Male elimination in the honeybee

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In a striking example of sex allocation modification, female social insect (hymenopteran) workers sometimes cannibalize a fraction of their colony’s immature males. The commonly cited explanation for this male elimination is that workers are in genetic conflict with the queen and are biasing the colony’s sex allocation in their favor. However, this behavior might allow workers to tailor their colony’s investment in reproduction to environmental conditions and therefore might play an important role even in the absence of queen–worker conflict. So far, male elimination has been demonstrated only in species where the potential for queen–worker conflict is high. Here we present experimental evidence for facultative male elimination in the honeybee, a species where queen–worker conflict is expected to be minimal or absent because of multiple mating by the queen. We manipulated the abundance of older male brood in colonies and found that survival of younger male larvae was lower when we increased the abundance than when we decreased it. Survival of worker larvae was high across colony conditions. These results suggest that genetic conflict is not a necessary precondition for male elimination in social insect societies. Instead, male elimination might sometimes reflect adaptive adjustment of male reproductive function, potentially increasing colony efficiency in the interests of all colony members. Key words: cooperation, kin selection, male elimination, male reproductive function, queen–worker conflict, sex allocation. [Behav Ecol 19:1075–1079 (2008)]

INTRODUCTION

Cooperation and conflict between subunits of a group are pervasive forces at multiple levels of biological organization, from genomes to organisms to animal societies. Understanding how cooperation arises and is maintained in spite of the potential for intragroup conflicts is a central quest in evolutionary biology (Queller and Strassmann 1998; Reeve and Hölldobler 2007). In animal societies, individuals often have competing interests that can lead to potential conflicts over the division of resources or reproduction among society members (Ratnieks et al. 2006). However, it can be tricky to determine the extent to which group member interactions are shaped by conflict because an observed behavior might be influenced by multiple environmental, genetic, and evolutionary factors (Chapuisat and Keller 1999). In this paper, we examine a behavior that is often assumed to spring from genetic conflict between the queen and the female workers in a social insect colony; the elimination of immature males by the workers. Most studies of male elimination have focused on species in which queen–worker conflict is expected to be high. To determine whether genetic conflict is a necessary precondition for male elimination, we studied honeybees (Apis mellifera), a species where such conflict is expected to be minimal, and in which any evidence of male elimination is likely to reflect cooperative resource allocation to male reproductive function.

In the social Hymenoptera (ants, bees, and wasps), the queen and workers are potentially in conflict over their colony’s sex allocation, or investment in males versus females. Although there are several types of queen–worker conflict (Ratnieks et al. 2006), we consider only conflict over sex allocation in this paper. The potential for this conflict arises from haplodiploid sex determination (females are diploid; males are haploid), which causes workers to be, on average, more closely related to their sisters than to brothers, whereas the queen is equally related to her daughters and sons (Hamilton 1964). These patterns of relatedness lead to the prediction that workers should generally prefer a less male-biased sex allocation than the queen (Trivers and Hare 1976).

Workers might bias sex allocation in their favor by eliminating a portion of the colony’s immature males. Male elimination has been inferred to occur in several ant species, based on observations that the secondary sex ratio was less male biased than the egg sex ratio (Aron et al. 1994, 1995; Keller et al. 1996; Passera and Aron 1996; Sundström et al. 1996; Chapuisat et al. 1997; Helms et al. 2000; Rosset and Chapuisat 2006). Because male elimination allows workers to bias sex allocation in their favor, these examples are, perhaps not surprisingly, consistently cited as evidence of a manifest queen–worker conflict (Meišlė et al. 2003; Ratnieks et al. 2006).

Although male elimination is consistent with the predictions of queen–worker genetic conflict, it might also serve a cooperative purpose. Workers have an opportunity to modify their colony’s investment in reproduction when they rear brood; these brood-rearing decisions might allow the colony to adaptively tailor reproductive effort to resource availability or other environmental conditions (Wharton et al. 2007). For example, workers might eliminate some immature males when colony resources are limited or if the colony is already well supplied with males. By eliminating males before they extract full developmental costs and begin extracting high adult maintenance costs, workers could enhance overall colony efficiency and success (Chapuisat et al. 1997).

So far, all reported cases of male elimination have been in species where the queen is singly mated and so the potential for conflict is high. Is genetic queen–worker conflict therefore a necessary precondition for male elimination? To determine this, it is important to investigate whether there is selective male elimination in species in which the queen mates multiple times. Multiple mating by the queen reduces the average relatedness among workers causing workers to become nearly equally related to their sisters and brothers, and consequently, lowering the potential for queen–worker conflict (Moritz 1985; Pamilo 1991a, 1991b). If male elimination was solely a consequence of queen–worker conflict, then we should not see it
in such species. Only one study examined a case in which conflict was expected to be low (in wood ant colonies headed by a multiply mated queen); it found no evidence for selective male elimination (Sundström et al. 1996). Clearly, however, there is a need for data from other species.

Honeybees are a good candidate species for testing whether male elimination might spring from cooperation. Honeybee queens are highly polyandrous (Tarpy and Nielsen 2002), so queen-worker conflict is expected to be very low or absent (Moritz 1985; Ratnieks et al. 2006). Additionally, colonies do not rear as many drones (males) as possible but rather regulate their investment in drones (Free and Williams 1975), presumably because drones are costly to produce (Seeley 2002; Seeley and Mikheyev 2003; Hrassnigg and Crailsheim 2005). Conveniently, good estimates of brood survival are possible in honeybees because researchers can reliably and nondestructively determine the sex of eggs: drone eggs are found in large "drone" cells, whereas worker eggs are found in small "worker" cells (Ratnieks and Keller 1998). This difference in cell size also lends itself to experimental manipulations; researchers can limit or manipulate a colony’s production of drones by controlling the abundance of drone cells in the colony.

In this study, we compared the survival of young immature drone and worker honeybees under 2 different experimental conditions: 1 in which colonies had an excess of older drone brood (D+ colonies) and 1 in which colonies had virtually no additional drone brood (D− colonies). See Figure 1 for the experimental design. This design was chosen because the presence of drone brood (immature drones) inhibits a colony’s future production of drones (Free and Williams 1975). The treatments were induced after the queen had laid both drone and worker eggs in order to isolate the influence of worker actions on brood survival. We predicted that if workers adaptively influence the colony’s investment in drones by eliminating immature drones, the survival of drone but not worker eggs would be lower in colonies that already were rearing large numbers of drones.

**METHODS**

**Honeybee colonies**

We conducted this study on the Michigan State University campus in East Lansing, Michigan, in May and June 2007. Twenty colonies of Italian honeybees, *Apis mellifera ligustica*, were used. Each colony contained the mother queen and roughly 15,000 workers and was housed in a standard Langstroth hive consisting of one deep hive body with 10 frames of worker comb. By initially providing the colonies only with worker comb, we ensured that drone production occurred mainly during the experiment.

**General methods**

Our general strategy was as follows. First, in each of the study colonies, we allowed the queen to lay drone and worker eggs in the presence of older drone brood (immature drones). Then, we divided the colonies into 2 groups, one from which drone brood was removed and the other to which drone brood was added. Finally, we compared the survival of the new worker and drone brood during the period in which the colony’s workers rear the larvae. The experimental setup is presented in Figure 1.

To set up the experiment, we placed one frame of drone comb and one frame of worker comb (the "treatment" frames) into each colony. These frames were used to manipulate the abundance of drone brood for the experimental treatments. After 10 days, these frames were largely filled with brood consisting of older larvae and some pupae.

To obtain eggs so that we could monitor the brood-rearing decisions of the workers, we inserted into each colony a "focal frame" consisting of drone and worker combs in blocks of equal area. On day 0 of the experiment, we caged the queen on this comb and allowed her to lay eggs for 24 h. The cage was made of queen excluder material, which consists of small openings that prevent the queen but not the workers from passing through. At the end of this 24-h egg-laying period, we placed the cage elsewhere in the colony and took a series of photographs that facilitated counting of numbers of worker and drone eggs laid by the queen. We then returned each of the focal frames to the cage in its colony, allowing worker access but this time with the queen excluded so that no further eggs could be laid on this comb.

To test whether the workers would adaptively modify their colony’s investment in drones, we randomly assigned the colonies to 1 of 2 treatments, drone brood added (D+) or drone brood removed (D−). Each D+ colony received a drone treatment frame from a D− colony and gave a worker treatment frame to that D− colony in return. Thus, after this treatment was applied, the D+ colonies had 2 frames of older drone brood, whereas the D− colonies had none. We applied this treatment over the course of days 2 and 3.

To quantify larvae for estimating brood survival, we took photographs of the focal frame when the brood had reached the late larval stage and were just prior to capping (day 8; 8–9 days after oviposition). In order to quantify the pupae on the treatment frames, we took photographs of the treatment...
frames twice: once on day 3 (shortly after the treatment had been applied) and once at the conclusion of the experiment, on day 8 (for the worker treatment frames) and day 9 (for the drone treatment frames).

Before the start of the experiment, our colonies had built small patches of drone comb in gaps within the hive. Consequently, our colonies contained a small background number of adult drones during the experiment. Because adult drones might influence drone production (Rinderer et al. 1985), we recorded the number of drones embarking on mating flights to determine whether the D− recorded the number of drones embarking on mating flights might influence drone production (Rinderer et al. 1985), we recorded the number of drones embarking on mating flights to determine whether the D+ colonies and the D− colonies had similar numbers of adult drones (see Seeley 2002). On day 8, an observer sat near the entrance of a hive, recorded the number of drones that left the colony during a 5-min interval, and visited each hive in a fixed order until each was visited for 10 min total. We used these counts to calculate a rate of drone departures per minute.

Survival of immature drones and workers

To calculate survival of brood on the focal frame, we used photographs to quantify (blindly) both the eggs initially present and the larvae that remained after our experimental manipulation. We used photographs because direct counts would have taken too long and increased the likelihood that the eggs would die from exposure to the summer heat. The larvae we counted were at a late (5–6 days) larval stage; nearly all larvae that old survive to eclosion (Woyke 1977; Schmickl and Crailsheim 2001, 2002).

Counting eggs from photographs is prone to error because of the small size of the eggs, their lack of color contrast with the wax cells, their position at the bottom of a cell, and various irregularities of the cells themselves. We expected to undercount the actual number of eggs on the focal frame. However, because the tendency to undercount depends mostly on wax comb properties, it should not be systematically biased across treatment groups.

Drone or worker survival on the focal frame was estimated from the proportion of eggs in each type of cell that reached the late larval stage. These counts did reveal that we may have undercounted eggs at the beginning. We counted more worker larvae than worker eggs in 3 of our colonies (467 larvae, 443 eggs; 593 larvae, 588 eggs; and 225 larvae, 74 eggs), and we counted more drone larvae than drone eggs in 2 of our colonies (728 larvae, 634 eggs and 584 larvae, 573 eggs). For these cases, we assigned a worker or drone survival value of 1.

Statistical analysis

There were no drone eggs on the focal frame in 2 of our 20 colonies, so we excluded those colonies from the analyses. Within each treatment group, all dependent variables were checked for normality using a Lilliefors test. For any dependent variable that did not meet the criterion for normality, the appropriate nonparametric test was used for hypothesis testing. All analyses were performed using MATLAB 7.0, and results are reported as mean ± 1 standard deviation.

RESULTS

Comparisons of the number of eggs on the focal frame and adult drones in the colony

Because the experimental treatment was randomly assigned after the queen had laid eggs on the focal frame, we did not expect any differences between the treatment groups in the number of eggs laid. Consistent with this, D− colonies and D+ colonies did not differ in the number of drone eggs (D− colonies: 456.2 ± 318.8, D+ colonies: 383.7 ± 277.5; t = 0.868, degrees of freedom [df] = 16, P = 0.398) or in the number of worker eggs (D− colonies: 797.8 ± 473.9, D+ colonies: 619.1 ± 275.8; t = 0.978, df = 16, P = 0.345) on the focal frame. Furthermore, there was no difference in the egg sex ratio (proportion of drone eggs, D− colonies: 0.38 ± 0.24, D+ colonies: 0.30 ± 0.21, Mann–Whitney U test, P = 0.489).

We also found that the abundance of adult drones (produced prior to the beginning of the experiment) did not differ between treatment groups (drone departures per minute, D− colonies: 0.51 ± 0.60, D+ colonies: 0.60 ± 0.83, Mann–Whitney U test, P = 0.774).

Because colonies did not differ significantly in the production of drone or worker eggs on the focal frame, or in the abundance of adult drones, any treatment differences in colony brood-rearing patterns cannot be attributed to those factors.

Comparisons of brood survival

D− colonies and D+ colonies differed with respect to brood survival (consisting of 2 variables: drone survival, worker survival) on the focal frame (multivariate analysis of variance, Wilks’ Λ1,16 = 0.566, P = 0.014) (Figure 2). Specifically, a greater proportion of drone eggs were reared to the late larval stage in D− colonies than in D+ colonies (drone survival, D− colonies: 0.83 ± 0.18, D+ colonies: 0.43 ± 0.54; F1,16 = 9.29, P = 0.008). D− colonies and D+ colonies did not differ in the proportion of worker eggs that were reared to the late larval stage (worker survival, D− colonies: 0.78 ± 0.16, D+ colonies: 0.72 ± 0.25; F1,16 = 0.22, P = 0.642). This indicates that our
treatment affected the survival of immature drones but not workers.

To further investigate how our colonies reared worker brood versus drone brood on the choice frame, we examined the relationship between drone survival and worker survival within each treatment group. There was no correlation between drone survival and worker survival in the D− colonies (r = 0.168, P = 0.666). However, in the D+ colonies, drone survival and worker survival tended to be negatively correlated, although this tendency was not statistically significant (r = −0.565, P = 0.120).

Our D+ colonies varied in the number of drone pupae on the treatment frames. We tested whether this variation across the D+ colonies (9 colonies) explained any variation in drone survival on the focal frames in those colonies. We found no correlation between drone survival and the number of drone pupae on treatment frames at the end of the experiment (D+ colonies: r = 0.496, P = 0.175).

To test whether the treatment caused a shift in sex ratio from the egg stage to the larval stage, we calculated the change in sex ratio for each focal frame by subtracting the larval sex ratio from the egg sex ratio. This shift in sex ratio was not significantly affected by the treatment (change in sex ratio; a positive change indicates a less male-biased larval sex ratio, D− colonies: −0.05 ± 0.05, D+ colonies: 0.05 ± 0.15, Mann–Whitney U test, P = 0.063).

**DISCUSSION**

This study provides evidence for conditional male elimination by honeybee workers and is one of only a few studies to date that uses an experimental approach to investigate the expression of this behavior. In colonies with an excess of older drone brood, the survival of newly produced drone brood was lower than in colonies with an absence of older drone brood. There was no effect of treatment on the survival of worker brood (Figure 2). These patterns of brood survival indicate that honeybee colonies, like individual organisms, regulate investment in male reproductive function.

As for how this regulation occurs, this study points to an important role for the workers. Previous work has shown that the presence of drone brood suppresses the further production of drone brood in honeybee colonies (Free and Williams 1975), suggesting that colonies regulate drone production via a negative feedback mechanism. Colonies manage to do this through some combination of queen and worker decisions. In this study, we isolated and examined worker influences on brood patterns by removing any direct influence of the queen: treatment groups were assigned after the queen laid eggs, and the queen was prevented from laying additional eggs on the focal frame following treatment assignments. Furthermore, the treatment difference in drone but not worker survival indicates that brood-rearing decisions by the workers rather than an overall difference in brood viability was responsible for the patterns. In short, the treatment differences in drone survival demonstrate that honeybee workers can modify their colony’s investment in immature drones after the queen lays eggs.

From the perspective of the colony, brood production should be an efficient process if both the workers and the queen are able to respond actively and separately to environmental conditions that convey information about the value of rearing males. The results of this study, together with a previous study by Wharton et al. (2007), suggest that both the honeybee queen and the workers can influence their colony’s investment in males. There is evidence that this shared or joint control over brood production is found in other species, including the harvester ant *Messor pergandei* (Ode and Rissing 2002) and the fire ant *Solenopsis invicta* (Aron et al. 1995; Passera et al. 2001). Shared control might also ensure that the ways in which resources are distributed to brood reflect an evolutionary tug-of-war between the queen and the workers for control over colony sex allocation (Trivers and Hare 1976; Reuter and Keller 2001). This shared control might arise because neither the queen nor the workers are able to gain complete control over sex allocation in species in which queen–worker conflict is expected. For example, in the fire ant, it appears that the queen is constrained by the ability of the workers to eliminate males and that the workers are in turn constrained by the primary sex ratio produced by the queen (Aron et al. 1995; Passera et al. 2001). In other cases, including species without queen–worker conflict, shared control might allow colonies to respond flexibly and efficiently to changing environmental conditions such as fluctuations in food availability (Ode and Rissing 2002; Wharton et al. 2007). This cooperative view might help to explain various phenomena such as why the *Linepithema humile* queen continues to produce male eggs outside the period of sexual production even though workers eliminate the vast majority of these eggs (Aron et al. 1994; Passera and Aron 1996).

Sibling cannibalism occurs in other animals, including amphibians and predatory birds, during periods of low food availability. Although this sibling cannibalism is traditionally considered a form of parent–offspring conflict, researchers suspect that it might serve an adaptive purpose for both parents and offspring in that it promotes a better distribution of limited resources among offspring (Mock and Forbes 1995). Similarly, parent–offspring conflict between a social insect queen and workers has typically served as an explanation for the ability of social insect workers to modify their colony’s investment in males (Mehdiabadi et al. 2003; Ratnieks et al. 2006). Indeed, the results from Sundström et al. (1996) lend strong support to a role for such conflict in some colonies of the wood ant *Formica exsecta*. Until our study, all known instances of male elimination in social insects have been reported in cases of limited resources among offspring (Mock and Forbes 1995).

Sibling cannibalism might provide fitness benefits to society members through enhanced colony efficiency and performance (Chapuisat et al. 1997; Aron et al. 2001; Linksvayer 2008). Our results are consistent with the notion that male elimination in honeybees allows workers to modify the queen’s egg-laying decisions in a way that increases colony efficiency. By eliminating excess male brood, the workers might channel resources to more pertinent aspects of colony reproduction or survival, such as additional females or an increased ability to retain colony food reserves. Although we did not measure such life history measures of colony performance in our study, previous studies with honeybees suggest that rearing drones does not trade-off with a colony’s production of workers but might negatively impact the colony’s ability to stockpile honey for winter survival (Allen 1963; Seeley 2002).

A major question emerging from our study is whether an ability of the social insect queen or workers to influence colony sex allocation reflects within-colony genetic conflict or shared interests over the colony’s resource allocation to male reproductive function. Because a mixture of the 2 explanations...
might apply in a species with a high potential for queen–worker conflict, disentangling the relative effects of each might be challenging. To do so, researchers must consider both environmental and genetic factors that favor conflict or cooperation within groups (Ratnieks and Reeve 1992; Reeve and Hölldobler 2007; Linksvayer 2008). These environmental and genetic influences on colony dynamics should be teased apart and investigated using manipulative experiments (Sundström et al. 1996; Chapuisat and Keller 1999). Finally, researchers should consider proximate factors, such as the costs of brood manipulation, that might constrain the ability of the queen and workers to influence sex allocation (Nonacs and Carlin 1990; Chapuisat et al. 1997; Chapuisat and Keller 1999; Beekman and Ratnieks 2003; Beekman et al. 2003; Reeve et al. 2004).

In conclusion, social insect biologists should exercise caution when interpreting male elimination as being indicative of a manifest queen–worker conflict. We echo the plea of Chapuisat and Keller (1999) for additional manipulative experiments, which should lead to a better understanding of the evolutionary factors that promote conflict and cooperation and the ways in which those forces shape social dynamics.

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