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Naturwissenschaften
The Science of Nature

ISSN 0028-1042

Naturwissenschaften
DOI 10.1007/s00114-013-1025-6



Springer

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No facultative worker policing in the honey bee (*Apis mellifera* L.)

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Received: 7 December 2012 / Revised: 11 February 2013 / Accepted: 14 February 2013
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Abstract Kin selection theory predicts that in colonies of social Hymenoptera with multiply mated queens, workers should mutually inhibit (“police”) worker reproduction, but that in colonies with singly mated queens, workers should favor rearing workers’ sons instead of queens’ sons. In line with these predictions, Mattila et al. (Curr Biol 22:2027–2031, 2012) documented increased ovary development among workers in colonies of honey bees with singly mated queens, suggesting that workers can detect and respond adaptively to queen mating frequency and raising the possibility that they facultative police. In a follow-up experiment, we test and reject the hypothesis that workers in single-patriline colonies prefer worker-derived males and are able to reproduce directly; we show that their eggs are policed as strongly as those of workers in colonies with multiply mated queens. Evidently, workers do not respond facultatively to a kin structure that favors relaxed policing and increased direct reproduction. These workers may instead be responding to a poor queen or preparing for possible queen loss.

Keywords Worker policing · Polyandry · Social conflict · Social insect · Kin selection theory

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-013-1025-6) contains supplementary material, which is available to authorized users.

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Introduction

In many social insect species, workers favor rearing eggs that are laid by queens and eat eggs that are produced by other workers (Ratnieks and Visscher 1989; Ratnieks et al. 2006; Wenseleers and Ratnieks 2006). Such mutual inhibition of worker reproduction is called policing and is thought to have contributed to the evolution of complex insect societies by reducing conflict over who produces males (Ratnieks et al. 2006; Wenseleers and Ratnieks 2006) and aligning workers’ interests in rearing their queens’ offspring (Bourke 1999; Wenseleers et al. 2004; Ratnieks and Helanterä 2009). Because workers are more related to their own sons than to the sons of other colony members, kin selection theory predicts that workers may attempt to reproduce directly, even at the expense of colony productivity (Hamilton 1972). However, mutual eating of worker-laid eggs is also predicted to be advantageous for workers if they are more related to their queen’s sons than to the sons of other workers (Starr 1984; Woyciechowski and Lomnicki 1987; Ratnieks 1988). For species with one queen per colony, worker–worker policing is favored when queens have an effective mating frequency (m_e) greater than two because multiple mating dilutes worker relatedness to nephews but not to brothers. Conversely, worker policing is not favored when m_e is less than two, because workers are more related to nephews than to brothers. An alternative to this relatedness hypothesis for worker egg eating is the colony productivity hypothesis, which suggests that workers eat eggs to increase total colony output regardless of kin structure, by avoiding either costly laziness by egg-laying workers (Ratnieks 1988) or costly investment in low viability brood produced by workers (Pirk et al. 2004; Nonacs 2006).

Empirical support for the relatedness hypothesis for worker policing comes largely from interspecific comparisons showing that the incidence of policing increases and percentage of males produced by workers decreases with

decreasing worker–worker relatedness (Wenseleers and Ratnieks 2006). However, kin selection theory also predicts between-colony differences in policing for species in which some queens mate multiply and others mate singly (and for species with variable queen numbers; Hammond et al. 2003). The evolution of facultative policing based on relatedness differences requires (1) natural between-colony variation in intracolony relatedness that spans the predicted policing threshold, (2) information detectable to workers that indicates intracolony relatedness, and (3) absence of other benefits of policing (such as avoiding reduced colony productivity) that would favor it regardless of relatedness benefits. If these requirements are met, then the relatedness hypothesis predicts that policing will be reduced or absent in colonies possessing a queen with $m_e < 2$.

Although worker policing was first described in honey bees (Ratnieks and Visscher 1989), they are not an obvious choice for studying facultative policing in relation to queen mating frequency, primarily because honey bee queens nearly always mate multiply (Tarpy and Nielsen 2002) and, thus, likely do not meet the aforementioned first requirement. As for the second and third requirements, it is unclear whether kinship information is available to and detectable by workers (Visscher 1986; Arnold et al. 1996) or whether there are productivity benefits of policing in this species (see Pirk et al. 2003 and “Discussion”). However, Mattila et al. 2012 recently found that honey bee workers develop their ovaries more and work less in colonies headed by single-drone inseminated (SDI) queens versus colonies headed by multiple-drone inseminated (MDI) queens. This discovery raises the possibility that workers in colonies with singly mated queens can detect and can respond adaptively to low queen mating frequency. This, in turn, suggests the intriguing hypothesis that honey bee workers in SDI colonies have reduced policing and, as a result, facultatively activate their ovaries to produce sons (Mattila et al. 2012; Van Zweden et al. 2012). We tested this explanation for worker ovary activation in SDI colonies by comparing policing rates among colonies headed by SDI queens, MDI queens, and naturally mated queens. If honey bee workers do not relax policing when queens are singly mated, then this would suggest that one or more of the requirements for the evolution of facultative policing are not met for this species. It would also suggest that worker ovaries are activated in colonies headed by singly mated queens for another reason that is independent of colony kin structure, such as the presence of a weak queen whose death could lead to reproductive opportunities for ovary-activated workers (Visscher 1989; Mattila et al. 2012).

Methods

Our test of policing involved a standard assay (Ratnieks and Visscher 1989) where worker-laid and queen-laid eggs from

source colonies are transferred into test colonies, and egg survival is measured over 24 h.

Source colonies for eggs

To acquire worker-laid and queen-laid eggs, we divided queenright colonies in Ithaca, NY, to form pairs of queenright and queenless colonies. We split each colony by removing its queen, two to four frames of brood and adult bees, and several frames of food from the original hive and installing them in a new hive. The queenless portion remained in the original hive with most of the brood and adult bees. All colonies were checked weekly for eggs. Queen cells were removed from the queenless colonies to ensure that they remained queenless. We split five colonies on June 4, 2010, and four more on June 18. On July 11, we selected as our source colonies the three pairs of colonies that had strong egg laying by both queens (queenright colonies) and workers (queenless colonies).

Test colonies for policing assay

To compare egg eating in colonies with singly mated (SDI) and multiply mated (MDI) queens, we obtained nine queens of each type from a queen breeder (Glenn Apiaries, Fallbrook, CA) who performs instrumental inseminations. All the test-colony queens were full sisters of *Apis mellifera carnica* and drones were of mixed ancestry. The semen for the instrumental inseminations was collected from drones chosen randomly from a pool of 1,000 drones that came from 20 unrelated colonies. SDI queens received semen from one drone, while MDI queens received semen from 15 drones (mixed by stirring with a glass rod before insemination). SDI and MDI queens received the same volume of semen (1 μL per queen). We introduced the 18 queens into test colonies that were maintained in Wellesley, MA. These introductions were performed on May 18, 8 weeks prior to the policing tests, which ensured that each test-colony workers were daughters of the colony's queen at the time of the assay. To check for possible effects of working with instrumentally inseminated queens, we also performed tests of policing with nine colonies headed by naturally (multiply) mated queens.

Policing assay

On July 12, the three pairs of egg-source colonies were moved from Ithaca, NY, to Wellesley, MA, and the policing assay was performed from July 14 to July 19. Twenty-four hours prior to egg transfer, frames of empty drone comb (comb built of drone cells) were inserted into source colonies (queenright and queenless) from a single pair, as well as into a trio of test colonies (SDI, MDI, and naturally mated). In the test colonies, each frame of comb was placed inside a

cage with walls made of queen excluder screen, enabling workers to clean the comb's cells but preventing the queen from laying eggs in them (these frames would receive eggs from the source colonies shortly). In the source colonies, workers (in queenless colonies) and the queen (in queenright colonies) were free to lay eggs on the drone comb frames for the 24-h period, ensuring that all eggs were less than 1 day old. The next day, these frames were removed from all hives, so that eggs could be transplanted out of frames from source colonies into frames from test colonies. Each test colony was given 30 eggs from both source colonies in a pair (queenright and queenless); the eggs were deposited in two adjacent rows of cells using modified forceps (Taber 1961). When frames were not being handled, they were placed in an incubator (34 °C); combs containing eggs were covered with damp paper towel to prevent desiccation. Frames with rows of eggs were then placed back inside the queen excluder cages inside the test colonies. These cages ensured that any missing eggs were removed by workers and not the queen, who was also prevented from laying eggs in the focal cells. The number of eggs remaining in each row was checked after 24 h.

We assayed nine trios of test colonies (sets with SDI, MDI, and naturally mated queens; 27 colonies in total). Source colony pairs A, B, and C provided the eggs for four, three, and two of the test trios, respectively. In the second trial using eggs from colony pair C, all queen-laid and worker-laid eggs were removed by workers, possibly due to egg desiccation during transplanting. This trial was removed from the analysis.

Statistical analysis

To determine whether mating frequency affected the propensity of workers to favor queen-laid over worker-laid eggs, we analyzed the effect of queen type (SDI, MDI, and naturally mated) and egg type (queen-laid and worker-laid) on the number of transferred eggs that survived the 24-h assay using a two-way ANOVA, with a random effect of source colony pair. This test was performed in R 2.9.2 (R Development Core Team 2012). Because our data violated the assumption of homogeneity of variances for ANOVA, we performed an alternative test that did not; this alternative test yielded similar results (see Supplementary Materials).

Results

Mating frequency of queens did not affect survival of queen-laid or worker-laid eggs after 24 h in colonies (Fig. 1; two-way ANOVA; effect of queen type: $F_{2, 40}=0.38$, $p>0.5$; queen type \times egg type interaction, $F_{2, 40}=0.41$, $p>0.5$). Worker policing was strong across all colonies, as many more queen-laid eggs survived than worker-laid eggs (Fig. 1; two-way

ANOVA; effect of egg type: $F_{1, 40}=106.52$, $p<0.0001$). Of the 810 worker-laid eggs that were transplanted into test colonies, only two eggs remained after 24 h (0.08 ± 0.06 eggs per colony, SEM), whereas 340 of the 810 queen-laid eggs remained after 24 h (12.6 ± 1.2 eggs per colony).

Discussion

This study was done to determine whether facultative worker policing based on relatedness can explain increased development of workers' ovaries in honey bee colonies with SDI queens (Mattila et al. 2012). Our results show unambiguously that workers in the colonies that we studied did not facultatively police as a function of their colony's kin structure. Workers in colonies with singly inseminated queens removed worker eggs at a high rate that was similar to colonies with multiply inseminated and naturally mated queens.

The absence of facultative policing in honey bees is consistent with what has been found in the other two social insect species that have been tested for this phenomenon. In the wasp *Dolichovespula saxonica*, Bonckaert et al. (2011) found no evidence for facultative policing based on mating frequency and concluded that earlier hints of facultative policing in this species (Foster and Ratnieks 2000) were likely due to a small sample size and a confound of relatedness with colony developmental stage. Similarly, in the ant *Leptothorax acervorum*, Hammond et al. (2003) found that variation in intracolony relatedness due to differences in queen number did not predict attempts by workers to reproduce. It appears, therefore, that social Hymenoptera typically do not meet the requirements that are necessary for the evolution of facultative policing, and that predictions regarding levels of policing based on relatedness may be upheld only for interspecific comparisons based on average relatedness structure.

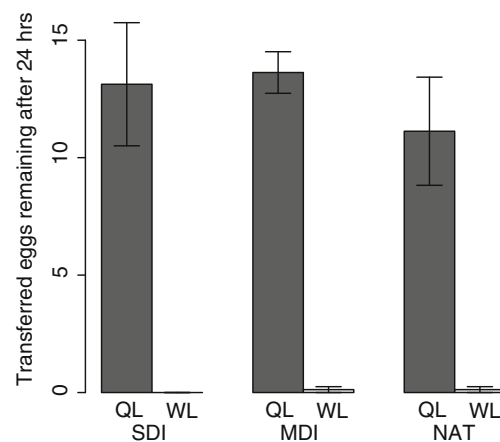


Fig. 1 Mean (\pm SEM) number of queen-laid (QL) and worker-laid (WL) eggs remaining 24 h after transfer into colonies with SDI, MDI, and naturally mated queens. For each colony, 30 eggs of each type were transferred ($n=8$ colonies for each type of queen)

Why honey bees do not exhibit facultative policing? Observations of non-policing colonies suggest that natural genetic variation for policing exists and could be a target for selection (Beekman et al. 2002). The evolution of a facultative response to mating frequency would require natural variation in m_e spanning the policing threshold, but such variation is evidently not present in *A. mellifera*. Honey bee queens nearly always mate multiply. Of the 113 colonies summarized by Tarpy and Nielson (2002), only eight had $m_e < 2$, and estimates of m_e may be artificially low for these colonies because only a small number of workers were genotyped. Even if variation in mating frequency was sufficient for facultative policing, its evolution would still require a mechanism for workers to assess mating frequency (or patriline diversity). Theoretical arguments suggest that this information may not be evolutionarily stable (e.g., Ratnieks 1991), though sufficient discriminatory information is present in some species (Sundström et al. 1996; Boomsma et al. 2003; van Zweden et al. 2010; Nehring et al. 2011) and may be present in the honey bee (Arnold et al. 1996).

Alternatively, it is possible that honey bees can detect and respond to mating frequency, but that selection maintains policing even in colonies with singly mated queens because colony-level productivity benefits discourage workers from reproducing (Ratnieks 1988; Pirk et al. 2004). This is an attractive but difficult-to-test hypothesis that is often proposed to explain the presence of worker policing in species in which colony kin structure does not predict that it should exist (e.g., Hammond and Keller 2004), and it has been invoked to explain why *A. mellifera capensis* workers police worker-laid eggs in the absence of relatedness benefits (Pirk et al. 2003).

Regardless of the selective basis for policing in honey bees, our results refute the hypothesis that workers activate their ovaries in queenright colonies with singly mated queens because there is reduced worker policing in these colonies and thus increased opportunities for direct reproduction by workers in these colonies. This finding points to an alternative hypothesis to explain why more workers undergo ovary development in colonies headed by singly inseminated queens: workers assess a singly mated queen as one who is weak and likely to fail (Mattila et al. 2012). The weak-queen hypothesis proposes that queen reliability, not colony kin structure, influences worker ovarian development. Workers may prime themselves for direct reproduction as the possibility of being able to reproduce directly in a queenless colony increases, even at a cost to the still queenright colony (Visscher 1989; Mattila et al. 2012).

Acknowledgments We thank Michael Smith for help with fieldwork; Michael Smith and Elizabeth Hunter for feedback on the manuscript; and the US National Science Foundation (Graduate Fellowship to KJL), Cornell Agricultural Experiment Station (grant NYC-191522 to TDS), and a Brachman-Hoffman Grant (to HRM) for financial support.

Conflict of interest The authors declare that they have no conflict of interest.

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