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Nepotism and brood reliability in the suppression of worker reproduction in the eusocial Hymenoptera

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In many eusocial Hymenoptera, workers prevent each other from producing male offspring by destroying worker-laid eggs. Kin selection theory predicts that such 'worker policing' behaviour can evolve by increasing the average relatedness between workers and their male brood. Alternatively, if worker-laid eggs are of low relative viability, their replacement would increase the developmental reliability of the brood. Less colony investment in terms of time and resources would be lost on poor males. This gain is independent of the relatedness of the males. Unfortunately, both nepotistic and group efficiency benefits can simultaneously accrue with the replacement of worker-laid eggs. Therefore, worker behaviour towards eggs cannot completely resolve whether both processes have been equally evolutionarily important. Adequate resolution requires the presentation of worker-produced brood of various ages. The stage at which brood are replaced can discriminate whether worker policing occurs owing to a preference for closer genetic kin, a preference for the more reliable brood or both.

Keywords: group selection; inclusive fitness; kin selection; nepotism; sexual deception; worker policing

1. INTRODUCTION

Nepotistic suppression of worker reproduction in eusocial Hymenoptera is potentially one of the most convincing examples of kin selection in action (Whitfield 2002; Gadagkar 2004; Sugden 2004; Wilson & Hölldobler 2005). It is possible for workers to gain inclusive fitness by this behaviour, because haplodiploidy creates a complex pattern of genetic relatedness across the sexes. Queens are always more related to their sons ($r=0.5$) than to male offspring of any other individual. Workers genetically prefer their own sons over queens' sons (which would be brothers to the workers, with $r=0.25$) and also prefer full-sisters' male offspring ($r=0.375$) over brothers. However, a worker would not genetically favour the male offspring of half-sisters ($r=0.125$) over brothers. If queens mate multiply, most worker-produced males fall into the last category from the perspective of any given worker. This results in the average worker-produced male having a lower expected relatedness than a queen-produced brother. If workers cannot discriminate across patrilines of male origin, kin selection predicts that workers should replace other workers' male eggs with queen-produced eggs (Woyciechowski & Łomnicki 1987; Ratnieks & Visscher 1989). Therefore, workers should police each other into sterility in the presence of a fecund, multiply mated queen.

Although data across many species are consistent with worker policing (Beekman & Oldroyd 2005), nepotism may not always be evolutionarily responsible (Gadagkar 2004; Hammond & Keller 2004). If worker-laid eggs have very low viability relative to queen-laid eggs, then the replacement of dead eggs would be strongly biased towards worker eggs (Pirk *et al.* 2004). Also, eusocial colonies may be at risk of being parasitized by unrelated workers entering the nest and laying male eggs (Neumann *et al.* 2001). Therefore, workers may be removing all eggs not coming from their queen as a colony defence mechanism (Pirk *et al.* 2006). Overall, the removal of dead or foreign eggs may increase the worker inclusive fitness more through improved group-level productivity than from gains through increased relatedness to male brood. Thus, nepotism could play a secondary, and perhaps trivial, role in the evolution of worker sterility.

Beekman & Oldroyd (2005) convincingly rejected the 'dead egg' hypothesis by showing that honeybee (*Apis mellifera*) workers strongly discriminate between worker and queen eggs, but not at all between dead and live eggs (from any source). Therefore, they rejected relatedness–neutral group advantages in favour of nepotistic worker policing. However, I believe that this last conclusion is premature.

Consider that neither a queen nor a worker should deliberately lay a non-viable male egg when their intention is to produce a viable egg. Apparently, however, they often do. Thus, it is reasonable to assume that if the egg-layer is unsure about its egg's quality, the 'policing' workers also cannot definitively identify which eggs will imminently fail. If as a class, worker-produced eggs are statistically more likely to fail than queen-produced eggs; their global replacement will improve reliability of the entire system in terms of maximizing a robust brood. This assumes that the cost of individual eggs is small relative to the potential lost investment of time and resources in raising males of low future viability. Given the high fecundity of honeybee queens (the most commonly used species in worker policing studies), this assumption is likely to be met. Also, workers routinely destroy significant numbers of queen eggs (e.g. Pirk *et al.* 2004; Beekman & Oldroyd 2005). It appears that workers have a high-quality threshold for accepting eggs, which would be expected if eggs are cheap to produce.

In the Pirk *et al.* (2004) study, worker eggs had four times the failure rate of queen eggs. Such a difference in expected reliability is certainly sufficient to predict worker replacement of eggs in the absence of any gain in the average relatedness to brood. Workers should immediately replace eggs perceived as not coming from the queen even if this means occasionally viable worker eggs are replaced by non-viable queen eggs. Therefore, a group-level benefit is not inextricably linked to the ability to differentiate dead from living eggs, as inferred by both Pirk *et al.* (2004) and Beekman & Oldroyd (2005).

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Nevertheless, it is just as important to realize that any removal of worker-laid eggs (be it for increasing brood reliability or defence against unrelated parasites) will increase an average worker to male relatedness. Thus, nepotistic and group efficiency benefits can accrue simultaneously with the replacement of any non-queen-laid eggs. They are not alternative hypotheses, but instead, complimentary ones. The experiments of Pirk *et al.* (2004, 2006) cannot reject nepotism as a significant evolutionary force, and the experiments of Beekman & Oldroyd (2005) do not rule out substantial non-nepotistic, colony-level benefits.

The question of whether evolution occurs through kin-directed or whole group-directed benefits is not a dichotomous choice and both are often simultaneously manifest (Lehmann & Keller 2006). Even so, a spirited debate has arisen as to the degree that social behaviour is structured by kin nepotism or group advantage. This debate rages at both the general level as to how eusociality itself evolves (Wilson & Hölldobler 2005; Foster *et al.* 2006) and the specific level of whether worker policing behaviour is consistent with colony relatedness patterns across species (Hammond & Keller 2004; Wenseleers & Ratnieks in press). Indeed, conflict over male parentage is one feature of social life, where it may be possible to compare the relative importance of each factor. Unfortunately, this question may not be adequately addressed by worker responses to eggs owing to their low relative cost of replacement. However, as the brood matures and grows, the benefits of nepotism and reliability change in predictable ways that can lead to differing predictions. I will illustrate with a potential example based on the biology of honeybees.

2. MODEL AND RESULTS

Worker-laid eggs are removed quickly and efficiently in honeybees (Visscher 1989). However, a small percentage of worker-produced males escape policing and mature as reproductive drones. Therefore, there is the possibility that if a worker-produced male can survive long enough, it is no longer cost effective for workers to kill and replace it with a queen-laid egg. This is similar to sexual deception, where worker-laid males hide among higher relatedness females until past the point in development, where kin selection predicts replacement (Nonacs & Carlin 1990; Nonacs 1993). To a worker, the inclusive fitness value of a queen-laid male egg is $r_q b_q / x_q$. The robustness of the male (b) and its cost of production (x) can be set to unity for the comparison purposes. Thus, the inclusive fitness of a queen egg is equal to the worker–brother relatedness value of 0.25. The inclusive fitness value of a worker-produced male is $r_w b_w / x_w$, and there are three ways by which this value can exceed 0.25. The first way is that relatedness can be higher, which would be true for sons and males from full sisters. However, the average relatedness of a worker-produced male would be a value weighted across the patrilines (assuming a random sperm use and a trivial contribution of one's own sons by any single worker). The mean would be: $r_w = [0.375 + 0.125(n-1)]/n$, where n is the number of matings by the queen. Therefore, in any colony where queens mate more than twice, brothers will be of higher relatedness than the average worker-produced male (Nonacs 1993).

Table 1. The point in its development (given as a proportion of the total required investment) where the value of an average worker-produced male is equal to a queen-produced egg for a policing worker. (The points are a function of the number of times the queen has mated and the expected robustness of a worker-produced male relative to a queen-produced egg (b_w/b_q).)

number of mates	$b_w/b_q = 1$	$b_w/b_q = 0.90$	$b_w/b_q = 0.75$	$b_w/b_q = 0.50$
3	0.167	0.250	0.375	0.583
5	0.300	0.370	0.475	0.650
10	0.400	0.460	0.550	0.700
15	0.433	0.490	0.575	0.717
20	0.450	0.505	0.587	0.725
∞	0.500	0.550	0.625	0.750

The second way by which worker-produced males could result in higher inclusive fitness is if they were more viable than queen-produced males ($b_w > b_q$). The evidence is mixed on this point at the egg stage. In honeybees, worker eggs have been found to be less viable (Velthuis *et al.* 2002; Pirk *et al.* 2004), equally viable (Ratnieks & Visscher 1989) or more viable (Beekman & Oldroyd 2005) than queen eggs. Moreover, the longer any offspring survive, the higher their expected future survivorship becomes (i.e. b_w should increase as brood get older). At some point, the expected viability of worker-produced larvae could exceed that of queen eggs (which is what would replace them). Finally, the competitive robustness of drones is not known, although worker-produced drones in queenless colonies are smaller and have less sperm than drones from normal, queened colonies (Gencer & Firatli 2005).

The third way is for worker-produced males to be less costly to make than queen-produced males ($x_w < x_q$). This may not be true for the total investment, but if a worker-produced male escaped destruction at the egg stage, its remaining costs would be less than the costs associated with a new queen egg. Note that this cost assumes a colony that is not under a time constraint, so that if a partially developed male is replaced with a new egg, this new male still has the time to develop and enter the breeding population. If a colony faces a time constraint (e.g. an annual species or a species with a fixed mating time), then a worker-produced male may only have to survive until it is not feasible to rear new males from eggs.

We can easily calculate the point where a developing worker-produced male can no longer be replaced to gain inclusive fitness. When we assume that all males cost the same to raise from egg to adult ($x_w = x_q$), the gains through nepotistic worker policing will depend on the number of matings by the queen, the relative viability of males (b_w/b_q) and how much remains to be invested to produce a viable male. As the number of matings increases and their relative viability decreases, worker-produced males have to escape policing until later in development to make their replacement no longer nepotistically favourable (table 1).

3. CONCLUSION

To truly gauge the relative benefits of kin and group selection, the experiments of Pirk *et al.* (2004) and Beekman & Oldroyd (2005) should be replicated with a variety of brood at different stages in development. For honeybees, where queens mate 10–15 times, policing across levels of brood maturity would indicate the relative strength of each process. (i) Gains from nepotism alone predict that any worker-produced male that has not completed more than 40% of its development should be policed (table 1). Therefore, if only worker-produced eggs and the smallest of larvae are removed, this would strongly suggest that improving brood reliability is the strongest evolutionary force. Eggs that prove their reliability by hatching may be raised no matter the relatedness. (ii) If eggs and larvae are removed up to about 40–50% of total development, but larvae past this point are not, this would be consistent with the quantitative predictions of a nepotistic policing outcome. Benefits through increased brood reliability could be minor or completely absent (i.e. $b_w \approx b_q$). (iii) If worker-produced larvae are also removed well past a 50% stage in development, this would strongly suggest that both factors play important roles. For instance, if worker-produced males have half the expected viability of queen-produced eggs ($b_w/b_q = 0.5$), then replacement is favoured until more than 70% of the total needed investment has been used (table 1).

It is difficult to separate benefits that accrue across all individuals in a group from those that are biased towards closer genetic relatives, because the two are often complimentary. Furthermore, certain behavioural outcomes may superficially appear identical independent of whether they arose from nepotism, group efficiency or selfish conflict (e.g. lack of worker-produced males across many species of eusocial Hymenoptera; Hammond & Keller 2004). However, to understand social evolution and to predict its dynamics, it is important to estimate the relative strengths of the composite mechanisms (Wilson & Hölldobler 2005; Foster *et al.* 2006). If worker policing is truly the exemplar of kin-selected nepotism, it is incumbent to keep testing it adequately against the alternatives.

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