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## Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: a review

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**Abstract** Honey-bees, *Apis*, are an important model system for investigating the evolution and maintenance of worker sterility. The queen is the main reproductive in a colony. Workers cannot mate, but they can lay unfertilized eggs, which develop into males if reared. Worker reproduction, while common in queenless colonies, is rare in queenright colonies, despite the fact that workers are more related to their own sons than to those of the queen. Evidence that worker sterility is enforced by ‘worker policing’ is reviewed and worker policing is shown to be widespread in *Apis*. We then discuss a rare behavioural syndrome, ‘anarchy’, in which substantial worker production of males occurs in queenright colonies. The level of worker reproduction in these anarchic colonies is far greater than in a normal queenright honey-bee colony. Anarchy is a counterstrategy against worker policing and an example of a ‘cheating’ strategy invading a cooperative system.

**Keywords** *Apis mellifera* · Worker policing · Anarchy

### Introduction

In the eusocial Hymenoptera, worker reproductive ability has several distinct stages of loss (Ratnieks 1999). The most extreme stage, full sterility in which workers have vestigial ovaries, is rare and occurs only in a few genera of ants (Oster and Wilson 1978) and one of the meliponine bees (da Cruz-Landim 2000). The least ex-

treme stage, in which workers can found nests, mate and lay eggs, is widespread and occurs in stenogastrine and polistine wasps, and in halictid and anthophorid bees (Wilson 1971; Michener 1974). Here workers and queens differ little or not at all in morphology, but there may be great (but reversible) differences in behaviour and physiology. Many groups such as honey-bees, bumble-bees, vespine wasps and most ants and meliponine bees occupy an intermediate stage. Here workers are morphologically very distinct from queens and cannot mate, but retain functional ovaries and can lay unfertilised male eggs. In these species, the extent of male production by workers in queenright colonies (colonies with a laying queen) is highly variable (Beig 1972; van Honk et al. 1981; Machado et al. 1984; Bourke 1988). What is the cause of this variation? Inclusive-fitness theory suggests that there is much potential for intracolony conflicts over male production (Ratnieks 1988; Ratnieks and Reeve 1992), both among the workers and between the queen and the workers. But the theory also shows that under certain conditions of kinship, a worker can enhance her inclusive fitness by reducing the reproduction of other workers, a behaviour termed worker policing (Starr 1984; Ratnieks 1988; Crozier and Pamilo 1996).

Experimental evidence, mainly from honey-bees (*Apis*), shows that worker policing is an important cause of worker sterility. This review has two complementary aims. The first is to consider what is known about worker policing in honey-bees, including recent studies of Asian *Apis* and the thelytokous Cape bee *Apis mellifera capensis*. The second is to review a rarely encountered, but highly significant, variation in normal reproductive division of labour in the honey-bee, the anarchic syndrome, in which workers in queenright colonies produce large numbers of males.

Anarchy is a revealing counterpoint to the normal situation in queenright colonies, in which worker reproduction is rare, and provides unique opportunities for studying worker policing and the control of worker reproduction in *A. mellifera*.

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## Worker reproduction in queenless colonies

In queenless colonies, some workers activate their ovaries and lay male eggs (Ruttner and Hesse 1981; Page and Erickson 1988; Robinson et al. 1990). Within a colony, there are differences among worker subfamilies both in the timing of onset of oviposition (Page and Erickson 1988) and the probability that worker offspring will be successfully reared (Robinson et al. 1990; Page and Robinson 1994). Selective destruction of worker-laid eggs has been observed in queenless colonies with some subfamilies' eggs cannibalised more than others (Page and Robinson 1994).

There is almost certainly a benefit for workers reproducing quickly following the failure of worker policing in queenless colonies. Most of the drone brood that is reared is laid in the first days following the collapse of worker policing in a queenless colony (Page and Erickson 1988). This is a consequence of both the rapid decline of queenless colonies (Page and Erickson 1988) and the developing worker-laid brood inhibiting ovary activation in other workers (Jay and Nelson 1973).

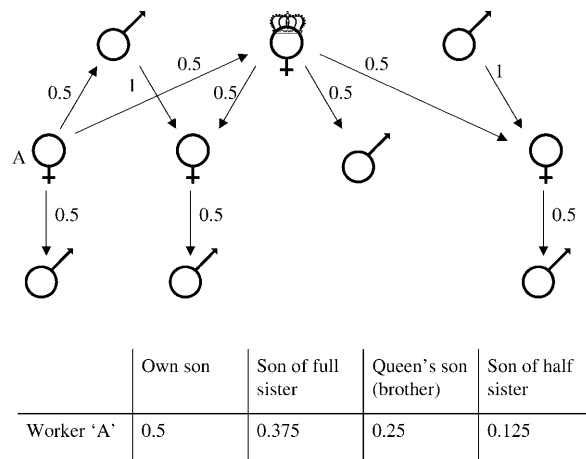
## Repression of worker reproduction

Given that *Apis* workers can produce males, why do they produce so few in queenright colonies? Microsatellite (Oldroyd et al. 1994; Oldroyd and Osborne 1999) and allozyme (Visscher 1996) markers can be used to identify the maternity of drones and male eggs in *Apis* colonies. Worker reproduction in queenright *Apis* colonies is rare. In queenright *A. mellifera* colonies, only 1 worker in 10,000 has full-sized eggs in her ovaries, showing that very few workers are capable of laying eggs (Ratnieks 1993; Visscher 1996). These few workers can lay a significant proportion (7%) of the total male eggs in a colony (Visscher 1996), but very few worker-laid eggs develop into drones, so that only about 0.1% of a colony's males are workers' sons (Visscher 1989, 1996; Ratnieks 1993).

Worker reproduction in queenright *A. florea* colonies is also very rare. No workers with active ovaries were found in a sample of 800 bees (Halling et al. 2001) and microsatellite analysis found no workers' sons in a sample of 564 drones.

Ovary activation is more common among *A. cerana* workers: up to 5% of workers have eggs in their ovaries (Oldroyd et al. 2001). However, microsatellite analysis of 652 pupal males from four colonies did not detect a single worker's son.

Kin selection theory (Hamilton 1964) predicts a conflict between females over the parentage of males. This conflict occurs among workers and between workers and the queen. The relative values to a focal worker of the different males that could be reared are simply the relatednesses of the worker to the males. These are illustrated in Fig. 1 for a Hymenopteran colony headed by a single queen mated to unrelated males, as occurs in *Apis*. For a



**Fig. 1** Pedigree showing the important life-for-life relatedness coefficients in a honey-bee colony. Here a single diploid queen is mated to two haploid males (normally there are many more males). The table gives the relatedness coefficients for the focal worker A to the possible males that could be produced in her colony

colony composed of several patriline, the mean relatedness of a worker to worker-laid males is  $0.125 + 0.25/n$ , where  $n$  is the effective number of males that father the workers (Hamilton 1964; Pamilo 1993; Boomsma and Ratnieks 1996). The relative kin values of a worker to the various males can be summarized by the following inequalities:

1.  $n < 2$ ; own sons > other workers' sons (nephews) > queen's sons (brothers)
2.  $n > 2$ ; own sons > queen's sons (brothers) > other workers' sons (nephews)

All *Apis* species that have been studied have population-wide effective paternity frequencies considerably greater than 2 (reviewed in Palmer and Oldroyd 2000) and thus are represented by (2) above. Inspection of (2) gives insight into one important aspect of the relative natures of the anarchic and normal reproductive syndromes, that of individual versus collective power. An individual worker is more related to her own sons than to any other males. But that same worker is also more related to the queen's sons than to other workers' sons (Fig. 1). Therefore, a worker can potentially enhance her inclusive fitness both by reproducing herself and by stopping other workers from reproducing. Inhibition of worker reproduction by other workers, worker policing, is more likely to evolve when workers are, on average, more closely related to the sons of the queen than to sons of their fellow workers (Ratnieks 1988). Worker policing can also be favoured when mating frequencies are less than 2 if it increases overall colony efficiency (measured as the production of reproductives; Ratnieks 1988). Conflict over sex allocation (with the workers 'preferring' a more female-biased sex ratio) can also favour worker policing because policing removes some male eggs (the worker's sons) (Foster 2000).

## Mechanisms of reproductive suppression

### Worker policing

In addition to being an important supported prediction of inclusive fitness theory (Krebs and Davies 1993; Bourke and Franks 1995; Crozier and Pamilo 1996), worker policing in the honey-bee is one of the best examples of social control overcoming individual selfishness (Williams 1992; Dugatkin 1997; Ridley 1997). Social control via worker policing is a process that helps stabilize the evolutionary transition to a colony with reproductive division of labour by reducing the ability of the potentially selfish subunits to reproduce independently (Maynard-Smith and Szathmáry 1985).

### Queen policing

In small-population colonies, the queen could use physical domination to prevent worker reproduction (West 1967; Wilson 1971; Ratnieks 1988), but this mechanism is considered unlikely in very large colonies such as those of *Apis* (Ratnieks 1988; Crozier and Pamilo 1996). Even the smallest honey-bee colonies, those of the dwarf honey-bees (*A. florea* and *A. andreniformis*) have around 6,000 workers on average (Seeley et al. 1982). *A. mellifera* colonies can have ten times this number (Winston 1987). Thus, for *Apis*, the regulation of worker reproduction is predominantly mediated pheromonally and through worker-worker interactions.

### Queen and brood pheromones

The presence of both the queen and brood inhibit worker reproduction. In *A. mellifera*, a pheromone from developing brood (Arnold et al. 1994) is the principle signal inhibiting worker ovary activation (Winston and Slessor 1998). Queens also inhibit worker ovary activation via a pheromonal signal (Free 1987). The queen's pheromonal signals have been interpreted as a form of control of worker reproduction (Wilson 1971; Michener 1974), but Seeley (1985) and Keller and Nonacs (1993) both argue that queen and brood pheromones are better interpreted as honest signals of queen fecundity. Rather than being manipulated against their own interests, workers use queen and brood pheromones as signals that the colony is queenright, and based on this information workers both refrain from personal reproduction and inhibit reproduction in other workers (Keller and Nonacs 1993). This is also referred to as self-policing (Ratnieks 1988).

### Mechanisms of worker policing in *Apis*: selective oophagy of male eggs

Worker honey-bees can discriminate between worker-laid and queen-laid eggs and eat the former (Ratnieks

and Visscher 1989). Worker-laid eggs cannot be interpreted as trophic eggs since they are as likely to develop as queen-laid eggs (Ratnieks and Visscher 1989). Workers do not seem to discriminate between queen- and worker-derived larvae (Ratnieks and Visscher 1989).

Selective oophagy of worker-laid eggs has also been demonstrated for *A. florea* (Halling 2001) and *A. cerana* (Oldroyd et al. 2001) suggesting that worker policing also occurs in these species. *A. florea* is the most basal extant species within *Apis* (Alexander 1991; Engel 1999) and since *A. cerana*, *A. florea* and *A. mellifera* all show discriminatory oophagy of worker-laid eggs, the most parsimonious inference is that this evolved before the divergence of the extant species (Halling et al. 2001; Oldroyd et al. 2001).

A queen-produced egg-marking pheromone identifies queen-laid eggs in *A. mellifera* (Ratnieks 1995). The pheromone seems to be applied to the surface of the egg during laying, since worker-laid eggs that have been touched to a queen-laid egg or to the sting sheaths of a queen lasted longer in queenright discriminator colonies than untreated worker-laid eggs (Ratnieks 1992, 1995). Further, treating worker-laid eggs with an ethanol extract of a queen's Dufour gland also reduces policing (Ratnieks 1995). This implicates the Dufour gland as a pheromone source, but does not rule out the involvement of the other glands in the honey-bee reproductive system. The egg-marking pheromone has not been identified, but the Dufour gland is more developed in queen honey-bees than in workers and the Dufour gland secretion of queens has a more diverse chemical composition than that of workers (Katav-Gozansky et al. 1997). The Dufour gland secretion of laying workers from queenless colonies is more diverse than that of workers with undeveloped ovaries, but still does not contain all the compounds isolated from queen glands (Katav-Gozansky et al. 1997).

When did oophagy of worker-laid eggs evolve as a policing mechanism in the genus *Apis*? The sister group to the honey-bees comprises the stingless bees (Meliponinae) (Schultz et al. 1999), of which most are believed to be monandrous (Peters et al. 1999). Worker policing is not predicted for the monandrous stingless bees, because workers are more related to the sons of their sisters than to sons of the queen (Ratnieks 1988). In accordance with theory, worker production of males is common in this group (Engels and Imperatriz-Fonseca 1990; Peters et al. 1999; Sommeijer et al. 1999) though not universal, as exclusive queen production of eggs has been reported for the monandrous *Austroplebeia australis* (Drumond et al. 2000).

In contrast, polyandry is ubiquitous within *Apis* (Palmer and Oldroyd 2000) and both polyandry and policing behaviour likely evolved after *Apis* and Meliponinae diverged, but before the divergence of *Apis* into the extant species. Since the most basal *Apis* species are extinct (Engel 1999), whether these behaviours are ancestral to all *Apis* or evolved early on in the genus is unclear. Thus, we suggest that the evolution of worker

policing behaviour followed the evolution of polyandry either in the ancestor of *Apis* or during the early radiation of the genus (Halling et al. 2001).

The evolution of worker policing may have been facilitated by a pre-existing queen-produced egg-marking pheromone (Ratnieks 1995). For bees in general, Dufour gland products are used in cell construction and social communication (Hefetz 1987) and in both parasitoids and social wasps, the Dufour gland is used in egg marking for intraspecific communication (Guillot and Vinson 1972; Downing 1991). In the stingless bees, queen policing of worker-laid eggs (Zucchi 1993) might be facilitated by a queen-produced egg-marking pheromone. In *Apis*, the egg-marking pheromone seems more likely to have been an existing cue recruited to a signalling role in worker policing rather than a signal that evolved as a response to worker policing to increase the efficiency of the behaviour.

#### Mechanisms of worker policing in *Apis*: worker-worker aggression

In queenless and queenright *A. mellifera* colonies, workers with activated ovaries are often attacked by their nest mates (Sakagami 1954; Evers and Seeley 1986; Visscher and Dukas 1995). This is potentially another mechanism of worker policing. In queenright colonies, aggression is directed towards workers with active ovaries (Visscher and Dukas 1995), suggesting that workers can perceive ovary activation possibly by associated changes in exocrine glands or cuticular compounds (Visscher and Dukas 1995).

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#### Cape honey-bees: the exception to worker policing in *Apis*

In the Cape honey-bee, *A. m. capensis* from South Africa, workers reproduce by thelytoky, laying eggs that develop into female workers (Anderson 1963). This highly unusual form of reproduction results in diploid female eggs following fusion of the nuclei formed by normal meiosis (Onions 1912; Verma and Ruttner 1983). Thelytoky of the Cape honey-bee contrasts with arrhenotokous male production of other subspecies.

In the Cape bees, daughters of workers are related to their mother and to their sisters by unity though they are not strictly clonal due to crossing over (Greef and Villet 1993; Greef 1996). This greatly alters the value of worker offspring to other workers. For example, if a worker in a queenright colony lays a diploid egg that turns into a worker there is no cost to other workers. If it turns into a queen, an average worker will be related to the new queen by  $1/4 + 1/2n$  (where  $n$  is the effective number of males that father the workers), which is exactly the same relatedness as they have to daughters of the queen (Greef 1996). Thus worker reproduction in thelytokous female-producing Cape worker bees will be less

costly to other workers than reproduction by arrhenotokous male-producing workers of other subspecies. This leads to the prediction that worker policing should be reduced in Cape bees, relative to other subspecies, and that worker reproduction would be more common (Greef 1996). Studies and reports support this prediction. Worker reproduction is sometimes apparent in *A. m. capensis* colonies, consistent with the absence or reduction of worker policing (Moritz et al. 1999 and references therein). Anderson (1963) and Hepburn et al. (1991) reported that about 2% of *A. m. capensis* workers had partially activated ovaries.

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#### The anarchic syndrome

Usually, worker policing and the pheromonal systems described above maintain the reproductive division of labour in queenright colonies, but in 'anarchic' colonies, these systems break down and worker reproduction is more common. These colonies are a revealing counterpoint to the normal situation in queenright colonies.

#### Finding anarchic colonies

A standard beekeeping practice is to confine the queen to one area of the hive using a queen excluder (a grid with holes large enough for workers, but not the queen, to pass through; Dadant 1975). Because honey-bees do not move brood around the nest, confining the queen also confines brood to one area of the hive. Honey is stored in the other areas and can be easily harvested. Colonies with male brood in areas of the hive off limits to the queen are therefore candidate anarchists. Further field observations, such as confirming the presence of a single queen on the correct side of the queen excluder, followed by genetic testing of the male brood are needed to confirm that the colony is rearing workers' sons.

Natural anarchic colonies are rare: we have seen very few, despite inspecting thousands of colonies. Oldroyd first saw anarchic colonies in 1987 in an apiary in New Zealand. Several colonies each had more than 500 drone larvae above the queen excluder. These colonies were part of a closed-population breeding program and the beekeeper claimed that the phenomenon was common in his stock. The beekeeper offered the unlikely explanation that worker bees carried queen-laid eggs through the excluder and licked off the sperm in the process so that the resulting brood were both male and above the excluder.

Since 1993, Oldroyd has been advertising in the Australian beekeeping press to hunt down more anarchic colonies. Two reported colonies [one in 1993 (Oldroyd et al. 1994) and one in 1995 (Montague and Oldroyd 1998)] were confirmed as anarchic. A third colony awaits confirmation using genetic markers. In 1995, Ratnieks observed anarchistic behaviour in one of his hives located in Riverside, California. In 1999, a British



beekeeper donated an anarchic colony to Ratnieks for both field and genetic testing. Page and Erickson (1988) reported a colony in which some workers were reproducing alongside the queen, but as this queen was an aged and failing cripple, this colony may not be equivalent to those described above. While it is impossible to say what proportion of natural colonies are anarchic, it is probably between one per several hundred to several thousand.

#### Characteristics of anarchic colonies

Anarchist worker reproduction is most pronounced in spring. This is the major period of drone rearing (Allen 1958), colony population growth and, normally, abundant food. The frequency of workers with developed ovaries in naturally occurring anarchic colonies is low (<1%; Montague and Oldroyd 1998; Oldroyd et al. 1999; B.P. Oldroyd, unpublished data). In Oldroyd's two Australian colonies, the majority of the workers' sons originated from a single patriline. In the first colony, 48 of 49 males analysed were from one subfamily even though at least 12 worker subfamilies were present (Oldroyd et al. 1994). In the second colony, at least 83% and up to 92% of 148 males analysed were from one subfamily and 20 worker subfamilies were present (Montague and Oldroyd 1998). This is strong evidence for a genetic basis to anarchistic behaviour. The production of males by a single (or few) subfamilies has also been seen in queenless colonies (Robinson et al. 1990), but the ability of a single (or few) subfamilies to evade worker policing in a queenright colony is what makes anarchy remarkable.

Queen excluders were present in two of the normally managed anarchist colonies. Queen excluders can interfere with the circulation of queen pheromone in a colony, which can sometimes result in worker laying above the excluder (Visscher 1998). We do not think the use of excluders provides an explanation for the anarchic syndrome because large numbers of workers' sons (at least 85% of total male brood) were reared below the queen excluder alongside the queen's sons, and not policed (Montague and Oldroyd 1998). Furthermore, we have frequently observed the rearing of worker-laid eggs in anarchic colonies kept in observation hives without excluders.

#### Selection for anarchistic behaviour

Breeding from an anarchic colony has resulted in a line of bees that reliably shows high levels of anarchistic behaviour. Daughter queens were raised from the naturally occurring anarchic colony studied by Montague and Oldroyd (1998) and instrumentally inseminated (Harbo 1986) with sperm from their nephews (the sons of anarchist sister workers). Repeating this process for a further two generations has resulted in a stock of bees in which all colonies are anarchic (Oldroyd and Osborne 1999).

#### Characteristics of selected anarchistic colonies

In a typical colony of our selected line, 5–10% of the workers have active ovaries (Oldroyd et al. 1999; Barron and Oldroyd 2001). Production of the selected line necessitated some inbreeding. Perhaps as a consequence, the brood pattern of the selected colonies is poor. A recent introduction of new genetic material to the line has restored brood viability.

Selected anarchic colonies have reduced survival that exceeds the normal difficulties in maintaining inbred stocks of bees with low brood viability (Laidlaw and Page 1986; Omholt and Adnoy 1994). They frequently make supersedure queen cells, but most of the larvae in these cells are male from worker-laid eggs, and so no queen develops. By the end of summer, workers oviposit in worker cells at such frequencies that 80% of the brood reared is male. Unless drastic action is taken by a beekeeper, such as combining it with another colony or adding combs of worker pupae from normal colonies, such colonies soon perish.

The anarchic syndrome is not due to queen failure or the queen running out of sperm, because if a queen from a declining anarchic colony is transplanted to a normal colony she produces normal patterns of worker brood in her new colony (B.P. Oldroyd, personal observation). This extreme colony decline has only been seen in the selected anarchic colonies and not in the original anarchic colonies reported to Oldroyd. This is probably related to the greater expression of anarchistic behaviour in the selected line.

As far as we can tell, anarchist bees in queenright colonies undertake normal tasks such as foraging, brood rearing and attending the queen's court. In wild-type colonies, workers with activated ovaries are often targeted and attacked by their nest mates (Sakagami 1954; Visscher and Dukas 1995), but laying workers in queenright anarchic colonies do not seem to attract extra aggression. Some laying workers in queenless wild-type colonies attract courts. These have been described as false queens (Sakagami 1958) and they produce queen-like secretions from their mandibular glands (Crewe and Velthuis 1988; Plettner et al. 1993). We have never observed false queens in queenright or queenless anarchic colonies.

#### How do they do it?

Anarchist bees are unusual in at least two ways. First, whereas ovary development is extremely rare among queenright wild-type workers (Visscher 1996), it is relatively common among queenright anarchist workers (Oldroyd et al. 1999; Barron and Oldroyd 2001). Second, their eggs are policed less (Oldroyd and Ratnieks 2000). The characteristics of the anarchic syndrome are summarised in Table 1. All these characters indicate a general breakdown of the pheromonal system that normally inhibits worker reproduction in queenright colonies, possibly involving changes in the production of pheromones, perception of pheromones, or both.

**Table 1** Characteristics of normal and selected anarchic colonies (*superscripts* indicate references: 1 Ratnieks 1995; 2 Barron and Oldroyd 2001, 3 Jay 1968; 4 Oldroyd and Osborne 1999; 5 Free 1987; 6 Jay 1970; 7 personal observations; 8 Oldroyd and

Ratnieks 2000; 9 Visscher 1998; 10 Sakagami 1954; 11 Oldroyd et al. 1999; 12 Page and Erickson 1988; 13 Robinson et al. 1990; 14 Ratnieks and Miller, unpublished data)

Character	Queenright wild-type colonies	Queenless wild-type colonies	Anarchic colonies
Percent ovary activation in workers	0.1–0.001 <sup>1</sup>	5–10 <sup>2,3</sup>	5–10 <sup>4</sup>
Brood or queen pheromones	Strong <sup>5</sup>	Decline with increasing time since queen removal. The rearing of worker-laid drones reintroduces brood pheromone to the colony <sup>6</sup>	Weak <sup>2</sup>
Queen cells	Rare and single supersedure cells	Several ‘emergency’ queen cells produced in response to queen removal <sup>5</sup>	Common multiple supersedure cells <sup>7</sup>
Production of egg-marking pheromone	Expressed only in queens <sup>1</sup>	Expressed only in queens <sup>1</sup>	Expressed in workers and queens <sup>8</sup>
Worker oviposition	Rare <sup>9</sup>	Frequently seen <sup>10</sup>	Frequently seen <sup>11</sup>
Response of workers to queen and brood pheromones	Strong in most workers, but some variation among subfamilies <sup>9,12</sup>	Strong in most workers, but some variation among subfamilies <sup>9,13</sup>	Weak <sup>2</sup>
Policing behaviour	Strong	Policing declines with increasing time since queen removal <sup>14</sup>	Permissive <sup>8</sup>

### Ovary activation

The anarchic genotype has a strong influence on ovary activation, since, regardless of their hive environment, anarchist bees are always more likely than wild-type workers to develop their ovaries (Barron and Oldroyd 2001). But a greater proportion of fostered wild-type workers have active ovaries in colonies from the selected anarchist line (SA line) than in wild-type hosts (Barron and Oldroyd 2001) and ovary activation is reduced in anarchist workers fostered into queenright wild-type hosts (Oldroyd et al. 1999). This shows that the genotype of the host colony also influences ovary activation.

There is genetic variation in the response of worker bees to queen pheromone (Pankiw et al. 1994, 1995). Selection for anarchistic behaviour has likely acted to produce sufficient mismatch between the production of ovary-inhibiting pheromones and worker sensitivity or response to the pheromones that workers are able to develop ovaries in a queenright condition.

### Evasion of policing

Eggs laid by anarchist workers are policed much less efficiently than normal worker-laid eggs (Oldroyd and Ratnieks 2000). In both wild-type and SA line discriminator colonies, eggs from anarchist workers are removed more slowly than eggs from wild-type laying workers from queenless colonies, and in SA line discriminator colonies, eggs from anarchist workers persist as long as queen-laid eggs (Oldroyd and Ratnieks 2000).

A likely hypothesis is that anarchist workers can counterfeit the queen-produced egg-marking pheromone thereby defeating the egg recognition mechanism on

which worker policing relies. Ratnieks (1992) predicted that this strategy would be a way to defeat worker policing, but he also considered it likely that workers counterfeiting queen pheromones could be detected and would be attacked by their nest mates. Workers often respond aggressively to workers contaminated with queen pheromones (Morse and Gary 1961; Yadava and Smith 1971), but as far as we know, laying anarchists do not attract excessive aggression from nest mates (Oldroyd et al. 1999; Barron and Oldroyd 2001).

Eggs laid by anarchist workers are acceptable in normal colonies. Therefore, anarchist workers should be able to reproduce successfully even if the other workers in their colony are not anarchistic. This is a situation that probably occurs in normally managed anarchic colonies when the workers of only one or a few subfamilies possess anarchistic tendencies.

Oldroyd and Ratnieks (2000) also examined the policing abilities of anarchist workers by giving SA line discriminator colonies eggs laid by normal (queenless) workers. They found that SA line colonies police less, either because they are more permissive of worker-laid eggs or because they are less good at distinguishing between wild-type worker-laid and queen-laid eggs. Oldroyd and Ratnieks (2000) caution that such ‘sloppy’ policing has been investigated in anarchic colonies from the selected line only. Whether reduced policing occurs in unselected anarchic colonies is not known.

### The development of anarchy

The anarchic syndrome has a strong heritable component. This is clearly indicated by the observations that anarchistic behaviour is often limited to a single sub-

family (Oldroyd et al. 1994; Montague and Oldroyd 1998) and that the syndrome can readily be propagated by breeding from colonies demonstrating anarchistic behaviour (Oldroyd and Osborne 1999).

Current evidence suggests that more than one locus is involved. During production of the selected line, some queens were inseminated with sperm from both sons of anarchist workers and sons of normal queens (Oldroyd and Osborne 1999). Given that workers of a single patriline produced almost all the workers' sons in the two normally managed Australian anarchic colonies (Oldroyd et al. 1994; Montague and Oldroyd 1998), in these mixed colonies the daughters of the anarchist males were predicted to reproduce successfully. However, no workers' sons were reared in these genetically mixed colonies (Oldroyd and Osborne 1999).

In the second generation, the same breeding scheme gave rise to four classes of colonies in terms of worker reproduction in queenright colonies:

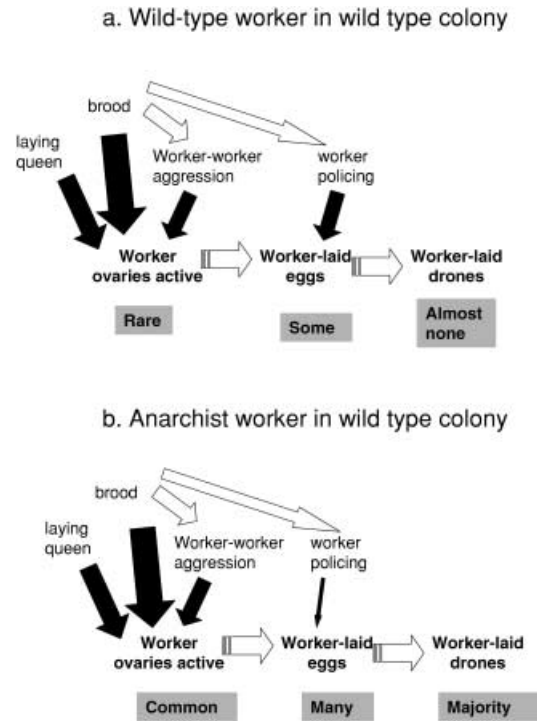
1. No ovary activation detected in any of the circa 100 workers per colony examined.
2. Ovary activation in the daughters of the anarchist males, but no detectable larval, pupal or adult male production by workers.
3. Ovary activation in workers from both anarchic and non-anarchic patrilines but no male production by either kind of worker.
4. Ovary activation and adult/pupal male production by worker daughters of anarchist males.

These observations suggest that in classes (2) and (3), worker-laid eggs were being effectively policed. In effect, during the production of the selected line, the two traits of ovary activation and the ability to lay eggs that are not policed segregate independently (Oldroyd and Osborne 1999). This may partly explain why reports of anarchist bees are so rare. Anarchy will only be visible when workers both have active ovaries in queenright colonies and lay eggs that are not policed (class 4).

#### A descriptive model of the anarchic syndrome

Figure 2 summarises our understanding of the regulation of worker reproduction for anarchist and wild-type bees. In wild-type colonies, strong pheromonal signals from both queen and brood inhibit ovary activation in wild-type workers (Winston and Slessor 1998). These signals are less effective at inhibiting anarchist workers and some anarchists have active ovaries.

In wild-type colonies, workers recognise most eggs laid by workers and eat them (Ratnieks and Visscher 1989). Anarchist workers are more permissive of worker-laid eggs (Oldroyd and Ratnieks 2000). Furthermore, anarchist workers lay eggs that are almost as acceptable as queen-laid eggs (Oldroyd and Ratnieks 2000). The net result is that the majority of males reared in anarchic colonies are workers' sons (Montague and Oldroyd 1998).



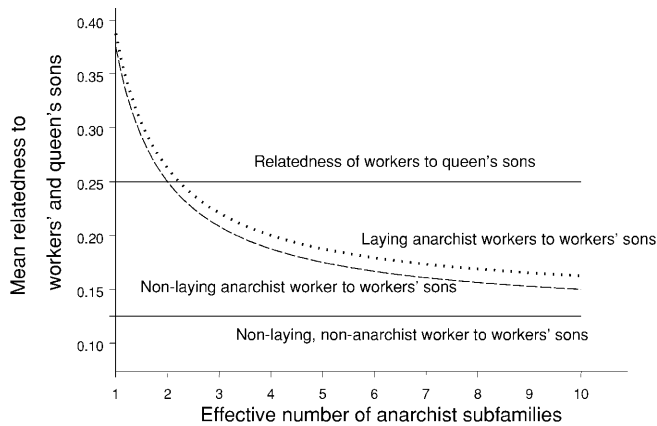
**Fig. 2** Descriptive models of the regulation of wild-type (a) and anarchist (b) worker reproduction in a wild-type colony. *White arrows* indicate positive stimuli, *black arrows* indicate negative stimuli. The *thickness* of the arrows indicates the strength of the interaction

#### Costs and benefits of anarchy

The comparison between anarchy and the reproductive monarchy of normal honey-bee societies is a striking example of an evolutionary tension between selection acting at the level of individual workers versus the colony level.

Anarchist workers probably reduce colony productivity. Excessive male production is certainly costly to a colony as males are both expensive to produce and do not forage. Anarchist workers could also reduce colony performance in other ways. Anarchists do forage (Oldroyd et al. 1999), but whether they forage as effectively as normal workers is not yet clear, and there is some evidence that anarchist workers have lower survival than normal bees (Oldroyd et al. 1999; Barron and Oldroyd 2001). Even so, colonies seem capable of supporting a small proportion of anarchists without an obvious reduction in fitness. The original anarchic colonies reported to Oldroyd were described by beekeepers as good colonies. These colonies do not exhibit the excessive, and ultimately lethal, male production found in the SA line colonies and there seems to be little impact on colony productivity and survival.

At the individual level, the benefits to anarchist workers can be great. For example, if one worker were mother of all the colony's males this would double her relatedness to the males reared (sons vs brothers). This extreme



**Fig. 3** Coefficients of relatedness of workers to worker-laid males. The model assumes that only anarchist workers can reproduce and that there are ten laying workers in the colony, all contributing equally to the worker-laid drones and distributed evenly across the subfamilies expressing the anarchic trait. As the number of anarchist subfamilies increases the mean relatedness of workers to worker-laid males decreases

situation does not occur, but in both Australian anarchic colonies, a majority of the workers' sons came from a single patriline. In this situation, workers from the anarchic patriline would be 50% more related to the worker-laid males than to queen-laid males, with an even greater benefit to the workers that actually laid the eggs (Fig. 3).

The situation in queenless colonies might present a further benefit for anarchy. There is almost certainly a benefit for workers reproducing early in queenless colonies (Page and Erickson 1988). Could anarchistic behaviour reflect selection for worker reproduction in queenless colonies? It seems not. While anarchist bees are more likely than wild-type bees to have active ovaries when queenless (Barron and Oldroyd 2001), they fail to contribute a substantial proportion of the male brood when queenless (Montague and Oldroyd 1998). This suggests anarchistic behaviour is not an epiphenomenon of queenlessness.

Natural anarchic colonies are rare and colonies whose reproduction is as extreme as those generated by artificial selection may never occur naturally. Anarchy is also a complex syndrome, which likely has a complex genetic architecture, and this complexity might contribute to its rarity. The benefits of anarchistic behaviour are only gained if the full syndrome is expressed (ovary activation leading to the laying of eggs by workers that are reared). Expressing any subset of the syndrome will accrue no benefit and will most likely be costly (for example, laying eggs that are policed represents a cost to both the individual and the colony). If the component traits of anarchy are under independent genetic control, and are rare, they will seldom co-occur within workers in a combination that confers a selective individual benefit.

Anarchy can be viewed as a social analogue of a selfish genetic element (Werren and Beukeboom 1993) distorting the production of male gametes (drones) by the colony such that they all carry the anarchy trait. The

negative impact of anarchist bees on colony fitness and the genetic complexity of the trait most likely limit the spread of anarchy.

## Summary

In the genus *Apis*, workers' sons are rarely produced in queenright colonies. Honey-bee colonies are too big for direct queen control (queen policing). Instead, pheromonal systems and worker-worker interactions regulate worker reproduction.

Worker policing, the selective destruction of worker-laid eggs by other workers has been observed in *A. mellifera*, *A. florea* and *A. cerana*. Worker policing probably occurs in all *Apis* species and likely evolved basally and after the evolution of multiple mating in *Apis* (Halling 2001). Aggression towards laying workers is, potentially, a second worker policing mechanism in *A. mellifera* (Visscher and Dukas 1995), but it has not yet been investigated in other honey-bee species.

In anarchic *A. mellifera* colonies, many workers activate their ovaries and many workers' sons are reared. Colonies from the selected line maintained at the University of Sydney are less inhibitory of worker ovary activation and anarchist workers are less sensitive or less responsive to inhibitory signals. Anarchist workers also evade worker policing, possibly by counterfeiting the queen-produced egg-marking pheromone.

Anarchy is an effective counter strategy to worker policing, but it is a complex syndrome. Several (perhaps many) genetic changes are needed to overcome the multiple mechanisms that otherwise maintain the reproductive status quo. Anarchy and worker policing illustrate the tensions that arise when selection at the level of the group (colony) is not coincident with selection on the individual. The benefits to anarchist workers of personal reproduction are offset by reductions in colony fitness, but normal colonies appear able to support a few anarchists without an obvious fitness reduction.

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