

Why do honey bee workers destroy each other's eggs?

Hamilton's theory of inclusive fitness predicts that in polyandrous colonies of social Hymenoptera, workers should prevent each other from reproducing and prefer to rear the queen's sons rather than their nephews. This is because, in polyandrous colonies, unlike in monandrous colonies, workers are expected to be more closely related to their brothers than to their nephews. Honey bees clearly fulfill this expectation – queens mate multiply, worker reproduction is rare and the few eggs laid by workers are usually destroyed by other workers. Such mutual destruction of each other's eggs by honey bee workers has been called worker policing and has achieved the status of a classic example of kin selection. However, recent evidence indicating that worker-laid eggs may be much less viable than queen-laid eggs opens up the possibility that worker policing may simply be a matter of destroying dead eggs and rearing live ones. If confirmed, this raises serious doubts about the widely accepted relatedness based argument for the evolution of worker policing. A major weakness of the kin selection-based explanation for worker policing is our ignorance of the proximate cues used by worker bees to distinguish between brother eggs and nephew eggs.

1. The argument

The two seminal papers by W D Hamilton which promulgated the concept that organisms maximize their inclusive fitness (Hamilton 1964a) and then explored its consequences for the haplodiploid (male haploid, female diploid) social Hymenoptera (Hamilton 1964b), have proved to be an inexhaustible source of new ideas for behavioural ecologists. One such idea, that follows from Hamilton's papers, although not explicitly mentioned in them, has now come to be known as worker policing. It was Starr (1984) who first pointed out explicitly that in large, polyandrous colonies of social Hymenoptera, such as those of the honey bee, workers would be expected to interfere with (police) each other's reproduction. The argument is a simple one and pertains only to conflict over male production; the focus is on species where workers have lost the ability to mate and produce diploid female offspring. If the queen mates with a single male, worker's would be more closely related to and hence would prefer their nephews (coefficient of genetic relatedness, $r = 0.375$) over their brothers ($r = 0.25$). If the queen mates with more than two males, workers would be more closely related to and hence would prefer brothers ($r = 0.25$) over nephews ($r < 0.25$). Although each worker's first preference would be her own sons ($r = 0.5$), workers cannot agree as to which one of them should lay male-destined eggs. Given a choice between eggs laid by another worker ($r < 0.25$) and the queen's haploid eggs ($r = 0.25$), a worker in a polyandrous colony should prefer to rear the queen's haploid eggs and destroy worker laid eggs.

Seeley (1985) and Woyciechowski (1985) made similar verbal arguments. Woyciechowski and Lomnicki (1987) were the first to confirm this verbal argument, using population genetic models, and they did so before the idea was made famous under the banner of worker policing. Their models considered two alleles at a single locus, one of which makes the workers rear brothers and the other makes them rear nephews. When the queen was assumed to mate singly, the allele that makes workers rear brothers decreased in frequency. On the other hand, when the queen was assumed to mate with three males, the allele which makes the workers rear brothers increased in frequency. The verbal, relatedness argument also suggests that multiple mating by queens can be adaptive because it seems to permit the queens to follow a strategy of 'divide and rule' – create discord among the workers and make them disagree as to which one of them should lay male eggs (Starr 1984). Woyciechowski and Lomnicki (1987) also tested this idea but found that it does not work in its simple form. Their model had two loci, one which governs whether the queens mate singly or multiply and the other which

governs whether workers rear brothers or nephews. The allele for multiple mating did not spread in spite of the advantage of workers potentially responding to it by preferring brothers over nephews. This counterintuitive result appears to be due to a quirk of the haplodiploid genetics of the Hymenoptera, which makes linkage disequilibrium higher among males compared to females. However, instead of simply considering two alleles, one which makes workers prefer brothers and another which makes them prefer nephews, irrespective of whether the queen is mated singly or multiply, one can imagine an allele that makes workers follow a conditional strategy – prefer nephews if the queen is singly mated and prefer brothers if the queen is multiply mated. With a third model that considers such a scenario, Woyciechowski and Lomnicki (1987) demonstrated a strong selection for multiple mating by queens. Thus, a divide-and-rule strategy is possible, but only if workers follow a conditional strategy, linked to the mating habits of the queen. An ability on the part of workers to detect whether the queen is singly or multiply mated implies a higher sensory ability. Woyciechowski and Lomnicki (1987) dramatically conclude that ‘the higher sensory abilities of workers bring about their sterility’.

Ratnieks (1988) introduced the phrase ‘worker policing’ and re-confirmed the verbal arguments of Starr (1984), Seeley (1985) and Woyciechowski (1985) and the modelling results of Woyciechowski and Lomnicki (1987), using another population genetic model. This model explored the conditions under which a worker ‘police allele’, that somehow causes a worker to favour the production of queen-produced males over worker-produced males, can spread in a population lacking the worker police allele and can then resist invasion by non police alleles.

If worker policing was selected because of such a relatedness argument, it follows that worker reproduction should be more common in monoandrous species as compared to polyandrous ones, and/or workers in polyandrous species should be seen to interfere with each other’s reproduction, by eating each other’s eggs, by being aggressive towards workers with developed ovaries or by some other means.

2. The evidence

While these models were being developed, it was already known that honeybees (which are highly polyandrous) confirm one prediction of the relatedness argument for the evolution of worker policing – that worker reproduction should be rare in polyandrous colonies than in monandrous ones. However, it was not clear if workers were also engaged in thwarting each other’s egg laying attempts. Visscher (1989) examined the extent of worker reproduction using a morphological marker and found that worker produced drones accounted only for 0.12% of all males produced in colonies with normal, healthy queens (queen-right colonies).

More direct evidence for worker policing came from a study by Ratnieks and Visscher (1989) who transferred queen- and worker-laid male eggs into queen-right colonies and compared their survival. In one experiment where queen- and worker-laid eggs were offered to workers of the same colony, only 2% of worker-laid eggs but 61% of queen-laid eggs remained after 24 h. The missing eggs were assumed to have been removed or eaten by the workers and not by the queens because the queen was excluded from the region where the eggs were presented. In a second experiment queen- and worker-laid eggs from one colony were offered to workers of a different queen-right colony. Even here less than 1% of worker-laid eggs but 59% of queen-laid eggs survived. While both experiments provide evidence for worker policing, the second experiment also makes an entirely new point. Policing of worker-laid eggs appears to have evolved as a constitutive trait, always expressed in honey bee colonies because honey bee queens are always polyandrous. Thus, workers do not appear to discriminate between queen- and worker-laid eggs by any direct assessment of their relatedness to the two classes of eggs. Both queen- and worker-laid eggs from the source colony were unrelated to them and yet they preferentially destroyed worker-laid and not queen-laid eggs. This is consistent with the idea that while the ultimate (evolutionary) reason why workers destroy worker-laid eggs and prefer queen-laid eggs may be their differential relatedness to the eggs, the proximate cue they use to do so may simply be olfactory or other cues provided by queen-laid eggs versus worker-laid eggs (Ratnieks and Visscher 1989). This is possible if queens mark their eggs by a queen pheromone which workers cannot mimic (Ratnieks 1988). Within a kin selection framework, such a mechanism would be expected to serve the

workers well in normal colonies where they can expect that queen laid eggs are more closely related to them compared to worker-laid eggs, unlike in experiment two of Ratnieks and Visscher (1989) where both queen- and worker-laid eggs were unrelated to them. There is some inconclusive evidence for a queen-produced, egg-marking pheromone: worker-laid eggs survived better when they were treated with an ethanol extract of the queens' Dufours gland while queen-laid eggs survived poorly when treated with polar solvents such as methanol and ethanol (Ratnieks 1995). Katzav-Gozansky *et al* (2001, 2002) dispute this claim. They show that worker-laid eggs are not protected even when treated with extracts from the queen's Dufour's glands and present evidence to suggest that these secretions are part of the queen signal but do not constitute an egg marking pheromone. Ignorance about the nature of the proximate cues that workers might use to differentiate between eggs that are to be destroyed and those that are to be reared, remains the Achilles heel of the entire worker policing story. Despite this ignorance, worker policing has continued to be investigated with much enthusiasm.

That workers lay eggs at a low rate and that most of these eggs are eaten, was confirmed using a more natural situation by Ratnieks (1993) without having to transfer eggs from one comb to another. Workers were shown to lay about one egg per 16,000 drone cells, 85% of which were removed within a day and only 2% of which hatched into larvae. Visscher (1996) has identified queen- and worker-laid eggs using isozyme markers and estimated that some 7% of the haploid eggs laid are worker-derived although most of them disappear within a few hours of laying. Evidence for a genetic basis of worker policing has come from two spontaneously occurring mutant colonies which produce large numbers of drone pupae above the queen extruder, i.e. where the queen cannot lay eggs (queen extruder is a wire-mesh screen that permits the workers but not the queen to pass through, which bee keepers use to exclude the queen from some portion of the hive so that they can harvest brood-free honey from there) (Oldroyd *et al* 1994). These two colonies and their derivatives have since been dubbed 'anarchistic' and have provided invaluable biological material for new genetic, molecular and behavioural studies of the phenomenon of worker policing. Polymorphic microsatellite markers confirmed that drone pupae appearing above the queen extruder in both the anarchistic colonies were worker produced, allaying any fears that workers may have carried queen-laid eggs across the queen extruder. But the more interesting result was that in both colonies, nearly all such worker produced drone pupae belonged to a single patriline, although the colonies had 12 and 20 different patrilines of workers, respectively (Oldroyd *et al* 1994; Montague and Oldroyd 1998). The anarchistic phenotype is now interpreted in terms of the ability of the workers to cheat, evade worker policing, presumably by counter-feiting the queen's egg-marking pheromone (whatever that is), and thus produce drones at rates far exceeding what wild type workers are capable (Oldroyd *et al* 1994; Montague and Oldroyd 1998; Oldroyd and Osborne 1999; Oldroyd and Ratnieks 2000).

The Cape honey bee (*Apis mellifera capensis*) is a remarkable subspecies found in the Cape region of South Africa. This is the only known honey bee where unmated workers produce diploid female offspring by a process known as thelytoky. In such a situation one would not expect worker policing to be favoured because a worker would be equally related to the queen's female offspring and to other workers' thelytokously produced female offspring ($r = 0.25$ in either case; Greeff 1996). Moritz *et al* (1999) have obtained genetic evidence that supports this theoretical expectation. Since the proximate cues used by the police workers in honey bees are not directly linked to their relatedness and must be linked instead to some queen or worker-related signal on the eggs, it must be assumed that laying workers in the Cape bee, like the laying workers in the anarchistic colonies, are capable of counter-feiting the queen-egg cue.

3. The doubts

Having now also been reported in ponerine ants (Monnin and Ratnieks 2001) and vespine wasps (Foster *et al* 2002; Foster and Ratnieks 2000), worker policing has attracted much attention as a clear-cut and experimentally tractable example of kin selection. However, our ignorance of the proximate cues used by the bees to detect queen-laid and worker-laid eggs is now catching up with us. A few years ago, I attended a symposium in the Netherlands, in honour of Hayo Velthuis who retired as Professor of Behavioural Biology from the University of Utrecht. The final talk was by Velthuis and

among the various things he talked about some work he had been doing on worker policing in honey bees. To my surprise he did not seem to believe in the idea of worker policing as we all knew it. Velthuis presented some data which suggested that worker-laid eggs were relatively less viable than queen-laid eggs (Velthuis *et al* 2002) and he argued that this might be the reason why worker-laid eggs are selectively destroyed. If that is correct, he went on to suggest that the egg eating workers should be labelled as janitors rather than as police! Ratnieks and Visscher (1989) had looked for but failed to find any significant differences in viability between queen- and worker-laid eggs under *in vitro* conditions. The question of differential viability has now been reexamined by Pirk *et al* (2004). The strengths of this latest study are that they are performed *in vivo* and that egg viability and egg removal are studied under the same conditions, using eggs from the same source colonies and using the same test colonies. Pirk *et al* (2004) report both a significantly lower viability and a significantly faster rate of disappearance of worker-laid eggs as compared to queen-laid eggs. They conclude that ‘to achieve an efficient removal of worker-laid eggs in a honey bee colony, it appears to be sufficient for a honey bee worker to simply discriminate between dead and live eggs’. The report by Pirk *et al* (2004) has elicited the comment that ‘a more parsimonious explanation for worker policing could be that the workers are simply discriminating between live and dead eggs’ (Sugden 2004)‘.

What neither Pirk *et al* (2004) nor Sugden (2004) explicitly emphasize is that if worker-laid eggs are being destroyed simply because they are less viable, the relatedness argument for the evolution of worker policing itself is called into question. In other words, the results of Pirk *et al* (2004), if confirmed, will not merely provide a more parsimonious proximate explanation for worker policing but will cast doubt on the widely accepted ultimate (evolutionary) explanation for worker policing. The relatedness argument for the evolution of worker policing predicts that worker-laid haploid eggs should be destroyed when the queen is multiply mated but not when the queen is singly mated. It seems very unlikely that the viability of worker-laid eggs will depend on whether the queen is singly or multiply mated. We must therefore conclude that if worker policing is maintained due to the relatedness-based inclusive fitness advantage and is operative in different taxa (polyandrous as well as monandrous), lower viability of worker-laid eggs is an inappropriate proximate cue. Conversely, if lower viability of worker-laid eggs is in fact the proximate cue used for policing, then relatedness based inclusive fitness advantage is an inappropriate explanation for the evolution of worker policing. A potential problem with the Pirk *et al* (2004) experimental design is that they measured viability after 96 h but worker policing (egg disappearance) after 2–4 h. Besides, we do not know whether dead, queen- and worker-laid eggs will be distinguished. Clearly, we need more studies that address the question of the proximate cues used by honey bee workers to police worker-laid eggs. More generally, this reiterates the point that, while formulating and testing hypotheses about the ultimate evolutionary forces that might have selected for a certain behaviour, we need to pay explicit attention to the proximate cues animals might use to achieve the fitness we postulate them to do (Gadagkar 1997).

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Note added in proof:

The relatedness-based explanation for worker policing has now received another, even greater challenge. In a comparative analysis of 50 species of social insects including 16 ants, 20 bees and 14 wasps that takes into account phylogenetic inertia, Hammond and Keller find no influence of intra-colony genetic relatedness on inter- and intra-specific variation in the proportion of worker-produced males, as opposed to queen-produced males. [Hammond R L and Keller L 2004 Conflict over male parentage in social insects; *PLoS Biol.* **2**(9), e248]

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