THE ROYAL SOCIETY

Development and behaviour of anarchistic honeybees

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Anarchistic honeybees result from extremely rare behavioural mutations which allow workers to lay eggs despite the presence of the queen. We investigated the behavioural development of bees derived from a line in which *ca.* 5% of workers have developed ovaries and lay viable eggs. Other than their developed ovaries and proclivity to lay eggs, the anarchistic workers we studied are apparently normal, performing normal worker-like behaviour. Unlike many laying workers in queenless colonies, they are not queen-like and are apparently not the objects of aggression. When day-old workers from anarchistic colonies were cross-fostered into anarchistic and wild-type host colonies, the frequency of ovary development was an order of magnitude higher in the anarchistic host (9.1%) than in the wild-type host (0.7%). This suggests that there is a policing mechanism that affects ovary development in honeybees. Thus, worker reproduction is probably suppressed at the level of ovary development as well as by oophagy of worker-laid eggs. Other mechanisms, such as aggression towards individuals with developed ovaries, may also exist, but we found no evidence for this.

Keywords: kin selection; worker policing; anarchy; laying workers; worker sterility

1. INTRODUCTION

The mechanisms by which worker sterility is maintained in eusocial insects such as honeybees remains an important question in biology. If workers in a colony lay eggs, they are related to the sons that arise from these eggs by 0.5. They are much less related to male offspring laid by the queen ($r=0.25$) and to those laid by most other workers ($r=0.125$) (Ratnieks 1988). This means that each worker should 'prefer' to rear her own sons rather than those laid by the queen or by other workers. However, in practice, most workers in most colonies enjoy little personal reproduction, despite their physiological ability to do so (reviewed in Visscher 1996). An attractive solution to this conundrum is the 'worker policing hypothesis' (Woyciechowski & Lomnicki 1987; Ratnieks 1988; Ratnieks & Reeve 1992; Visscher 1996, 1998). This idea suggests that worker sterility is maintained by reciprocal control of worker egg production. Ratnieks (1988) demonstrated theoretically that 'policing' alleles that cause workers to favour the production of queen-laid males over worker-laid males could spread in populations provided queens mate more than twice.

There is now convincing evidence that worker policing has indeed evolved in honeybees. Although all workers are physiologically capable of laying eggs, in normal colonies only *ca.* 7% of male eggs are laid by workers (Visscher 1996). Of these eggs, 99.88% are removed by worker policing (Visscher 1996). The mechanism by which workers are able to recognize eggs laid by other workers appears to rely on pheromones placed on queen-laid eggs by the Dufour's gland. Worker-laid eggs lack this pheromone and are therefore eaten (Ratnieks 1995).

The worker policing hypothesis also predicts the emergence of 'cheating' behaviour, in which workers introduce their own eggs into the pool laid by the queen (Ratnieks 1988). This predicted behaviour has also been demonstrated. In certain rare colonies, the majority of drones are offspring of workers not the queen (Oldroyd *et al.* 1994; Montague & Oldroyd 1998). Oldroyd *et al.* (1994) called this behaviour 'anarchistic' because it represented a failure of effective policing. Our laboratory has now bred a line of anarchistic bees in which up to 9% of workers have developed ovaries and the vast majority of male offspring of these colonies are worker laid (Oldroyd & Osborne 1999).

Although oophagy of worker-laid eggs appears to be an important mechanism by which worker sterility is maintained in honeybees, other as yet unidentified mechanisms of worker policing (any behaviour that reduces the reproductive potential of sibling workers; Ratnieks 1988) are also likely. A useful strategy for identifying these other mechanisms of worker policing is to study the effects of social environment on the development of reproductive behaviour in bees from anarchistic stock, because mechanisms of policing must be socially derived as they rely on workers performing acts that affect other workers.

In both queenless and queenright colonies, workers which develop ovaries are more likely to be attacked by other bees (Evers & Seeley 1986; Schneider & McNally 1991; Van der Blom 1991; Visscher & Dukas 1995) and this may serve to curtail the further reproductive dominance of these individuals, that is, workers attempt to police the reproductive efforts of their siblings. We therefore investigated whether bees from anarchistic stocks are

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subject to aggressive acts which may curtail the development of their ovaries. In doing so, we made the first observations on the behaviour of anarchistic workers.

2. MATERIAL AND METHODS

These experiments were conducted with three colonies of anarchistic bees resulting from four generations of selection for high rates of worker oviposition (Oldroyd & Osborne 1999).

(a) *Experiment 1. Observations of anarchistic workers*

An anarchistic colony containing *ca.* 4000 bees was placed in a two-comb observation hive in November 1998. The lower comb and the queen were separated from an upper comb by a queen excluder. The upper comb had worker and drone cells and workers were seen regularly laying eggs in this comb, despite the presence of a queen and an active brood nest beneath the queen excluder. Eggs, larvae and pupae were observed in the drone cells and these must have been worker laid. Montague & Oldroyd (1998) have shown that anarchistic workers lay many eggs in the brood nest alongside those laid by the queen. Thus, the behaviour we were observing was not an artefact of the use of a queen excluder, a necessary experimental convenience.

The Perspex walls of the hive were fitted with windows that could be opened to mark the bees with coloured paints. When a worker was observed with her abdomen in a cell (presumably laying an egg), we carefully opened one of the windows and attempted to mark her with paint. Success was infrequent, but seven laying bees were individually marked over a ten-day period. The behaviour of each of these seven bees was then observed for ten days, as was the behaviour of other bees towards these marked individuals. During this observation period, a sample of 105 randomly caught hive bees was obtained. These bees were dissected (Dade 1977) and classified into two classes for level of ovary development: fully developed eggs present and all others, which includes those bees with swollen ovaries but no developed eggs present.

(b) *Experiment 2. Cross-fostering of anarchistic workers*

Two observation hives were established, one containing an anarchistic colony (the one used for experiment 1) and the second a wild-type colony in which no eggs were observed in a drone comb placed above the queen excluder. The colonies were of similar strength (*ca.* 4000 bees). The colonies were separated by 5 m and the entrances conspicuously marked, so drifting of bees between the colonies is unlikely to have been significant. The environmental conditions were excellent for bees.

On 27 January 1999 combs containing sealed worker brood were obtained from two other anarchistic colonies and these were placed in an incubator at 35 °C. Workers were actively laying eggs in large numbers in both of the source colonies. Emerging workers were marked with coloured paints, such that the source colony and day of emergence of each bee could be determined. Bees were added to both the observation hives each day until 2 February. In total, 743 bees were introduced into each of the observation hives, with equal numbers from both source colonies added to each.

The observation hives were observed every second day on a 30 min rotation between 12.00 and 16.00 such that each colony was observed for a total of 2 h. (We chose this period for study as we knew from experiment 1 that this is the period in which we were most likely to observe worker oviposition.) Notes were made on the behaviour of marked bees.

On 12 February, a marked worker was seen with her abdomen inserted in a cell, presumably laying an egg. As other marked bees were beginning to forage, a decision was taken to terminate the experiment before marked bees were lost in foraging accidents. The colonies were killed at dawn the following day with ethyl acetate and all marked bees retrieved. A large, random sample of these bees was dissected and scored for ovary development as above.

3. RESULTS

(a) *Experiment 1*

Of the 105 workers dissected, four had highly developed ovaries, indicating that *ca.* 4% of workers in this colony were actively laying. This rate of ovary development is typical for our anarchistic bees (Oldroyd & Osborne 1999). On the assumption that a worker which had backed into a cell actually laid an egg, we estimated that more than 20 bees were laying in our colony, with over 50 ovipositions and hundreds of eggs seen. Only one marked bee laid eggs across two days, although six were observed to lay more than one egg on one day. One worker laid three eggs at *ca.* 10 min intervals. All eggs were laid in drone cells. Anarchistic workers were not observed to be aggressive nor were they the objects of aggression.

One anarchist was observed to lay an egg 10 min after attending the queen and licking her (presumably receiving queen pheromones). Other anarchists exchanged food, fed larvae, groomed other workers, were themselves groomed and, within one or two days of laying eggs, foraged for nectar and later pollen. Oviposition appeared to be only one of several activities done by anarchistic workers as they proceeded through a course of apparently normal temporal polyethism. Presumptive laying workers did not attract 'courts' as do some workers in queenless colonies (Sakagami 1958; Velthuis 1970), indicating that they did not develop queen-like mandibular pheromones (Plettner *et al.* 1993).

(b) *Experiment 2*

Marked bees appeared to follow a normal behavioural ontogeny (Seeley 1985). Bees which were two to four days old fanned, tended cappings, fed older larvae and cleaned cells. The first foraging trips were seen when bees were ten days old and the first (and only) oviposition was observed when bees were 14 days old. Again, marked bees appeared completely normal and there was no suggestion that these bees were subjected to unusual levels of aggression. (Some aggression is expected towards newly introduced non-nest mates for the first few hours and this was observed.)

Of the 743 bees of anarchistic stocks added to each observation hive, 343 were retrieved from the anarchist colony and 418 from the non-anarchist colony. Of these, 284 were scored for ovary development from the anarchistic observation hive and 298 from the wild-type colony.

Anarchistic bees from both source colonies were much more likely to develop eggs in the anarchistic host than in the wild-type host (tables 1 and 2), though this probability was greater in bees from source colony 1 than source colony 2.

Table 1. *Rates of ovary development of bees from two colonies of an anarchistic line cross-fostered into non-anarchistic and anarchistic host colonies*

source colony	host colony	ovary development		total
		developed	non-developed	
1	anarchist	22	124	146
	non-anarchist	2	115	117
2	anarchist	4	134	138
	non-anarchist	0	181	181
	total	28	554	582

Table 2. *G-tests of heterogeneity (Zar 1996) of equal ovary development in the different hosts*

source colony	G	d.f.	p
1	16.64	1	0.000
2	6.77	1	0.009
G of totals	26.68	1	0.000
total of Gs	23.41	2	0.000
heterogeneity	3.27	1	0.070

4. DISCUSSION

These results show that anarchistic bees behave apparently normally except for egg laying. Thus, anarchistic bees are very different from the 'false queens' observed in queenless colonies (Sakagami 1958). Some queenless workers become 'false queens', disengaged from normal worker behaviour. Both false queens (Sakagami 1958) and laying workers (Evers & Seeley 1986) are often aggressively mauled by nest mates. We never observed such behaviour in our anarchistic bees. Moreover, when the anarchistic colony studied by Montague & Oldroyd (1998) was made queenless, non-anarchist subfamilies were the first to commence egg laying. Thus, anarchistic behaviour does not arise from a simple failure to receive signals indicating the presence of the queen, nor is anarchistic behaviour queen-like, as laying was preceded and followed by normal worker activities, which are never performed by queens.

Experiment 2 demonstrates that the social environment is important in the development of the anarchist phenotype. Bees of anarchistic stock were much more likely to develop ovaries in the anarchistic host colony than in the wild-type colony. This probably represents a second mechanism of worker policing (the first being oophagy of worker-laid eggs; Ratnieks & Visscher 1989). This suggests that, in addition to reduced oophagy of worker-laid eggs (Oldroyd & Ratnieks 1999), our selection programme for anarchistic behaviour (Oldroyd & Osborne 1999) relaxed other mechanisms of worker policing. Note, however, that, because we were unable to replicate the anarchist line, we cannot exclude the possibility that the higher rate of ovary development in bees added to the anarchistic colony was due to a colony-specific effect unrelated to the anarchistic phenotype of the host. However, this remote possibility is not important to our contention that the social environment in which a bee is raised strongly affects the probability that that bee will develop ovaries, which we have unequivocally demonstrated.

As our colonies were >5 m apart, the entrances conspicuously marked and because the experiment was terminated soon after foraging commenced, drifting of marked workers between colonies is unlikely to have been significant. To the extent that drifting did occur between the colonies, this would only serve to lessen the extreme difference between the rates of ovary development observed in marked bees in the two colonies.

The rate of recovery of marked bees from the two colonies was lower than we expected. We do not know the fate of the unrecovered bees, but sources of loss may include removal of paint marks, attacks upon introduction, drifting to other colonies and premature death caused by handling. However, as we had similar recovery rates from the two observation colonies, the lower than expected recovery rate is extremely unlikely to be the cause of the effect we observed.

What other mechanisms of policing may exist in *Apis mellifera*? We found no evidence that the seemingly most obvious mechanism of attacking workers with active ovaries is used. Visscher & Dukas (1995) showed that, when queenless bees are reintroduced to their queenright colony, those individuals with developed ovaries are more often attacked than bees with undeveloped ovaries. This demonstrates that bees have the capacity to detect sisters with developed ovaries. A greater number of introduced anarchist workers survived in the non-anarchist host than the anarchist host and no attacks on marked workers were ever observed after introduction. Thus, the failure of almost all introduced workers to develop ovaries in the non-anarchist host does not appear to be because those that did so were killed or otherwise selectively attacked. Indeed, except for the initial introduction period, no aggressive acts were ever seen in our colonies. As aggressive acts towards laying workers are relatively common in queenless and queenright colonies (Evers & Seeley 1986; Schneider & McNally 1991; Van der Blom 1991; Visscher & Dukas 1995) we find it unlikely that we would have missed all aggressive acts towards our introduced bees had they occurred. This supports our notion that anarchistic bees are qualitatively different to laying workers from queenless colonies. This is not to say that aggressive acts towards individuals with developed ovaries is not an important mechanism for regulating worker oviposition under many circumstances.

We speculate that a second mechanism of worker policing in *A. mellifera* involves chemical signals produced by larvae which inhibit ovary development (Kropakova & Haslbachova 1971; Jay 1972; Arnold *et al.* 1994). These pheromones may be manipulative in that they chemically suppress the development of ovaries or merely signal (Keller & Nonacs 1993) that brood is present and worker ovary development is maladaptive because policing via oophagy will ensue. Our selection programme presumably reduced the production of these putative signals so that bees with a genetic predisposition towards anarchistic behaviour were able to develop ovaries.

What then is the origin of naturally occurring anarchist honeybees? It is doubtful that recent mutations could produce the total anarchistic phenotype. Appropriate cell size selection, egg laying as an element of otherwise normal behaviour and the lack of aggression related to egg laying all support the interpretation that anarchistic egg laying is complex, well-integrated behaviour and, quite possibly, an expression of the ancestral condition. Its expression in modern bees feasibly results from a disruption of genetically based systems such as brood pheromone production which normally suppress anarchistic behaviour. If we assume that monandry is the ancestral condition for bees, all workers were fathered by the same haploid male and were more related to each other's sons (0.375) than they were to their mother's sons (0.25). Monandry fosters a resolution of queen–worker and worker–worker conflicts regarding drone production in favour of workers collectively laying eggs and producing the colony's males (Moritz 1985). With polyandry, the resolution of these conflicts shifts in favour of the workers rearing the sons of the queen and workers not tolerating reproduction by other workers (Woyciechowski & Lomnicki 1987; Ratnieks & Visscher 1989). The fact that the anarchy phenotype can be readily selected (Oldroyd & Osborne 1999) further indicates that anarchy is an ancestral condition which is restrained in an evolutionary dynamic by coevolving suppression systems such as worker policing via oophagy (Ratnieks & Visscher 1989) and the chemical or other suppression of ovary development. Thus, at any one time, anarchistic behaviour is very rare in a population, as countervailing policing mechanisms are selected to reduce the expression of anarchistic behaviour when it arises.

Some authors (e.g. Ratnieks 1988; Montague & Oldroyd 1998) have theorized that one potential selective force against worker oviposition is the cost to colony fitness, based on the assumption that laying workers do less work than sterile ones. The observations reported in this study might suggest that worker oviposition need not have a great cost to colony fitness, as our observations of individual bees revealed a normal behavioural ontogeny. However, we would not like to leave this impression. Although our marked bees had a normal behavioural ontogeny, we cannot exclude the possibility that anarchistic workers perform necessary hive functions at a slower rate than normal bees and, thus, adversely affect colony fitness.

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