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SHORT COMMUNICATION

Irregular brood patterns and worker reproduction in social wasps

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Abstract The potential for reproductive conflict among colony members exists in all social insect societies. For example, queens and workers may be in conflict over the production of males within colonies. Kin selection theory predicts that in a colony headed by a multiply-mated queen, worker reproduction is prevented by worker policing in the form of differential oophagy. However, few studies have demonstrated that workers actually lay eggs within queenright colonies. The purpose of this study was to determine if workers laid male eggs within unmanipulated queenright colonies of the polyandrous social wasps *Vespula maculifrons* and *V. squamosa*. We focused our analysis on an unusual brood pattern within colonies, multiple egg cells. We were primarily interested in determining if individuals reared in these irregular circumstances were queen or worker offspring. To address this question, we genotyped 318 eggs from eight *V. maculifrons* and two *V. squamosa* colonies. No worker reproduction was detected in any of the queenright colonies; all of the eggs found in multiple egg cells were consistent with being queen produced. However, the frequency of multiple egg cells differed among colonies, suggesting that queens vary in the frequency of errors they make when laying eggs within cells. Finally, we suggest that workers may not be laying eggs within queenright colonies and that worker reproduction may be controlled through mechanisms other than differential oophagy in polyandrous *Vespula* wasps.

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Introduction

The potential for conflict exists in societies composed of group members with different interests (Keller 1999). Within social insect groups, conflict may exist over reproduction (Foster and Ratnieks 2001a; Hammond and Keller 2004; Wenseleers et al. 2004; Ratnieks et al. 2006). In many social Hymenoptera, workers, although unable to mate, have the ability to lay unfertilized male eggs. Consequently, there may be conflict between queens and workers over the production of males (Ratnieks 1988). Worker policing can resolve conflict and can take the form of differential egg eating (oophagy) or behavioral aggression towards reproductive workers (Ratnieks and Wenseleers 2005). According to kin selection theory, worker policing should occur in nests headed by queens effectively mated to two or more males, when there is no cost to policing behavior (Ratnieks 1988). In contrast, in nests headed by queens effectively mated to fewer than two males, worker policing is not expected.

Many studies have documented worker policing by introducing foreign worker produced eggs from queenless colonies into queenright colonies (e.g. Ratnieks and Visscher 1989; Foster and Ratnieks 2001b; Halling et al. 2001; Pirk et al. 2004; Wenseleers et al. 2005a). In cases where foreign eggs disappeared over time but queen-produced eggs were still present, worker policing was assumed to be the cause. However, these manipulative studies did not address the important question of whether workers actually reproduce in the presence of the queen under natural conditions.

The purpose of this study was to determine if workers laid male eggs within unmanipulated queenright colonies of the social wasps *V. maculifrons* and *V. squamosa*. As queens of both species mate multiply (Ross 1985), worker policing is predicted. Accordingly, no worker produced males have been found in queenright colonies. However, a small percentage (2%) of *V. maculifrons* workers have activated ovaries (Ross 1985). Therefore, workers are either failing to reproduce or their eggs are being policed. For this study, we were interested in determining if workers laid eggs in unmanipulated natural colonies, as opposed to previous studies that used artificially manipulated colonies to detect policing.

We specifically focused our analysis on eggs found in multiple egg cells (MECs), brood cells in which two or more eggs or larvae were present (Fig. 1). MECs are found at low frequencies within many *Vespula* nests. However, only one wasp reared in a MEC will survive, and therefore, extra eggs within a cell represent a waste of resources by the queen and a cost to the colony. We focused on MECs both because they have been associated with worker reproduction in queenless colonies and because they are commonly found in colonies headed by more than one reproductive queen (Spradbery 1973). In this study, we wanted to determine if eggs within MECs were worker produced.

Materials and methods

In 2005 and 2006, we collected eight *V. maculifrons* and two *V. squamosa* colonies in Atlanta, GA, USA. The reproductive queen was collected with three of these colonies (Table 1; Colonies 48, 53, and 62). Additionally,

Fig. 1 *Vespula* wasp nest containing a high proportion of multiple egg cells

10 to 40 workers were sampled from nine colonies (excluding Colony 62) for later genetic analysis.

Eggs were removed from MECs using sterile toothpicks. Eggs from single egg cells the same age and from the same section of comb as the MECs were also sampled. Fifty microliters of 5% Chelex solution was added to the egg samples; the tubes were vigorously vortexed and centrifuged for 5 min. DNA was extracted from adults following the methods of Goodisman et al. (2001).

Genotypes were determined at eight microsatellite loci: LIST2003, LIST2004, LIST2013, LIST2019, LIST2020 (Daly et al. 2002), RUFA 5 (Foster et al. 2001), VMA 3, and VMA 6 (Hasegawa and Takahashi 2002). The genotype of the queen for each colony was determined using direct analysis of eggs and adult workers. Eggs were deemed to be haploid (male) if they displayed a single allele at all amplified microsatellite loci. At least three successfully amplified loci were necessary for sex determination. In fact, males amplified at a mean of 7.4±1.06 loci and females amplified at a mean of 6.8±1.66 loci. Worker produced males were identified as haploid individuals not exhibiting one of the queen's alleles at one or more loci.

To determine our ability to detect worker produced males, we calculated the probability that a worker's son possessed at least one allele inherited from the queen's mate(s) that was distinct from the alleles inherited from the queen (P_j; Foster and Ratnieks 2001a). High values of P_j indicated a high probability of detecting worker produced males due to the high genetic variability of individuals within colonies.

We used two tailed Fisher's exact tests to determine whether males or females were found more frequently in MECs than in single egg cells; Fisher's method of combined probability was used to combine information across all colonies.

Results

In eight of the ten colonies, MECs were found at low frequencies (Colonies 48, 52, 49, 50, 51, 53, 57, and 58; Table 1). In these colonies, the majority of MECs contained two eggs, while a few contained three eggs. The remaining two colonies (55 and 62) contained higher frequencies of MECs.

In total, we successfully amplified microsatellite loci from 303 of 441 *V. maculifrons* eggs and 45 of 84 *V. squamosa* eggs. We detected 153 male and 134 female eggs in the eight *V. maculifrons* colonies, and 7 male and 28 female eggs in the two *V. squamosa* colonies. We were unable to assign a sex to 26 individuals based on the loci that successfully amplified. The probability of detecting a worker's son (P_j) ranged between 0.836 and 0.972 (Table 1). These results indicate that we had sufficient power to

Table 1 The number of eggs analyzed, male and female eggs detected, multiple egg cells (MECs) observed, males and females detected within MECs, and total number of cells in ten *Vespula* colonies

Species	Colony ID	Number of eggs analyzed	Number of male eggs	Number of female eggs	Number of males in MECs	Number of females in MECs	*P* value	Number of MECs	Number of worker cells	P_j	Number of worker produced eggs
V. squamosa	48	22[a]	1	18	1	3	0.2727	4	N/D	0.972	0
V. squamosa	52	23[a]	6	10	0	5	0.0934	4	5730	0.836	0
V. maculifrons	49	44	17	27	9	11	0.7640	10	4988	0.939	0
V. maculifrons	50	11	9	2	1	1	0.3454	1	4800	0.952	0
V. maculifrons	51	17[a]	6	9	4	4	0.9999	6	6521	0.954	0
V. maculifrons	53	25	5	20	2	5	0.6080	5	3681	0.969	0
V. maculifrons	55	107[a]	103	3	83	0	N/A	53	6302	0.885	82
V. maculifrons	57	19	5	14	0	8	0.0445	5	4524	0.950	0
V. maculifrons	58	36	8	28	4	21	0.2137	15	2990	0.955	0
V. maculifrons	62	44[a]	0	31	0	31	N/A	26	1923	0.924	0

P–values are reported for Fisher's exact tests, which were used to determine if the proportion of male and female eggs in MECs and single egg cells differed. P_j is the probability of identifying a worker–produced male within each colony.
N/D Data not recorded; *N/A* not applicable
[a] The number of eggs analyzed was greater than the sum of the number of male and female eggs because sex could not be assigned to all eggs.

identify nearly all worker produced males present in the colonies.

In nine of the ten colonies, all eggs were consistent with being queen produced. In contrast, in Colony 55, only 18% of eggs had genotypes consistent with being queen produced, and all but three eggs were haploid (Table 1). We also found that MECs contained both male and female eggs. In nine of the ten colonies there was no significant difference in the proportion of male and female eggs in MECs and in single egg cells (two-tailed Fisher's exact tests, Table 1). Moreover, when all colonies were combined, male and female eggs were found just as frequently in MECs as they were in single egg cells (Fisher's combined probability, P=0.37624).

Discussion

We found no evidence of worker reproduction in nine of the ten colonies collected. Colony 55, the only colony with worker produced eggs, had the most MECs, many of which contained three to seven eggs. Genetic analysis of Colony 55 eggs revealed that all but three were male (Table 1). In addition, the queen was not found at the time of collection. Thus, Colony 55 was most likely queenless. Consequently, our data provide no evidence of worker reproduction within queenright *V. maculifrons* and *V. squamosa* colonies.

Both worker reproduction and worker policing have been directly observed in unmanipulated queenright colonies of the monandrous social wasp *Dolichovespula sylvestris* (Wenseleers et al. 2005b). In this species, 49% of the observed policing events were performed by the queen, demonstrating the effect of queen control on worker reproduction (Foster et al. 2001;Wenseleers et al. 2005b).

However, a study by Pirk et al. (2004) suggested that worker policing in polyandrous *Apis* colonies may not be influenced by variation in relatedness as predicted by kin selection theory but rather by egg viability. It was found that worker produced eggs experienced higher mortality than queen produced eggs. Specifically, it was suggested that the high rate of worker laid egg removal may be due to worker hygienic behavior, not worker policing. Although criticisms have been leveled at this study (Beekman and Oldroyd 2005), the possibility remains that the high levels of egg removal seen in worker policing studies using foreign worker and queen produced eggs may be due to low egg viability or the foreign origin of the eggs. This leaves the possibility that hygienic behavior may still explain cases of egg removal in manipulated colonies.

It has been hypothesized that worker reproduction is generally not detected in queenright colonies due to the rapid policing of worker laid eggs (Foster and Ratnieks 2001a). Although our study cannot rule out the possibility that worker produced eggs are policed immediately, the absence of worker produced eggs and the presence of energetically expensive MECs suggests that policing is not occurring. Therefore, worker reproduction may be controlled through mechanisms other than differential oophagy.

For example, it has been hypothesized that the absence of worker reproduction in some ants (Dietemann et al.

2005) is due to “self restraint” in response to honest queen fertility signals. Perhaps in highly polyandrous social wasps, like *V. maculifrons* and *V. squamosa*, worker reproduction is suppressed not by worker policing, but rather through worker abstinence.

MECs were found in all nests. All males identified in both MECs and single egg cells in the nine queenright colonies had genotypes consistent with being queen produced. Consequently, our results suggest that MECs are not the result of worker reproduction in queenright colonies. Rather, they result from multiple eggs being laid in the same cell by the queen.

Both males and females were found in MECs. Moreover, neither sex was found in MECs more frequently than in single egg cells. This suggests that MECs in queenright colonies are not the result of an adaptive egg laying strategy by the queen, as might be expected if males had particularly low viability and multiple haploid eggs were laid within a cell to ensure that a single male emerged. In addition, MECs do not seem to be trophic eggs, as they persist in colonies for extended periods of time. Consequently, we suggest that MECs result from queen error during egg laying.

Colonies 55 and 62 exhibited high numbers of MECs as the result of two different mechanisms. Colony 55 supports the long standing assumption that MECs are the result of worker reproduction by multiple workers (Spradbery 1973). MECs are commonly found in *Vespula* colonies containing multiple reproductives. In rare polygyne *Vespula* colonies, reproductive conflict between two or more queens can lead to a high frequency of MECs. MECs in queenless colonies can be explained by the increased conflict between potential reproductive workers.

In contrast, Colony 62 demonstrates that MECs can occur at high frequencies without worker reproduction. Colony 62 contained 26 MECs, all of which held two diploid eggs. In addition, the queen was collected with the nest, and all eggs in MECs were consistent with being queen produced. Consequently, some queens apparently produce a high proportion of MECs. In monogyne queenright colonies, queens have been observed laying a second egg in some cells (Spradbery 1973). Therefore, it cannot be assumed that MECs, even at rather high frequencies, serve as evidence of worker reproduction or queenlessness. The reason for the variation in the number of MECs between queenright colonies is unclear, but we suggest that it may be due to constraints on the size of the nest, with queens heading colonies that are unable to expand their nest producing more MECs than queens heading colonies that are easily expanded.

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