drone and worker pupae we counted. Specifically, the WC colonies failed to rear 54.2 ± 13.9% of the drone eggs and 53.2 ± 12.0% of the worker eggs on the choice frames, and the DC colonies failed to rear 57.0 ± 28.7% of the drone eggs and 60.0 ± 24.1% of the worker eggs on the choice frames. Some of this estimated mortality is most likely a by-product of our overestimation of the number of eggs (see Methods), which is expected to be equal for the 2 treatment groups. The remainder of the estimated mortality might have arisen from the failure of the worker honeybees to rear some of the drone and worker larvae. If workers were failing to rear brood in a treatment-dependent manner, we should see a difference in mortality between the treatment groups. However, treatment had no effect on the amount of drone mortality ($t = 0.190$, df = 7, $P = 0.855$) or the amount of worker mortality ($t = 0.619$, df = 10, $P = 0.550$). This suggests that any decisions that the workers made about drone or worker removal were independent of the queen's recent egg-laying decisions. Therefore, the net differences between treatments in the pupae-rearing patterns of the colonies can be at least partially attributed to actions of the queens.

Did the colonies differ in their investment in drones throughout the study?

We measured a drone departure rate at the beginning of the experiment of 0.16 ± 0.37 drones per min in the WC colonies and 0.27 ± 0.25 drones per min in the DC colonies. These rates are not significantly different ($t = 0.816$, df = 22, $P = 0.423$), indicating that the 2 treatment groups had roughly the same number of adult drones present at the beginning of the experiment. Therefore, any treatment differences in queen egg-laying decisions or colony brood-rearing patterns did not result from a difference in abundance of adult drones in the colonies.

By the end of the experiment (day 20 in 2004 and day 15 in 2005; see Methods), we could determine the total number of drone pupae reared throughout the study by summing the drone pupae on both the treatment and the choice frames. WC and DC colonies did not differ in this total (total number of drone pupae produced during the experiment, WC colonies: 564.8 ± 343.4, DC colonies: 593.3 ± 548.5; $t = 0.153$, df = 18.5, $P = 0.880$). Therefore, even though WC colonies were given only one half of a frame of drone comb whereas DC colonies were given 3 times that amount, the treatment groups produced a similar final number of pupal drones. This suggests that the colonies regulated their production of drones.

DISCUSSION

It is often assumed that worker honeybees regulate their colony's investment in drones because they build the wax cells for rearing drones and also tend to the larvae. The results of this study suggest that honeybee queens also contribute to the regulation of their colony's drone production through modulation of their egg-laying decisions. Queens that were prevented from laying drone eggs for a brief period of time (WC queens) later produced more drone eggs than queens who had not been prevented from laying drone eggs (DC queens) (Figure 2). As the eggs on the choice frame matured into pupae, there is no evidence that the workers selectively killed the larvae in a treatment-dependent manner (Figure 4), so the heightened production of drone eggs by WC queens (when given a choice of egg type to lay) allowed the WC colonies to "catch up" on the number of drones that were being reared. As a result, both treatment groups reared approximately the same total number of drone pupae throughout the experiment.

Our finding that the colonies invested equally in pupal males over the duration of the experiment is consistent with studies indicating that colonies across a population produce similar amounts of drone brood (Page and Metcalf 1984; Henderson 1991). Our colonies were approximately equal in size and therefore are expected to invest equally in drones. It is remarkable that our colonies were able to accomplish this even though they differed greatly in the amount of drone comb available (DC colonies were provided with 3 times the amount of drone comb as WC colonies). Therefore, our study provides further evidence that honeybee colonies are adept at regulating drone production.

As for how this regulation of drone production occurs, our study suggests a greater role for the queen than is often assumed. Previous studies have shown that the presence of drone brood suppresses the further production of drone brood in honeybee colonies (Free and Williams 1975); brood production might also be suppressed by a large quantity of adult drones in the colony (Rinderer et. al. 1985; but see Henderson 1991). Together, these studies suggest that colonies regulate drone production via a negative feedback mechanism. However, these previous studies assessed colony drone production by counting numbers of drone larvae or pupae, which may reflect a combination of queen and worker decisions. Therefore, these studies do not directly address the role that the queen plays in regulating colony drone production. Our study fills this gap by focusing on whether the queen regulates her drone egg production. We found that the queen compensates for her own production of drone eggs based on the amount of drone eggs she recently laid. Furthermore, the workers in our study did not block (via using cells for nectar storage) or alter (via failing to rear larvae) the queen's decisions in any sort of treatment-dependent manner. Taken together, these results suggest that the regulation of drone brood production at the colony level may emerge at least in part by a negative feedback process of drone egg production by the queen.

Queens might increase their drone egg production by laying a greater total number of eggs, by creating a more drone-biased egg sex ratio, or some combination of these 2. In our study, queens did differ in the total number of eggs they laid, which was due to a difference in the number of drone but not worker eggs that were laid. The difference in egg sex ratio was not statistically significant, but we did find a trend in the predicted direction, in that queens who were prevented from laying drone eggs subsequently produced a more drone–biased egg sex ratio. Because we had a relatively low sample size and queen behavior was quite variable, we suspect that this sex ratio trend may reflect a biologically real behavior. In short, honeybee queens can modulate their investment in drones by altering the total number of drone eggs they lay and possibly also by changing their egg sex ratio.

Whether social insect queens can adaptively regulate the egg sex ratio is an important line of future research. This ability has been found in queens of species that have a high potential for queen–worker conflict over sex allocation (Aron et al. 1995; Keller et al. 1996; de Menten, Cremer, et al. 2005; de Menten, Fournier, et al. 2005). In species that experience this queen–worker conflict, the queen may manipulate her egg sex ratio in order to exercise power, where power is defined as the ability to control reproduction when conflict exists (Beekman and Ratnieks 2003; Beekman et al. 2003). Alternatively, she might manipulate her egg sex ratio in order to influence the colony's allocation to reproduction in agreement with the workers. Future studies that examine whether queens can influence colony male production should attempt to distinguish between these 2 functional hypotheses.

Our study opens up some fascinating questions that deserve further work. For example, what proximate cues does the queen use to alter her egg-laying decisions in relation to previous egg-laying decisions? As one possibility, a queen might use some type of memory to keep track of previous ovipositions and could stop producing more drone eggs after she remembers laying many drone eggs. Alternatively, queens might use brood pheromone cues to indicate the amount of drones in her colony and could stop producing more drone eggs when her chemical sensory system detects a large amount of drone brood. In our study, we did not remove any eggs before allowing queens to make egg-laying choices, so our experimental design does not allow us to distinguish between the hypotheses that queens are using memory or brood pheromones. Identifying the proximate mechanisms by which social insect queens keep track of the colony's abundance of males is an attractive study for future research because it has implications for the ability of queens to react to male removal by the workers. In species that experience conflict, for example, if workers selectively remove males, we predict that the queen might not rely on oviposition memory because it will mislead her estimate of the number of developing male larvae.

This work sets the stage for examining how the actions and interactions of the queen and workers influence a colony's investment in reproduction. Investing in males can be likened to a supply chain management issue for the colony, where a major goal should be to produce an adaptive amount of a product (males) through the most efficient use of resources. Brood production should be an efficient process if both the workers and the queen are able to actively and separately respond to environmental conditions that convey information about the value of rearing males. In honeybees, some of these conditions include season, colony size, availability of food, whether or not a queen is present, and the number of drones present (Free and Williams 1975; Page and Metcalf 1984; Seeley and Mikheyev 2003). How do colony members perceive information about these conditions? Which conditions are assessed by the queen, and which are assessed by the workers? Additionally, environmental conditions that favor drone production might change after the queen has laid drone eggs. Will workers modify the previous egg-laying decisions of their queen if environmental conditions suddenly become unfavorable for rearing drones? These questions, which resemble questions from supply chain management, must await further work.

In conclusion, our study identifies a role for the honeybee queen in the regulation of colony drone production. This demonstrates that a social insect queen can influence colony male production even when conflict with the workers is absent. In order to better understand the functional significance of queen and worker decisions, future work should attempt to distinguish between actions that arise from conflict over sex allocation and those that arise from cooperation and shared control over the colony's investment in reproduction.

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