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Reproductive restraint without policing in early stages of a social insect colony

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Punishment of cheaters is a widespread means of promoting cooperation in social groups. In the eusocial Hymenoptera, punishment (i.e. 'policing') of reproductively active workers is thought to have been essential for the evolution of near-sterile worker castes. Many studies have investigated the evolutionary origin of policing behaviour in social insects, but few have considered the factors that affect the expression of policing behaviour in extant species. One factor that is predicted to affect the expression of policing behaviour is colony age. Specifically, policing is expected to be strongest at early stages of colony growth, when the cost of worker reproduction is high, and weaker at later stages. In a previous study, we found that egg-eating behaviour (i.e. 'policing', where workers eat worker-laid eggs) is not expressed in early stages of colony growth in the carpenter ant *Camponotus floridanus*, contrary to theoretical predictions. Here we test two other mechanisms of policing in incipient colonies: queen policing and destruction of male larvae. We found no evidence of either. We also found that workers from incipient colonies were capable of activating their ovaries and laying eggs. In the absence of evidence of policing in incipient *C. floridanus* colonies, we suggest that reproductive restraint by workers in early colony stages can arise because the inclusive fitness benefits of helping outweigh the direct benefits of reproducing.

Punishment of social cheaters can suppress within-group competition and promote cooperation, as has been observed in animal species as diverse as humans (Fehr & Gächter 2002; Hauert et al. 2007), fish (Raihani et al. 2010) and insects (Ratnieks & Wenseleers 2005, 2008). According to current theory, social sanctions penalizing undesired reproductives were essential for the evolution of near-sterile workers in eusocial insect colonies (Wenseleers et al. 2004a, b; Ratnieks & Wenseleers 2005, 2008; Ratnieks & Helanterä 2009). These sanctions, known as 'policing', include any behaviour that interferes with a worker nestmate's reproductive success (Ratnieks 1988). Because policing behaviour was crucial for the evolution of eusociality in ants, bees and wasps, there has been much interest in explaining the origin of policing. Two nonmutually exclusive hypotheses have been advanced: the relatedness hypothesis and the colony efficiency hypothesis. The relatedness hypothesis argues that policing evolves when workers are, on average, more closely related to the queen's sons than they are to the sons of workers, as when the colony has a queen that has mated more than twice (Ratnieks 1988; Wenseleers & Ratnieks 2006). The colony efficiency hypothesis states that worker policing evolves when worker reproduction reduces colony productivity, thus lowering workers' inclusive fitness (Cole 1986; Ratnieks 1988; Hammond & Keller 2004). In the rush to test these hypotheses concerning the evolution of policing behaviour, the factors that modulate the expression of policing behaviour in modern social insect species have been neglected (Ohtsuki & Tsuji 2009; Bonckaert et al. 2011b). Failure to consider factors that may influence policing behaviour in present-day species interferes with our ability to draw sound conclusions about the original evolution of policing.

The evolution of policing behaviour can allow for the subsequent evolution of social systems in which the importance of policing is significantly diminished. Effective policing means that worker-produced offspring are unlikely to survive. Under these conditions, reproductive restraint (i.e. 'self-policing') can spread if the increase in workers' inclusive fitness from self-policing outweighs the time and energy costs of attempted reproduction that are typically thwarted by policing by others (Ratnieks 1988). Self-policing, in turn, reduces selection for policing behaviour. Policing behaviour also promotes the evolution of larger colony sizes, which are associated with a suite of mutually reinforcing traits such as loss of reproductive potential for workers and increasing morphological skew between queens and workers (Bourke 1999). These traits lower the profitability of worker reproduction and, potentially, the importance of policing in enforcing worker sterility.

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One consequence of increased colony size is a prolonged phase of ergonomic growth in which new workers are added to the colony but no sexuals are produced (Oster & Wilson 1978). Ohtsuki & Tsuji (2009) created a model to explore how a colony's developmental stage affects the expression of policing behaviour. According to their model, it is in interest of both queens and workers to produce workers instead of sexuals during the ergonomic growth phase. Because worker offspring are males and, therefore, sexuals, workers should refrain from reproducing at this stage. Ohtsuki & Tsuji predicted that if worker reproduction occurs during ergonomic growth, it will result in a strong policing response. This prediction has been upheld in recent studies of the ant *Temnothorax unifasciatus* (Walter et al. 2011) and of the wasp *Dolichovespula saxonica* (Bonckaert et al. 2011b), where policing of reproductive workers was found to be stronger in earlier stages of colony development. However, Ohtsuki & Tsuji's insight is also compatible with an alternate evolutionary outcome that is not explicitly stated in their paper: if self-restraint is sufficient to prevent worker reproduction early in colony development, then policing behaviour may not need to be expressed in this stage. This alternative may explain the behaviour observed in the ant *Camponotus floridanus*.

Camponotus floridanus is a monogynous species in which queens are singly mated (Gadau et al. 1996). *Camponotus floridanus* workers do not lay eggs under queenright conditions, but they can be induced to begin egg laying if separated from the queen for at least 60 days (Endler et al. 2004). In large *C. floridanus* colonies (1000–5000 workers), workers destroy worker-laid eggs (Endler et al. 2004, 2006). This behaviour has been interpreted as 'policing'. In a recent study, we showed that workers from incipient colonies (60–80 workers) do not destroy worker-laid eggs (Moore & Liebig 2010a). This contradicts the explicit prediction of Ohtsuki & Tsuji (i.e. worker policing should be strongest in the earliest phases of colony growth) and is consistent with the implicit alternative (i.e. self-restraint is in the worker's self-interest in early colony stages; therefore, policing is unnecessary). Of course, absence of egg policing in incipient colonies does not preclude the existence of an alternative policing mechanism. In the present study, we test two alternate mechanisms that could enforce worker sterility in incipient *C. floridanus* colonies: egg policing by the queen and male larvae elimination.

Queen policing is widespread among social insects (Nakata & Tsuji 1996; Monnin & Peeters 1997; Kikuta & Tsuji 1999; Saigo & Tsuchida 2004; Wenseleers et al. 2005a, b; Bonckaert et al. 2011a; Smith et al. 2011). Queen policing is most often described in species with relatively small colony sizes (but see Smith et al. 2011), consistent with the idea that larger colonies cannot be effectively patrolled by the queen, and therefore require indirect mechanisms to limit worker reproduction (Bourke 1999; Kikuchi et al. 2008). *Camponotus floridanus* colonies are large at maturity (>8000 workers), but the queen may be important in preventing worker reproduction at early stages of colony development, when the colony is small. Furthermore, there is evidence that queens are sensitive to more subtle cues than are workers when making policing decisions (Smith et al. 2011), meaning that queens may be able to discriminate between reproductive and nonreproductive workers or between worker-laid and queen-laid eggs, even when workers cannot (Moore & Liebig 2010a).

Elimination of male larvae has been reported in a number of social insect species (Passera & Aron 1996; Helms et al. 2000; Wharton et al. 2008) and implied in several others (Aron et al. 1995; Keller et al. 1996; Sundström et al. 1996). Most of these studies interpret male larvae elimination as evidence of queen/worker conflict over sex allocation (Trivers & Hare 1976; Ratnieks et al. 2006), but the elimination of male larvae can also be explained as a mechanism to adjust investment in sexual reproduction (Reuter & Keller 2001; Wharton et al. 2008) or as a mechanism to prevent worker reproduction (Ratnieks 1988). The potential for differential treatment of male larvae in *C. floridanus* was investigated by Nonacs & Carlin (1990) using a larval retrieval assay. They found that workers retrieved male and female larvae at similar rates, suggesting that workers do not discriminate between male and female larvae in the context of this emergency response behaviour. The long-term fate of male larvae in queenright *C. floridanus* colonies, however, has not been explored.

In addition to testing these two mechanisms of worker policing, we tested whether workers from incipient colonies are capable of activating their ovaries and laying male-destined eggs. This study is the first test of the idea that policing may not be necessary to enforce worker sterility in incipient colonies.

METHODS

Study Species and Culturing Conditions

We used *C. floridanus* colonies collected as founding queens or incipient colonies with fewer than 10 workers from the Florida Keys, U.S.A. The incipient colonies in this study were collected between August and November 2009 and in November 2011. Animals were transported to the laboratory via passenger aircraft in ventilated 50 ml centrifuge tubes with moistened paper towel and peanut butter. Established colonies, which were used as controls and sources of female larvae, were collected in 2007 and 2008. Queenless worker groups, which provided male larvae and worker-laid eggs, were orphaned from colonies collected between 2001 and 2007. Laboratory culturing conditions were as described in Moore & Liebig (2010b). Transportation and housing of study animals was in accordance with U.S. Department of Agriculture (permits P526P-12-04151 and P526P-11-01507).

Queen Policing

We tested for queen policing of worker-laid eggs when the incipient colonies contained 40–60 workers. Two groups were isolated from each of 12 incipient colonies. One group consisted of five workers and the queen; the other consisted of six workers. The isolates were housed in experimental chambers with moistened plaster floors and provided both sugar-water and water. After 1 h, we presented each group with 15 worker-laid eggs. Isolates from the same colony received worker-laid eggs from the same queenless worker group. Different queenless worker groups were used as worker-laid egg sources for each colony. In the hour immediately following the introduction of the eggs, we observed the behaviour of the queen, looking especially for interactions between the queen and the worker-laid eggs (e.g. antennation, carrying, eating). One hour is more than sufficient to see policing in groups of *C. floridanus* workers from established colonies (Endler et al. 2004). After 24 h, we counted the number of eggs in each experimental arena.

A direct comparison of the number of eggs recovered from groups with and without the queen was not possible because the queen continued to lay eggs during the experiment. To differentiate between queen-laid and worker-laid eggs, only worker-laid eggs with distinct embryonic development were used. *Camponotus floridanus* eggs hatch after approximately 20 days; the level of development of eggs used in this study was typical of eggs that are 7–14 days old. In pilot studies with workers from established colonies, we determined that freshly laid eggs are somewhat more likely to be eaten than are older eggs, but the effect was weak. A strong policing response has been observed in studies in which egg age was not controlled for (Moore & Liebig 2010a). We chose to use eggs with embryonic development because this allowed us to reliably distinguish introduced eggs from eggs laid by the queen.