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Worker bees and the fate of their eggs

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In this contribution worker egg-laying in a bumblebee, a honeybee and a stingless bee species is compared to sociobiological models concerning reproductive conflict in hymenopteran societies. It is argued that the models offer an incomplete insight in the phenomenon. Without studying the behavioural and physiological mechanisms involved, the biological meaning of worker egg-laying in these species cannot be fully understood.

Keywords: worker eggs, reproductive conflict, worker policing, polygyny, Hymenoptera

Since Hamilton (1974) published his famous paper on the indirect fitness consequences of the haplo-diploid system of the Hymenoptera, the theoretical developments in sociobiology have been much faster than the empirical analysis of the fitness components in hymenopteran colonies. In this paper we will discuss the direct and indirect contribution of worker bees to the production of males in relation to the genetic structure of the colony, and we will illustrate the existing disparity between the actual mechanisms and the theoretical predictions that follow from the concepts. This disparity emerges because mechanistic constraints are involved.

In many social insects the colony is headed by a single queen that has mated with a single male (Strassmann, 2001). This is the origin of the asymmetries in relatedness on which the evolution of the hymenopteran colony is supposed to be based. As Figure 1 shows, the worker gains more in inclusive fitness by rearing sisters than by laying eggs herself, because with a sister the relatedness is on average 75%, while through each offspring of her own only 50% of her genes are propagated. If numerical aspects remain the same, she should help her mother, as long as her mother produces more daughters than sons.

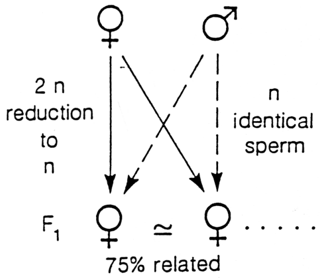

Figure 1. Females gain more inclusive fitness if they help their mother in producing daughters than if they would rear their own offspring.

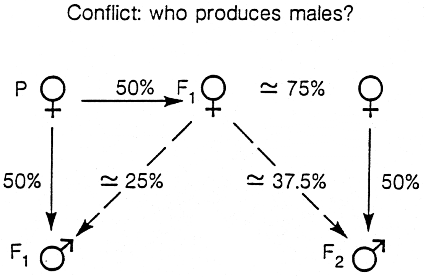

Figure 2. In monogynous, monandrous colonies workers are expected to compete with the queen over male production. Sterile workers would gain more if the males are nephews compared to brothers.

The latter restriction has to be made, because a worker is related to a brother by only 25% on average; the brother, being haploid, has no father. As Fig. 2 shows, workers should compete with their mother when it concerns male production by the colony and indeed, in many species workers maintained the capacity to lay unfertilized eggs. Workers that, for one reason or the other, are unable to reproduce, should support their reproductive sisters in their efforts, because their indirect gain is 50% higher if the males are nephews than if they are brothers.

BUMBLEBEES

The bumblebee *Bombus terrestris* is one of the many species in which conditions are met to test this prediction. The queen is singly mated. After hibernation she produces three broods, initially all females, then switches to laying only unfertilized eggs. Remarkably, workers do not respond to the production of these haploid eggs. Though workers are able to lay eggs, their moment to express this capacity is independent from the moment the queen switches to laying haploid eggs (Duchateau & Velthuis, 1988). Once workers start to lay eggs (a moment we coined the competition point), the social structure of the colony begins to fall apart, one of its consequences being that the foragers no longer bring in the food needed for temperature control and feeding the larvae. Eggs laid by the queen are eaten by laying workers and the queen eats most of the worker laid eggs, so that hardly any egg does make it to an adult male. However, some eggs do escape this ovivorous phase. Who is the mother of these eggs, a worker or the queen?

By crossing *B. terrestris terrestris* with *B. t. sassaricus*, subspecies with different colouration of the hairs, it was possible to distinguish sons of the *B. terrestris* queen from the sons of the hybrid workers, because half of the workers' sons have the colour pattern characteristic of *B. t. sassaricus* (Duchateau, 1996, in prep.). By feeding the colonies with ample food we could prevent the complete destruction of the eggs. This experiment showed that those eggs that escaped being removed practically all originated from the queen (Fig. 3).

In this case, two theoretical expectations are not met: the laying workers are unsuccessful, and the non-laying workers are not involved in this reproductive conflict.

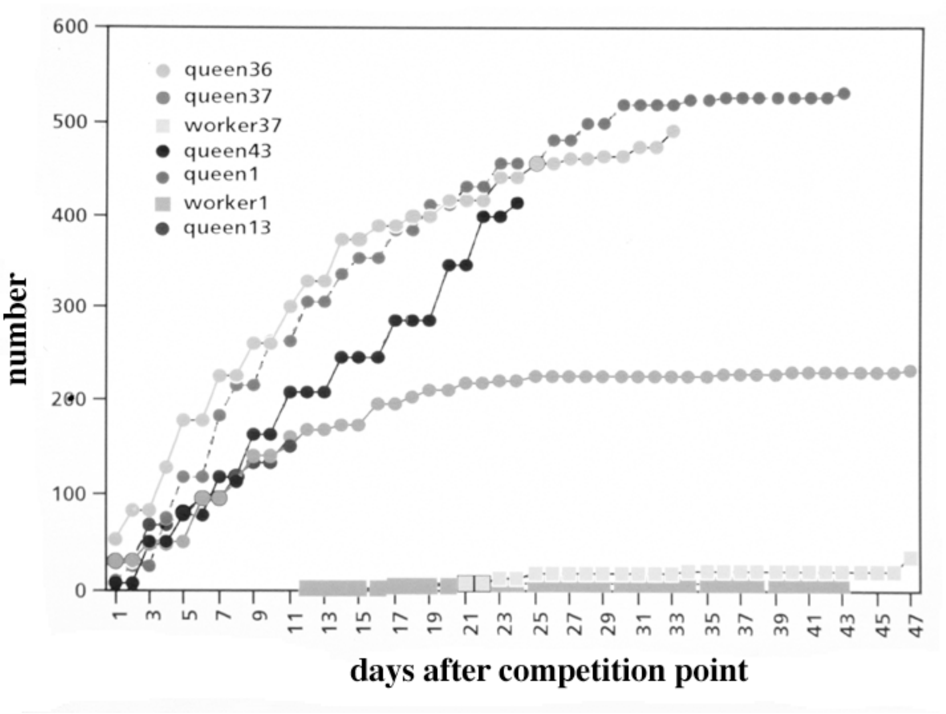

Figure 3. In colonies of *Bombus terrestris*, even after the competition point males are usually sons of the queen.

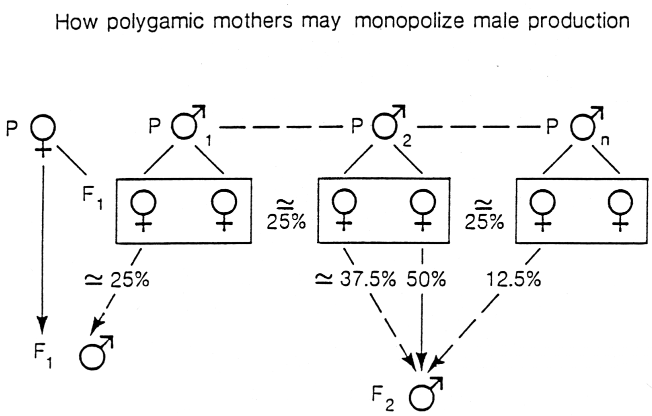

Figure 4. Honeybee colonies are monogynous but polyandrous. This has as a consequence, that we expect the males to be the sons of the queen, rather than of the workers, because the workers share more of their genes with their brother than with the average son of a fellow worker.

POLYANDRY IN HONEYBEES

The genetic structure of a colony becomes totally different if the queen mates with several males. This is the case in the honeybees, where the simultaneous presence of 20 or more patrilines in the colony is a quite common feature.

Fig. 4 gives the degrees of relatedness among the colony members. If workers could distinguish their full sisters from their half-sisters, subgroups of workers could exist trying to realize the reproductive gains depicted in Fig. 2; however, the subgroups would compete among each other. Half-sisters only share genes through the common descent from the mother therefore share on average 25% of their genes (provided their fathers are unrelated). If they cannot distinguish full sisters from half-sisters, there are no partners to rely on when a worker attempts to reproduce, because its relatedness with the average worker is just a bit above 25%, given the many patrilines. Now each worker trying to reproduce will be confronted by the other workers who prefer males from the queen (25% relatedness) above those from workers (12.5% relatedness).

In a normal colony reproductive worker bees occasionally occur. Their frequency varies according to the season and whether the colony is preparing to swarm or not (Verheijen-Voogd, 1959). However, only very rarely does a worker-laid egg result in an adult male. This led Ratnieks (1988) to conclude that non-reproductive workers responded to their inclusive fitness interests and ate these worker-laid haploid eggs. The phenomenon became known as worker policing. Interestingly, Visscher (1996) found that worker eggs were rapidly removed; he found a 50% survival time in the order of 30 min. Whether the probability of being removed varies among the eggs is unknown, but Katzav-Gozansky *et al.* (1997) reported variability in caste-specific odour marks on the worker-derived eggs. Is the removal of worker-laid eggs by worker bees necessarily worker policing?

We measured the survival of eggs in the absence of workers in nuclei of *Apis mellifera*. Mating nuclei, containing a few hundred workers, some with and others without a queen, were checked daily for the presence of eggs. Once eggs appeared laid by workers, a new comb was inserted. This comb was removed 24 h later, and with the eggs in its cells, placed in an incubator at 35°C and 50% RH, the climatic conditions they would experience in a hive (Büdel, 1960). Combs with eggs of queens served as controls. The eggs were inspected daily, and the emergence of the larvae from the eggs was noted. It appeared that many worker eggs were inadequate; they shriveled and apparently had insufficient protection against dehydration.

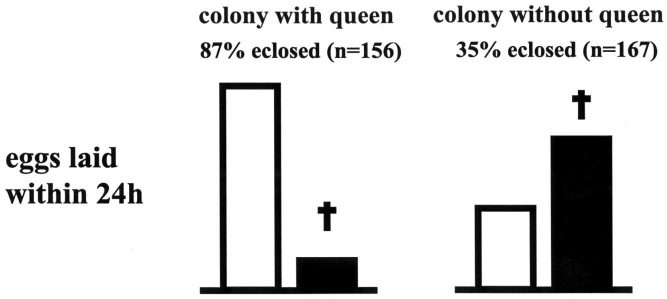

Figure 5. In the honey bee worker-laid eggs from a queenless colony suffer a considerably higher mortality than eggs produced by a queen.

Frequently, after one or two days, only the empty egg envelope was left. Only 35% of the worker eggs produced a larva, and of these about half of them emerged with a delay, compared to 87% normal emergence from the eggs laid by the queen (Fig. 5).

This observation leads to the question whether workers, removing worker-laid eggs, do so to improve their indirect fitness or whether they are removing debris. Is it a police or a janitor force? And is this removal done by non-reproductive workers or by reproductive ones? In the latter case, removal of genuine eggs would be a simple form of direct competition. The situation in the colony, therefore, might be much more complex than has been conceived before.

In the bumblebee example as well as in the case of the honeybee, workers do produce eggs but do not generate offspring. One might wonder why these eggs are produced at all. In case of most of the stingless bees (monogynous colonies, a single father, a situation fully comparable to the bumblebees) workers also produce eggs that are being eaten. It concerns their trophic eggs, which are eaten by the queen. The proteins invested in these worker eggs are being recycled and help the queen to increase her daily output of eggs. Such recycling, however, does not occur in the bumblebees, because those workers that eat the eggs are not the ones involved in rearing the larvae. For the honeybees it is not known if there are colony-level consequences of egg consumption by workers.

POLYGYNY IN A STINGLESS BEE

Still another genetic structure can be found among the bees. This concerns the polygynous condition that occurs in the stingless bee *Melipona bicolor*. Colonies of this species may contain from 1-5 laying queens (Bego, 1983; Velthuis *et al.*, 2001). Each queen mates with only one male (Peters *et al.*, 1999). If we suppose that the queens are related (young queens, after mating outside the colony, return to the colony of origin), several colony structures are possible. In case of a colony having three queens these may be a mother and two daughters, or three sisters, or queens with an even more distant relatedness, such as nieces and aunts. In any case, the relatedness between two average workers is reduced compared to a monogyne situation, as well as the asymmetry in relatedness of a worker with the female and male offspring of the colony. Also, on average, there is hardly any difference in relatedness with males derived from queens and males derived from workers. Therefore, if the degree of relatedness cannot be perceived, only the classic direct competition among egg layers can be expected.

Like in the monogynous *Melipona* species, in colonies of *M. bicolor* laying workers occur, producing trophic or reproductive eggs (Koedam *et al.*, 2001). Reproductive worker eggs are laid after the interactive process of cell provisioning and the oviposition by the queen has taken place (Sakagami, 1982). Such a worker positions herself onto the cell, often starts sealing the cell but then lays an additional egg during the sealing process. The larva from the workers' egg may eat the one emerging from the egg of the queen, thus securing all the food in the cell.

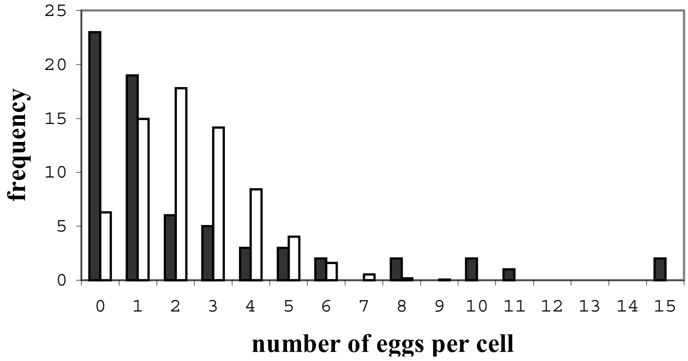

Figure 6. In *Melipona bicolor* the distribution of the number of reproductive worker eggs over the available cells is far from random, indicating that the workers compete more among themselves than with the queen (black: distribution found; white: Poisson distribution).

Males are produced in small number throughout the year, but during short periods their number might increase. In *M. subnitida*, Koedam *et al.* (in manuscript) found that during such a brief period the queen may produce about twice as many males compared to the workers, probably because she responds to the presence of laying workers by producing haploid eggs herself.

In *M. bicolor*, apart from the continuous but low rate of male production throughout the year, there occur periods with excessive egg-laying by reproductive workers. Such periods occur after the hibernation period or when a new queen becomes physogastric, circumstances that caused a reduced production of new brood cells. It then happens frequently that a laying worker, when she is closing the cell after her egg-laying, is replaced by another worker. The new one first eats the egg present in the cell before laying her own. Only the first laying worker puts her egg next to the one laid by the queen, but a subsequent worker usually eats both, so that at the end there is usually only one worker egg in the cell.

Interestingly, during such a period of excessive egg-laying by workers, a number of eggs laid by the queen escape being devoured. After she has laid such an egg, a non-reproductive worker seals the cell that is not being opened again by a reproductive worker.

During one such a period of worker egg-laying, we videotaped the occurrences at 68 brood cells in a single colony. Compared to the 68 eggs the queen laid in them, the workers laid 162 eggs, an average of 2.4 per cell. Nevertheless, the reproductive workers did not use 23 cells. This might indicate that in these cases the queen might have been able to protect her egg in some way. The distribution of the number of worker eggs over the cells is given in figure 6. The survival of worker-laid eggs is about 50% compared to the eggs of the queen. While 43% of the queen eggs developed into a larva, the rest being eaten by workers or destroyed by the emerging son of the workers, only 24% of the eggs laid by the reproductive workers escaped being eaten.

During most of the year, when there is no such excessive egg-laying by the workers, there seem to exist better conditions for the reproductive worker eggs. Our sample is still small, but from cells in which both a queen and a worker oviposited, so that there were two eggs inside the cell, we got 7 females (apparently descendants of the queen) and 7 males (supposedly sons of the workers). This suggests equal chances for the two eggs. This differs from the situation in *Scaptotrigona postica*, where the male larva usually wins (Beig, 1972).

To increase the proportion and the number of males produced by them, the workers have to lay a disproportionate higher number of eggs. They are competing more among themselves than with the queen for the sites to breed. In this way, the question which caste wins the reproductive competition, the workers or the queen, could get two different answers. It appears as if the queen, by varying the protection of her eggs, sacrifices part of them to get rid of an explosion of reproductive workers. In order to know how much she gave in, the proportion of males she produces has to be determined during the brief period of excessive worker egg-laying as well as during the rest of the year.

This has to be done using genetic markers, but behind the conclusions of gene frequency analysis are the mechanisms of behaviour, which could show us how the queen manipulated the workers during periods of adverse conditions for her own reproductive success.

CONCLUSION

Indirect measurements of the outcome of behaviour, such as DNA analyses, often produce an incomplete and distorted insight in the behavioural mechanisms involved. The random changes in these mechanisms during their evolutionary history, associated with variability at population level, may lead to several stable outcomes. Though theoretical investigations may predict where such stable moments may be found, they do not predict the route towards those points of equilibrium.

If it happens that further predictions of the theoretical concept are not confirmed, we usually speak of constraints without really knowing what this includes. The explanation probably would come from further analytical studies, rather than from defining the restrictions under which such predictions may come true. Modelling as well as reductionistic analyses are needed to understand the evolutionary process.

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