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Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing

Abstract Using electrophoretic markers, eggs laid by workers were identified in honey bee (*Apis mellifera*) colonies with a queen. Based on extrapolation, these represented about 7% of the unfertilized (male) eggs laid in the colonies. A very small proportion of workers (of the order of 0.01%) lay these eggs. Worker-laid eggs are rapidly removed, so that very few sons of workers are reared. Thus the reproductive cooperation in bee colonies is maintained by ongoing antagonistic interactions among the members of the colony, with worker laying and egg removal policing by other workers being relatively common.

Key words *Apis mellifera* · Policing · Worker egg laying · Electrophoresis

Introduction

Social insect colonies display elaborate group-level functional organization, in spite of kinship structure which leads to potential conflict among individuals. Evolution of cooperation is likely enhanced by strict reproductive division of labor (Seeley 1985; Ratnieks and Visscher 1989). Studies of honey bees (*Apis mellifera*) are providing insight on the evolution and maintenance of this division. Worker honey bees cannot mate, but can lay unfertilized (male) eggs. In colonies with a queen, workers do produce a few males (Mackensen and Roberts 1948; Page and Erickson 1988), but only about 1 in 1000 males reared to adulthood derives from a worker (Visscher 1989). Why is worker reproduction so rare? The cost to group efficiency of egg laying by workers might lower the payoff to these workers enough to prevent their laying (Seeley 1985),

but this cost is probably small relative to the benefit, and unlikely to be sufficient (West Eberhard 1981; Visscher 1989). The presence of worker policing – behavioral mechanisms of worker egg destruction by other workers (Starr 1984; Ratnieks 1988) – suggests that reduction of the benefits of worker laying due to other workers' manipulation, not efficiency costs, has been the driving force. The presence of policing mechanisms might promote a forced cooperation: abandonment of attempted reproduction without direct antagonist interactions. Alternatively, direct conflict could be common, with frequent worker oviposition and egg removal. Worker eggs were reported by Ratnieks (1993) in queenright colonies above a queen-excluding mesh. However, workers are known to rear queens more readily above a queen excluder than they will below it (Laidlaw 1979; Visscher 1986), suggesting that normal queen pheromone transmission may have been disrupted in Ratnieks' experiment, and, though unlikely, some queen-laid eggs could have been moved above the excluder (Winston 1987; Ratnieks 1993). This study was undertaken to demonstrate and quantify the occurrence of worker egg-laying and egg-removal policing in normal queenright honey bee colonies.

Materials and methods

The approach of this study was to use isozyme electrophoresis to identify the maternity of male eggs in honey bee colonies. This data, and the rate of egg removal under similar conditions, was used to extrapolate to the frequency of worker-laid eggs at the time of laying (W_0). Samples were collected at different intervals, to provide multiple estimates of W_0, and to document the changing frequency of worker-laid eggs as worker policing occurred.

Study colonies

The experimental colonies were full-sized (30,000–40,000 workers), occupying two standard Langstroth hive bodies ($\approx 84{,}000 \text{ cm}^3$).

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The bees were of mixed European ancestry, principally *Apis mellifera ligustica* headed by naturally-mated queens of unknown age (3 months to 2 years). The movement of workers and queens was unrestricted within the beehives. I identified colonies headed by queens which were homozygous for the less common alleles of malate dehydrogenase (MDH), and in which workers were all or mostly heterozygous, because the queen had mated mostly with males with MDH alleles different than her own. To estimate the proportion of workers of the same phenotype as the queen (i.e., whose egg could not be distinguished from those of the queen), I collected 48 workers from the broodnest of each colony at the beginning and end of egg collections. Egg collections were made during June, July, and August 1991. Throughout this period all colonies were rearing male brood on several combs.

Egg collections

To obtain newly laid male eggs, I introduced combs of male cells ("drone comb") into the middle of the brood rearing area of these colonies. Every two hours during the day or after 16 hours overnight, I inspected these combs for eggs. Usually there were none, but when there were eggs, I transferred all of them onto beeswax-coated black paper using specialized forceps (Taber 1961), placed this inside of a labeled vial, and stored it at $-80°C$ until processing for electrophoresis.

Rate of egg removal

Analysis of the data required quantifying the rate at which worker-laid eggs are removed by worker policing in colonies under the conditions of this study. To do this, I confined a queen on a single drone comb inside a cage which allowed workers, but not the queen, to pass through, and placed this into the broodnest of colony. After 24 h I released the queen. I removed some of the eggs which she had laid from cells of the comb and replaced them with worker-laid eggs from a queenless colony. I then put the comb back into the queen-right colony without the cage. Then at 15-min intervals for 2 h I removed the comb, and without dislodging adhering bees, quickly counted the number of worker-laid eggs remaining, and replaced the frame. This was done with two of the study colonies, one once and one twice.

Identification of egg maternity

To distinguish worker- and queen-laid eggs, I used cellulose acetate electrophoresis to identify the MDH phenotype of newly laid eggs. Honey bee eggs express exclusively the maternal MDH phenotype for more than 24 h after laying. The offspring phenotype gradually replaces the maternal phenotype after 36–48 h (P.K. Visscher, unpublished work).

I transferred frozen eggs individually into the wells of a Z-12 sample plate (Helena Laboratories), added 4 μl of extraction solution (0.1% Triton-X surfactant, 0.1% dithiothreitol, w/w in H_2O), and ground the egg with the tip of a stainless steel spatula. I applied this homogenate with a Z-12 applicator to a cellulose acetate gel. Procedures and stains were as described by Herbert and Beaton (1989).

Worker ovary dissections

To estimate the frequency of workers with developed ovaries, I dissected 3176 worker bees taken from the broodnest of three colonies unrelated to each other or the colonies used in egg assays. I fixed these bees in Kahle's solution, dissected them under a microscope, and scored the degree of development of their ovaries. I photographed those with any degree of ovary development to ensure consistency in scoring.

Results and analysis

Worker and egg phenotypes

Overall, 1950 eggs were identified in this study (Table 1). All eggs collected were in the normal position: standing upright in the bottom of the cell, one egg per cell. For every colony the inspections resulting in egg collections occurred on several different days, so the samples came from 3, 6, 5, and 4 different days for colonies 3, 30, 45, and 61, respectively, and the collections of non-Q-type eggs were distributed among these days. For none of the colonies did the distribution of workers among MDH phenotypes differ between the samples at the start and end of collections (χ^2 test, $P > 0.05$).

Accounting for Q-type worker-laid eggs

Only some proportion of the adult workers had MDH phenotypes different from the queen (i.e., W-type). This proportion of W-type adults in colony i is denoted P_{Ai}. Because 1-P_{Ai} workers had the same MDH phenotype as the queen (i.e., Q-type), only some worker-laid eggs would be W-type. Therefore, in Table 1 the proportions of W-type eggs for each colony i (P_{Ei}) can underestimate the true proportion of worker-laid eggs in the total egg collection for that sample interval ($N_{obs\ i}$). To correct for this, I assumed that the distribution of

Table 1 Workers and eggs with the queen MDH phenotype ($= Q$-type) and heterozygous phenotypes, ($= W$-type, one allele same as the queen, the other one of the other 2 MDH allozymes). Q-type eggs may be from the queen or workers, W-type cannot be from the queen

Colony	Queen MDH	Worker sample		2-h Egg collections		Overnight (16-h) egg collections	
		Q-type	W-type	Q-type	W-type	Q-type	W-type
3	FF	33	63	0	0	182	1
30	MM	0	96	17	1	875	3
45	FF	31	65	281	7	291	0
61	MM	0	96	107	0	197	0
Overall		64	320	405	8	1545	4

MDH phenotypes among workers laying eggs was the same as in worker samples (but see Discussion). For the 2-h and 16-h collection times I derived an estimate for the true worker-laid percentage for that interval (W_T*) This is a weighted average (by sample size) of the quotient of P_{Ai}/P_{Ei}. The standard deviation of W_T* is derived in the appendix.

Test for heterogeneity among colonies

To evaluate whether there was evidence of heterogeneity among colonies for the proportion of worker-laid eggs, a goodness-of-fit (GOF) test for heterogeneity was performed. The observed GOF statistic is generated by calculating GOF of the observed count in each cell with the corresponding expectation, and summing GOF over all cells [GOF = (obs − exp)²/exp]. Expectations are calculated as $E(W\text{-type}_i) = W_T* \cdot N_{obs\,i} \cdot P_{Ai}$ and $E(Q\text{-type}_i) = N_{obs\,i} − E(W\text{-type}_i)$.

Because many expected values for W-type are small, the assumptions under which this GOF statistic would follow the χ^2 distribution are violated. In particular, the statistic is likely to have larger values than a χ^2 distribution, so it would be more likely to reject the null hypothesis (that the data is homogeneous) than it should be (i.e., excessive Type I errors). To achieve an unbiased test, the distribution of the total GOF statistic under the null hypothesis was derived by simulation.

Simulation involved generating pseudoobservations under the null hypothesis that the probability of each egg being from a worker is W_T*, and the probability of a worker egg being of worker type is P_{Ai}. For each colony, a sample of $N_{obs\,i}$ "eggs" is generated, each "egg" has a probability of W_T* of being worker-laid, and if it is, a probability of P_{Ai} of being W-type, otherwise it is Q-type. This is repeated for each colony. The GOF statistic is computed for each cell with the same expected values as above, and summed over all cells. This process is repeated 1000 times, and the observed GOF is compared to the resulting distribution of total GOF under the null hypothesis, and the alpha level estimated by the proportion of the simulated GOF that are larger than the observed GOF. To estimate the variation of the alpha levels obtained under this procedure, the process is repeated five times, and the mean ± SD of the alpha level estimates is calculated.

These simulations yielded an estimated alpha level for the test of heterogeneity of the two hour observations, among hives, of 0.235 ± 0.020, and of the 16 h observations, among hives, of 0.538 ± 0.018. The observed values are thus compatible with the null hypothesis, so there is no evidence of heterogeneity among colonies for the proportion of worker-laid eggs. (The conclusion is the same if the statistic is assumed to be distributed as χ^2 with P = 0.13 and 0.48 for 2- and 16-h collections, respectively.)

Table 2 Worker- and queen-derived male eggs in honey bee colonies. W_T* and W_0 are extrapolations estimating true levels of worker laying (see text)

	2-h Egg collections	16-h Egg collections
Total Q-type	405	1545
Total W-type	8	4
W-type % ± SD	1.94% ± 0.68%	0.26% ± 0.13%
Worker-laid% (W_T*) ± SD	2.75% ± 0.98%	0.29% ± 0.15%
Initial% worker-laid (W_0) ± SD	9.0% ± 3.2%	7.3% ± 3.7%

Comparisons between collection intervals

Table 2 shows the average (weighted by sample size) of the proportions of W-type eggs found in the study (non-Q%) and the corrected average, W_T* (worker-laid%). To compare the observed proportions of W-type eggs in the samples from 2 h and 16 h, and of W-type adult males, I used simulations similar to those above. Here the null hypothesis is that W_T* was the same for both intervals (estimated by a pooled $W*$). This null hypothesis is rejected in a comparison between the 2-h and 16-h samples (α-level = 0.001 ± 0.001), indicating a higher proportion of worker-laid eggs in the 2-h sample. Again, the conclusions of a χ^2 test, which is biased toward rejection of the null hypothesis, would be the same.

Estimating W_0

Policing would reduce worker egg percentages even during short sample intervals. To estimate the proportion of worker eggs at laying (W_0), the proportions in the samples can be divided by the probability (P_s) that an egg laid during the sample interval T is present at the end of the interval. I estimated P_s from the rate at which worker-laid eggs that I presented to queenright colonies were removed. Figure 1 shows this data for 92 eggs in two of the study colonies.

These data fit quite well to an exponential decay curve, with a constant proportion (r) of remaining worker eggs being removed each time interval, and the proportion remaining at time t after laying equal to e^{-rt}. If during the interval T between inspections, eggs have a constant probability $1/T$ of being laid at any time, and are subject to removal after laying at the rate r, then

$$P_s = \frac{1}{T} \int_{t=0}^{T} e^{-r(T-t)} dt = \frac{e^{-rT}}{-rT} - \frac{1}{-rT} \quad (1)$$

As shown in Table 2, worker-derived eggs composed 2.75% of the 2-h sample, and 0.29% of the 16-h sample. Using r = − 0.026/min from the curve in Fig. 1, these yield estimates of the worker contribution of male eggs

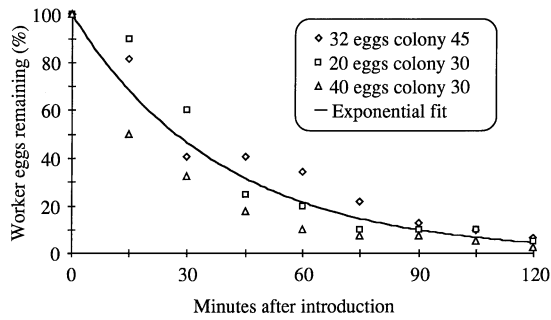**Fig 1** Rate of removal of worker-laid honey bee eggs in a queenright colony. The *curve* is an exponential decay curve fit to all the data

Fig. 2 Range of levels of worker laying (W_0) consistent with observed data. α-levels are of goodness-of-fit tests between observed data and expectations if workers laid a given percentage (horizontal axis) of their colonies' male eggs (*solid line* α-levels $\pm$ SD from simulation, *dashed line* α-levels from (biased) χ^2 distribution. The region with y values above $P = 0.05$ (*horizontal line*) is consistent with the observed data for 3 exposure intervals

at laying of 9% and 7%, for the 2-h and 16-h sample, respectively.

Evaluating range of W_0 consistent with data

The estimates of W_0 are fairly similar for 2- and 16-h collections. This led to the question of whether the data was consistent with a model of a uniform percentage of male eggs laid by workers in all the colonies observed, followed by policing behavior removing eggs at a constant rate r for at least the first 16 h (i.e., whether these two assays of worker reproduction could share a single initial W_0). I tested this null hypothesis with simulations similar to those above. As above, P_{Ai} is the proportion of workers in colony i with phenotypes distinguishable from the queen, and $N_{obs\,i}$ is the size sample collected from colony i. N_{0i} is the number of male eggs presumed laid in colony i during the interval T between inspections. W_0 is the proportion of these eggs laid by workers, and P_T the probability that a worker egg survives to the end of the interval T, with exponential decay through policing with parameter r (see Fig. 1 and Eq. 1). Expected values are derived as follows:

$E(W\text{-type}_i) = N_{0i} W_0 P_T P_{Ai}$

$E(Q\text{-type}_i) = N_{0i}(1 - W_0) + N_{0i} W_0 P_T (1\text{-}P_{Ai})$

$N_{obs\,i} = E(W\text{-type}_i) + E(Q\text{-type}_i)$

$\qquad = N_{0i} W_0 P_T P_{Ai} + N_{0i}(1 - W_0)$

$\qquad\quad + N_{0i} W_0 P_T(1 - P_{Ai}) = N_{0i}[1 - W_0(1 - P_T)]$

so $N_{0i} = N_{obs\,i}/[1 - W_0(1 - P_T)]$

$E(W\text{-type}_i) = W_0 P_T P_{Ai} N_{obs\,i}/(1 - W_0(1 - P_T)]$ and

$E(Q\text{-type}_i) = N_{obs\,i} - E(W\text{-type}_i)$

The expected values $E(W\text{-type}_i)$ and $E(Q\text{-type}_i)$ were calculated for each colony and summed to give an experiment-wise expected value for W-type and Q-t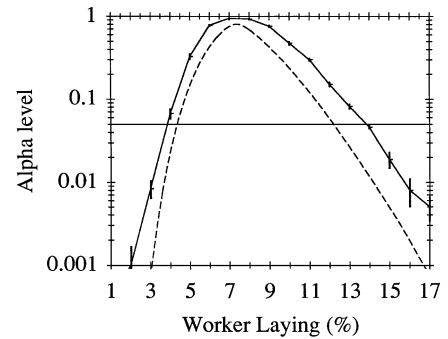ype for each of the two sampling intervals (with sample sizes as in the corresponding experiment). The observed and expected values were compared with a 2×2 goodness-of-fit statistic. This was done for each of a range of values of W_0. As W_0 varied, the GOF statistic reached a minimum (0.061) when $W_0 = 7.4\%$, indicating that the data best fit the model when this level of worker laying is assumed. Each value of W_0 was examined to determine whether it is consistent with the observed results (Fig. 2). To do this, the GOF statistic for each W_0 is compared to its distribution under the null hypothesis. Conventionally this is done with a χ^2 distribution (dashed line in Fig. 2). As before, however, low expected values violate the assumptions, and the test is biased toward rejecting the null hypothesis, so it would result in too narrow a range of values of W_0 consistent with the model. Instead, I determined an unbiased distribution of the statistic under the null hypothesis by simulation. For each W_0, 1000 simulated datasets were generated with Bernoulli trials and the parameters W_0, P_T, and P_{Ai}. The GOF statistic was calculated between each simulated dataset and the expected values for that W_0. The observed-versus-expected GOF was compared to the resulting distribution to determine the frequency under the null hypothesis of results as extreme as those observed. This process is then repeated five times to estimate mean and SD of the alpha level for each W_0 value (solid line and error bars in Fig. 2).

Worker ovary development

Of the 3176 workers dissected, 96.5% had completely undeveloped ovaries, 3.5% had some degree of ovary development, and 1% had ovaries in which separate oocytes could be distinguished, but none of these had full-sized ova.

Discussion

The proportion of worker laying

Identification of egg maternity with electrophoresis shows unequivocally that a substantial proportion of the male eggs in honey bee colonies are laid by workers. About 2% of eggs in the 2-h collections could not have been laid by the colonies' queens. Extrapolating to initial laying, using the best estimates available, suggests that W_0 is in the vicinity of 7%. This result confirms predictions based on earlier studies. A small percentage, about 1 in 1000, of adult drones are worker-derived (Page and Erickson 1988; Visscher 1989), and there are strong policing mechanisms which allow only fewer than 1 in 100 worker-laid eggs to develop (Ratnieks and Visscher 1989). From these facts it seemed likely that W_0, the proportion of male eggs laid by workers, was of the order of 1 in 10. The results of this study confirm this prediction. The two assays here, with eggs exposed to policing for different periods, were consistent with a single uniform proportion of worker laying, followed by a uniform rate of egg removal for different intervals. Under a model with these assumptions, Fig. 2 shows that the data fit very well with the expectations of 7.4% of male eggs being laid by workers in all colonies ($P \approx 0.95$), but would be consistent (i.e., not rejected at $P < 0.05$) for a range from about 4% to 14% worker laying.

Is r constant?

Ratnieks and Visscher (1989) showed that workers do not discriminate between queen-laid and worker-laid larvae, so policing is confined to the egg stage. While the results of this study are consistent with a constant removal rate during the first 16 h of the egg stage, other results indicate that the rate of removal must not remain constant throughout the entire egg-development period of about 72 hours. Visscher (1989), from adult drones whose maternity could be identified, estimated $W = 0.06\% \pm 0.01\%$ and $W^* = 0.13\% \pm 0.02\%$ (SD calculated with the methods of this study), but did not estimate W_0. Since these eggs were exposed to policing throughout their development period of about 72 h, if policing were constant, then only a proportion e^{-rT} would be expected to survive. This differs from Eq. 1 because all eggs were present throughout the interval T, not assumed to have been laid uniformly throughout the interval. Using the value of r from Fig. 1, the probability of escaping policing throughout the interval would be negligibly small ($\approx 10^{-48}$), and no value of W_0 would be consistent with such a model. Data from Ratnieks (1993) also suggests that eggs which escape policing for a period of time do become more acceptable, though, as Ratnieks noted, his data could be confounded by moved, queen-laid eggs.

Several mechanisms would be expected to lead to diminishing removal with time. The differences between worker and queen laid eggs (perhaps a queen-applied pheromone as suggested by Ratnieks 1995) may diminish with time. If some worker eggs are always more acceptable than others the exponential decay would also flatten out at later times. Alternatively, workers may simply be less selective with older eggs.

If r reduces with time, then samples with longer periods of exposure to policing, provided they were long enough that the removal rate was decreasing, would be expected to increasingly overestimate W_0. However, in this study the 2-h sample led to an estimate of a higher W_0 than the 16-h sample, which is the opposite from the expectation if r reduced during the first 16 h. This result suggests that within 16 hours r did not decrease significantly, and that the estimates of W_0 are reliable.

Generality of the results

These calculations are dependent on the rate of egg removal derived from Fig. 1 and Eq. 1. This data was gathered from two of the same colonies used for egg collections, for which it was fairly consistent, and provides the most appropriate estimate for this study. However, similar data from Ratnieks and Visscher (1989) show a slower rate of removal (more eggs were presented at once by Ratnieks and Visscher, and it seems reasonable that this might lead to a slower removal rate). On the other hand, observations by Ratnieks (1993, 1995) suggest a greater rate of removal than observed here. So, the r value associated with worker policing may be subject to a variety of colony-specific conditions.

Subfamily biases in the likelihood of laying eggs, reported in queenless colonies with a small number of subfamilies by Robinson et al. (1990), would cause under- or overestimates in calculating W^*, but these would be expected to cancel on average, especially with naturally mated queens, whose colonies contain a large number (10–20) of subfamilies. There was no evidence of heterogeneity in the proportion of eggs laid by workers between the colonies in this study.

It could be that worker laying and worker policing vary seasonally in honey bees (see references in Ratnieks 1993). The evolutionary payoffs of these strategies may vary, with possible seasonal differences in the reproductive value of males, and costs to the colony of any diversion of worker effort to reproduction. The data from Ratnieks (1993) do not suggest a pattern of different levels of worker laying during the swarming season. There was active rearing of males in all colonies during this study, though it did not include the swarming season.

In sum, this study provides the only quantitative estimate of the proportion of worker laying in honey bees. This proportion should not be taken as exact; it is an extrapolation using the most appropriate data obtainable. Seasonal variation may occur, but the available evidence does not suggest that it does. There are several sources of variability that may affect these estimates, but they are not systematically biased upward or downward. Data from both assays suggest similar levels of worker laying. With some caution, the levels observed here should be assumed to be general for European honey bees, but further studies are needed to clarify this, and it would also be of interest to compare other species and subspecies of honey bees, which may differ in the level of worker laying.

Worker laying

A much larger proportion of the male eggs laid in a colony derive from workers than has heretofore been appreciated. Until recently, workers in queenright colonies were considered completely sterile, or nearly so (Butler 1975; Winston 1987; Bourke 1988). The levels of worker offspring found among adult drones by Visscher (1989) were so small that mutation rate in the marker system and rare genetic anomalies had to be tested as confounding factors. (Van der Blom 1991 dismissed these as possible paternate males, from fertilized eggs where the maternal nucleus died, overlooking that this possibility was tested and rejected by Visscher 1989). The data here, in contrast, show that the presence of worker-laid eggs in colonies is not a rarity, but apparently a common event.

Most inspections resulted in no eggs, but when eggs were present there were nearly always many. On only one occasion was a single egg present in the comb at inspection, and it was worker-laid. This may suggest that worker laying ordinarily takes place in the combs in the nest in which the queen is actively laying. Workers may increase the chance of their eggs escaping detection by such behavior. It may also be that policing is much quicker on isolated eggs, so they were removed before I could detect them. Ratnieks (1993) found apparently worker-laid eggs near brood where the queen could not lay, but he also showed that workers were much less likely to lay outside of the brood nest.

Worker oviposition clearly is done by a small minority of the workers in a honey bee colony. I found no workers bearing fully developed ova among more than 3000 dissected, and very few with any degree of ovary development at all. Ratnieks (1993) reported similar results, though with a sample of $\approx 10,000$ workers he did find two workers with well-developed eggs in their ovaries. Consideration of vitellogenesis (Engels 1973) and worker ovary development (Velthuis 1970) suggest that a worker would produce a maximum of 5 to 10 eggs per day (Pereplova 1928 estimated greater rates). If a colony of 40,000 bees produces up to several hundred adult queen-derived males per day during the summer (Visscher 1989), and worker oviposition were about 10% of that (prior to policing), then fewer than 0.01% of the workers in a colony could account for all worker oviposition.

These results confirm that the eggs found by Ratnieks (1993) most likely were from normal worker laying, not experimental artifacts or moved queen-laid eggs. They suggest that the adult males found in queen-right colonies by Page and Erickson (1988) and Visscher (1989) were the small number which escaped policing on a larger number laid, rather than an aberration in receiving signals of queen presence, or failure of ovary suppression mechanisms (Visscher 1989).

Worker policing

In addition to worker laying, the results of this study (Table 2) give a clear picture of worker policing taking place actively in normal, unmanipulated bee colonies. There is a dramatic difference in the frequency of worker-laid eggs among eggs up to 2 h after laying and those up to 16 h after laying, because a larger proportion of the latter had been exposed to a long period of selective policing. At the end of development, this proportion is even smaller, about 0.1% (Visscher 1989). Though other models would also be possible, this attrition fits a parsimonious model of constant-rate exponential decay in the early egg period, but it is apparent that it is not constant throughout the egg period.

Conflict and cooperation

Honey bee colonies and other advanced social insect colonies have been characterized as "superorganisms" in which reproduction is channeled exclusively through the queen, and selection on workers is restricted to maximizing their efficiency as parts of coordinated whole (Seeley 1989). While honey bee reproductives, both female and male, do derive almost exclusively from the queen, this study clarifies several considerations of this condition which bear directly on questions of the evolution of cooperative behavior. The value of a son to a worker is large relative to the component of fitness she gains through helping rear the queens offspring (West Eberhard 1981; Visscher 1989). A number of explanations have been put forward to explain why worker reproduction is not common. The queen might directly suppress worker reproduction, and queen pheromones have often been interpreted in this way (Winston 1987) but the details of response to queen pheromone suggest this is not its current function in honey bee colonies (Seeley 1985; Keller and

Nonacs 1993). Workers might forgo personal reproduction, with its attendant cost to group efficiency, in order to maximize their inclusive fitness (Seeley 1985). The manipulation by other workers, through policing, of the payoffs of individuals attempts at reproduction make them nearly unproductive (Ratnieks and Visscher 1989; Visscher 1989), and might shift the balance between efficiency costs and benefits of direct reproduction to favor worker sterility. The forces hypothesized above probably are important in keeping many workers sterile, but it is now clear that there is a continuing high frequency of active attempted reproduction by workers even when a queen is present, and direct active conflict in the form of egg policing between workers.

One of the outstanding problems that remains in evaluating the questions discussed here is the quantification of the costs of various behavioral alternatives. It may be that the cost of worker laying is very low, since it may not substantially diminish a worker's contribution to the colony labor. On the other hand, perhaps laying workers do considerably less other work, and ovary development may have nutritional costs as well. Worker policing may have costs in terms of mistakes resulting in destruction of queen-laid eggs, or in efficiency of brood rearing (Ratnieks and Reeve 1992). The aggression received by workers with developing ovaries from their sisters (Visscher and Dukas 1995) may or may not impose high costs. In general, quantifying costs of individual behaviors within insect societies has received less attention than has benefits, and may be more difficult, but such studies are required to complete our understanding in these areas.

The results of this study reemphasize how, in contemplating the evolution of highly cooperative social behavior, attention must be directed to several levels: DNA, genes, cells, organisms, and colonies. Conflict between subunits at a lower level of biological organization probably is one of the chief constraints on the evolution of higher levels. Even in species such as honey bees where group-level coordination is highly developed, individual genetic interests continue to play a role in shaping interactions, even at a possible cost to group efficiency.

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Appendix: derivation of variance of $W_{T}*$

The estimate of $W_{T}*$, the corrected worker-laid proportion of eggs, accounting for Q-type worker-laid eggs, and its standard deviation, are derived below. The estimate and SD for initial worker laying (W_0) were obtained by dividing $W_{T}*$ and its SD by the estimated

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probability of escaping policing for the time interval during which each treatment was exposed, calculated as described in the methods.

$$W_{T}* = \frac{\sum\limits_{i=1}^{k} \frac{p_{E}}{p_{Ai}} N_{obs\,i}}{\sum\limits_{i=1}^{k} N_{obs\,i}} \qquad (2)$$

To estimate the variance of W_T*, let $R = P_{Ei}/P_{Ai}$, and expand R in a bivariate Taylor series

$$\frac{\partial R}{\partial P_E} = \frac{1}{P_A} \quad \frac{\partial^2 R}{\partial P_E{}^2} = 0 \quad \frac{\partial R}{\partial P_A} = \frac{P_E}{P_A{}^2}$$

$$\frac{\partial^2 R}{\partial P_A{}^2} = \frac{2P_E}{P_A{}^3} \quad \frac{\partial^2 R}{\partial P_E \partial P_A} = \frac{1}{P_A{}^2}$$

$$R = \frac{\pi_1}{\pi_2} + \frac{P_E - \pi_1}{1!}\frac{1}{\pi^2} - \frac{P_A - \pi^2}{1!}\frac{\pi_1}{\pi_2^2} + \frac{(P_E - \pi_1)^2}{2!} - 0$$

$$+ \frac{(P_A - \pi_2)^2}{2!}\frac{2\pi_1}{\pi 2^3} - (P_E - \pi_1)(P_A - \pi_2)\frac{1}{\pi_2^2}$$

Using only a first-order approximation:

$$Var(R) = E\left((P_E - \pi_1)\frac{1}{\pi_2} - (P_A - \pi_2)\frac{\pi_1}{\pi_2^2}\right)^2$$

$$= \left((P_E - \pi_1)^2 \frac{1}{\pi_2^2} - (P_A - \pi_2)^2 \frac{\pi_1^2}{\pi_2^4} - 2(P_E - \pi_1)(P_A - \pi_2 \frac{\pi^1}{\pi_2^2}\right)$$

$$= Var(P_E)\frac{1}{\pi_2^2} + Var(P_A)\frac{\pi_1^2}{\pi_2^4} + 0$$

and, since $Var(P) = \pi(1 - \pi)/n$, from the binomial distribution:

$$= \frac{\pi_2^2}{\pi_2^2}\left(\frac{Var(P_E)}{\pi_1^2} + \frac{Var(P_A)}{\pi_2^2}\right) = \frac{\pi_1^2}{\pi_2^2}\left(\frac{\pi_1(1-\pi_1)}{n_1\,\pi_1^2} + \frac{\pi_2(1-\pi_2)}{n_2\,\pi_2^2}\right)$$

Finally, from Eq. 2 above:

$$Var(W_T*) \approx \frac{\sum\limits_{i=1}^{k} n_{1i}^2 \left(\frac{\pi_{1i}}{\pi_{2i}}\right)^2 \left(\frac{(1-\pi_{1i})}{n_{1i}\,\pi_{1i}} + \frac{(1-\pi_{2i})}{n_{2i}\,\pi_{2i}}\right)}{\left(\sum\limits_{i=1}^{k} n_i\right)^2}$$

which is estimated with observed Ps in place of the population πs in the above expression. The standard deviation is the square root of this variance.

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