

Worker policing in the honeybee

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In most species of social Hymenoptera with queen-worker dimorphism, workers cannot mate but retain functional ovaries¹; because males arise from unfertilized haploid eggs, workers can potentially produce males. Worker-derived males are frequent in some species, but in others occur only in queenless colonies^{2,3}. Workers are more related to their own sons (coefficient of 0.5) than to the queen's sons (their brothers; 0.25); they are also more related to nephews (0.375) than brothers if queens mate with one male, but if queens mate with more than two unrelated males a worker's mean relatedness to nephews is less than to brothers³⁻⁵. In this case workers could benefit by 'worker policing'^{3,5}: preventing each other from producing males, perhaps by destroying worker-laid eggs or by aggression toward reproductive workers. Worker reproduction is rare in queenright colonies of species with multiply mated queens (such as honeybees⁶ and some yellowjacket wasps⁷), but is common in some monandrous species (bumblebees and stingless bees³). Here we describe experiments showing strong discrimination by honeybee (*Apis mellifera*) workers against worker-laid male eggs, supporting the worker-policing hypothesis. The honeybee was studied because queens mate with 10–20 males⁸, making worker policing seem likely as a cause for the rarity of worker-derived males (about one in a thousand males is worker-derived⁶).

We introduced queen- and worker-laid (from queenless colonies) male eggs to queenright colonies, and compared the numbers removed over 24 h (2×2 χ^2 test for independence of egg source and removal). Colonies used had naturally mated queens, were of mixed European races, and were rearing drones. In experiments 1 and 2, colonies were divided with the queenless part above the queenright part, separated by two screens 1 cm apart, to minimize colony odour differences but prevent exchange of bees, food, or queen pheromones. The two parts had entrances facing opposite directions, and were moved at the time of division to a new apiary, so that bees would orient to their own part. Once workers in the queenless part began to lay, male eggs were obtained by introducing combs of drone cells to each part. (Queens were caged on these combs using queen excluder mesh, which allows workers but not the larger queen to pass through; these queen-laid eggs were apparently all male, see Table 1). After 24 hours, combs were removed and, using special forceps^{9,10}, queen-laid eggs were removed and replaced with either another queen-laid egg from the same comb,

or, in alternate adjacent rows, with a worker-laid egg from the queenless part. The comb with transferred eggs was then put into the brood area of a queenright colony, but separated from the queen by queen-excluder mesh to prevent the queen removing or depositing eggs, and scored for egg removal 24 hours later.

Experiment 1 compared removal of mother-queen-laid and sister-worker-laid eggs (that is, eggs were reintroduced to the queenright part of their source colony). Workers accurately discriminated between these classes of eggs (Table 1): only 4 of 204 worker-laid eggs (2%), but 150 of 237 queen-laid eggs (61%) remained ($P < 0.0001$) after 24 hours.

This discrimination against worker-laid eggs could result from assessment of relatedness to eggs, preference for queen-laid over worker-laid eggs, or differences in colony odor despite the double screen. To differentiate between these, experiment 2 compared the treatment of eggs laid by the queen and by her worker daughters, all unrelated to discriminator bees (that is eggs were introduced to an unrelated colony). The results were as in experiment 1, with 59% of queen-laid and <1% of worker-laid eggs remaining (Table 1.2), indicating that the caste of the egg's mother was responsible for differential removal. In trial 4 of experiment 2, inspections were made more frequently. Removal of worker-laid eggs was rapid, with half removed in 2 hours and 90% in 6 hours (Fig. 1).

In experiment 3, an additional 11 colonies were tested using similar methods, except that queenless colonies were not kept above queenright colonies, queen- and worker-laid eggs used in a trial were not always from the same source colony, and not all eggs were transferred to a cell which previously held a queen-laid male egg. All colonies showed significantly greater removal of worker-laid eggs, except one which removed both worker- and queen-laid eggs (Table 1.3).

If worker eggs were not viable, this could explain the differential removal. However, queenless honey bee colonies often rear many males¹¹ so at least a proportion of worker-laid eggs must be viable. We compared the *in vitro* viability of queen- and worker-laid eggs by transferring 0–24-hour-old eggs of each type into a beeswax-lined Petri dish also containing a water-saturated piece of cotton, incubating them at 35 °C for four days, and comparing the numbers hatching. In five trials, 81 of 207 worker-laid eggs (39%) and 86 of 215 queen-laid eggs (40%) hatched ($P = 0.90$). (These viabilities are probably abnormally low because of dehydration or damage during transfer and incubation.). Once hatched, there seems to be no discrimination between queen- and worker-derived larvae. We transferred one-day-old larvae from their natal combs, following the methods of experiment 2 (using a 'grafting' tool as in commercial queen-rearing¹²). In one trial 47 of 57 queen derived larvae (82%), and 24 of 33 worker derived larvae (73%) remained after 24 hours ($P = 0.70$).

These results provide strong support for the worker-policing

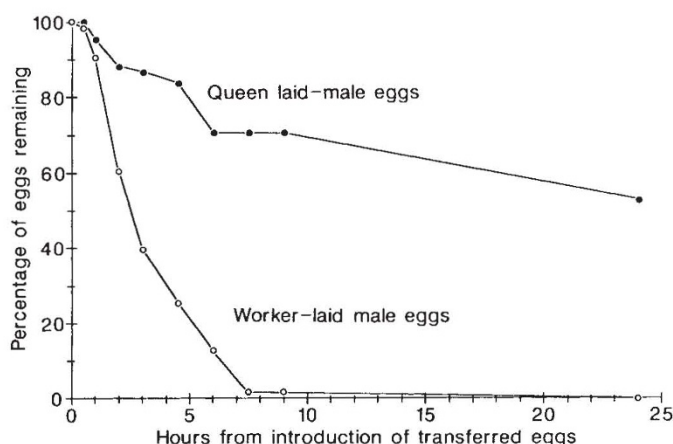


FIG. 1 Time course of egg removal for worker- and queen-laid male eggs within the brood area of a populous queenright honeybee colony. (Data from trial 4, experiment 2.)

TABLE 1 Worker policing of eggs in honey bee colonies.

Date	Source Colony		Rearing colony	Queen-laid		Worker-laid		P
	W	Q		N ₁ /N ₀	%	N ₁ /N ₀	%	
Expt 1: eggs reintroduced to queenright part of colony from which eggs came								
28 March 1989	A	A	A	5/32	16%	0/33	0%	0.028
*31 March 1989	B	B	B	56/89	63%	0/60	0%	<0.0001
31 March 1989	C	C	C	23/34	68%	1/34	3%	<0.0001
1 April 1989	A	A	A	31/40	78%	3/44	7%	<0.0001
3 April 1989	B	B	B	35/42	83%	0/33	0%	<0.0001
Expt 2: eggs introduced to queenright colony unrelated to egg-source colony								
*23 March 1989	C	C	D	30/96	31%	1/88	1%	<0.0001
1 April 1989	A	A	C	39/41	95%	0/31	0%	<0.0001
6 April 1989	B	B	D	47/51	92%	0/44	0%	<0.0001
7 April 1989	B	B	C	36/68	53%	0/63	0%	<0.0001
Expt 3: worker-laid eggs and queen-laid eggs from separated colonies								
*21 June 1989	E	F	L	50/59	85%	4/45	9%	<0.0001
*21 June 1989	E	F	M	29/58	50%	0/48	0%	<0.0001
*21 June 1989	E	F	N	3/46	7%	0/52	0%	0.07 NS
23 June 1989	G	G	O	8/46	17%	0/48	0%	0.006
24 June 1989	G	G	G	4/46	9%	0/48	0%	0.045
24 June 1989	G	G	F	16/46	35%	0/46	0%	0.0002
*26 June 1989	H	F	G	25/42	60%	0/43	0%	<0.0001
*26 June 1989	G	G	P	25/58	43%	0/48	0%	<0.0001
*26 June 1989	G	G	R	7/58	12%	0/60	0%	0.0089
*5 June 1988	H	I	S	19/66	29%	0/67	0%	<0.0001
*5 June 1988	J	K	T	29/69	42%	0/71	0%	<0.0001
*5 June 1988	H	I	U	42/65	65%	0/119	0%	<0.0001
*5 June 1988	J	K	U	31/128	24%	0/72	0%	<0.0001
*1 June 1988	J	I	T	59/104	57%	0/73	0%	<0.0001
*1 June 1988	J	I	U	12/77	16%	0/70	0%	0.0014
TOTAL				661/1461	45%	9/1340	1%	

All trials resulted in significantly ($P < 0.05$) higher removal of worker-laid male eggs than queen-laid male eggs in queenright honey bee colonies, except colony N, in which both classes were removed. Letters A-U (excluding Q) designate different, unrelated honey bee colonies. For each type, N₁ is the number of eggs remaining after 24 hours, and N₀ is the number of eggs introduced. P is the alpha level of a chi-squared test for independence of treatment by the rearing colony and caste of the eggs' mothers. Trials marked * refer to cases in which brood were observed to pupation. In all such cases queen-laid eggs gave rise only to males. Experiments were conducted in Florida (March/April) and New York (June).

hypothesis. Effective removal of worker-laid male eggs occurred in all colonies. In experiments 1 to 3 only 0.7% worker-laid versus 45.2% queen-laid male eggs remained after 24 hours. Since the egg stage lasts three days, actual removal of worker-laid eggs could be still greater.

The results also allow inferences concerning the cue used in discrimination. Worker-laid eggs, but not worker-derived male larvae, are strongly discriminated against, suggesting that cues are found on the egg itself or possibly on the inner surface of the cell. The latter idea is rejected because worker-laid eggs transferred into cells previously containing queen-laid male eggs were removed (experiments 1 and 2). Because differential removal of worker-laid male eggs is not due to relatedness differences (experiments 2 and 3) the idea that there is a queen-specific egg-marketing pheromone by which workers can determine maternal origin of the egg^{3,13} is supported. Such a pheromone could evolve because both the signal¹⁴-producing queen and the signal-receiving police workers would benefit by

increased relatedness to the colony's males³. (A mechanism would also be required to detect and prevent workers cheating by producing the pheromone.) Ultimately, worker policing is based on relatedness-based discrimination, but cues or signals of maternal origin, not relatedness differences, are apparently used proximally.

Worker policing may be important in the evolution of highly cooperative worker behavior. Intra-colony conflict is inherent in insect societies because of genetic differences between colony members^{4,13,15}. However, effective worker policing causes male-production to reach a state of forced cooperation, with workers effectively sterile⁶, and the queen the sole reproductive by default³. By denying individual workers the alternative of 'selfish' reproduction, in which they could increase their inclusive fitness by manipulating colony resources, worker policing aligns the genetic interests of all workers and the queen, enhancing selection for workers to increase their inclusive fitness by increasing colony resources through cooperation. □

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