

How the production patterns of sexuals may contribute to genetic variation in *Melipona favosa*

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In this paper we present a hypothesis for a function of the typical patterns of the production of males and gynes in *Melipona favosa* about which we reported in earlier papers. These studies dealt with the maternity of males, the occurrence frequency of males and young queens ('gynes') in a large series of brood cell samples taken over the years, and detailed observations on the behaviour of gynes and drones inside the nest and in the field. For this paper the following observations are important: Males are for a large percentage sons of workers; The production of males by workers is strongly batched in time; Several 'male production periods' (MPPs) occur over time in each colony; MPPs of neighbouring colonies do not occur synchronously; At the peak of a MPP hardly any female brood is produced; Drone congregations consist of numbers of drones that often exceed the numbers produced by one colony.

Since the queen does almost not oviposit in a MPP, there will hardly be any gynes emerging during a period of male emergence. This implies that within a single colony, the production of gynes will not coincide with the production of males. However, the asynchronous production of drones in neighbouring colonies will lead to the simultaneous occurrence of both sexes from different colonies in the field. From this, we may hypothesise that a function of these typical patterns for the production of the sexuals of *Melipona favosa* is to contribute to genetic variation within the population.

Keywords: Queen-worker conflict, stingless bees, gynes, drones

Queen-worker conflict in stingless bees

The eusocial stingless bees (Apidae, Meliponini) are different from the honeybees because of the common occurrence of laying workers in queen-right colonies of many species. The diversity of this behaviour among the hundreds of stingless bee species makes this group of social insects an interesting object for evolutionary studies on reproductive strategies in social Hymenoptera. The

haplo-diploid sex determination system of the Hymenoptera is considered to lead to a so-called queen-worker conflict for the production of males in highly social species. Because of specific degrees of genetic relationship, workers collaborate with the queen for the production and care of her female offspring. However, for the production of (haploid) males, workers are considered to be more inclined to promote their own offspring, instead of collaborating in the production of sons of the queen.

The genus *Melipona* forms a special group within the tribe of stingless bees because of its unique method of queen production. Young queens are not reared in typical queen cells, as in nearly all other stingless bees as well as in the Honeybees (*Apis* spp.). *Melipona* queens emerge from the standard brood cell type that also serves for the rearing of workers and males. The process of queen determination in *Melipona* has been a subject of controversy and is still not fully understood (Velthuis & Sommeijer 1991).

Various theoretical studies have been published on reproductive strategies in social Hymenoptera and also specifically of *Melipona* bees (Ratnieks & Reve 1992, Peters *et al.* 1999, Ratnieks 2001, Paxton *et al.* 2001, Toth *et al.* 2002, Wenseleers & Ratnieks 2004, Wenseleers *et al.* 2004). Our reports on this topic mainly concern direct and detailed observations on the reproductive behaviour of *Melipona favosa*. We specifically reported on the production of sexuals and the behaviour of males and gynes (young queens) in this species (Chinh *et al.* 2003, Sommeijer *et al.* 2003a, b, c, 2004, Sommeijer & De Bruijn 2003). The objective of the present paper is to discuss the impact of observed patterns of the production of sexuals in *M. favosa* on the genetic variation in the population.

OBSERVATIONS ON THE PRODUCTION AND THE BEHAVIOUR OF SEXUALS

Gynes

Initial analyses of the production of young queens and males revealed that *Melipona* queens are produced in large numbers (Imperatriz & Zucchi 1995, Ratnieks 2001). In our study of 167 brood samples (16342 pupae) of *M. favosa*, taken from 78 colonies in the natural environment over the years 1993–2001, gynes represented 5.1% of all female brood (Sommeijer *et al.* 2003a). There was considerable variation in the percentage of gynes among these samples. The proportion gynes-of-females ranged from 0 to 22.5% (Sommeijer *et al.* 2003a). Although we found 14% of the samples to contain no gynes at all, at the population level gynes were found to occur throughout the year. Figure 1 is an illustration of the common occurrence of gynes among emerging bees: two gynes are visible all set for emergence in nearby already opened cells on the same comb.

Previously it was commonly accepted that gynes, if not used for swarming or supersedure, are each and every one being killed by workers (Imperatriz &



Figure 1. Gynes of *Melipona favosa* are born from the same type of brood cell as workers and drones. Often gynes are not leaving directly from their cells that may already be open and where they can be observed ready for emergence. This figure illustrates the high frequency of gynes among the cells with workers and males: two gynes are visible all set for emergence in nearby already opened cells on the same comb.

Zucchi 1995, Ratnieks 2001). However, Sommeijer *et al.* (2003b) found that 57 % of the non-accepted queens of *M. favosa* are not being killed but are able to depart from the nest. Before leaving the nest, gynes were alternatively fed and aggressively chased by their sister workers. Gynes that do not depart from the nest are indeed killed. Departed gynes were observed foraging on flowers, visiting drone congregation sites and penetrating weak nests (Sommeijer *et al.* 2003c).

Drones

In previous publications we reported about the behaviour of drones of *Melipona* (Van Veen *et al.* 1997, Sommeijer *et al.* 2004). They leave the nest at an average age of 18 days, never to return. Drone congregations are not commonly observed in *Melipona*. The first description was of a congregation near a nest in an old wall in Panamá (Michener 1946). Over the years, we were able to study several large congregations of drones of *M. favosa* (Sommeijer & De Bruijn 1994, Van Veen *et al.* 1997, Sommeijer *et al.* 2004). It was remarkable that drones congregated at substrates and not in the air. At those sites, individual drones can be observed for periods of several days. Drones gather at the sites from 0600 to 1800 hrs. The large number of drones at certain congregation sites suggests the origin of these

drones from different colonies. We have no reliable observations of matings at these drone congregation sites.

Like gynes, males were also found during all months of the year in brood samples but the numbers of males were susceptible to seasonal influences, reaching peak values in the months of July and August. Minimum values were found in May and October (Sommeijer *et al.* 2003a). We assume that the fluctuation in the number of males over the year can be explained by the seasonally varying food stores of the colonies, as has also been indicated by Moo-Valle *et al.* (2001). At the population level, males occurred in 73.5% of the samples. Their occurrence within these brood samples from individual colonies is characterized by occasional very high values (maximum 74.2% of the cells in a sample) next to a frequent complete absence.

The behavioural studies revealed that in individual colonies the emergence of males occurs according to a remarkable pattern. We found that considerable numbers of males were born concentrated in distinct periods of time (Chinh *et al.* 2003). We designated these periods as ‘Male Emergence Periods (MEPs)’. They were characterised by a gradual increase of numbers of emerging drones. At the peak of a MEP about 100% of the emerging bees consisted of males with a maximum of 12 emerging males per day, which is a normal number of emerging bees per day for this species. MEPs varied in duration; the average duration was 32.8 days (18.3 standard deviation). In all colonies, periods with drone emergence alternated with periods without emerging drones. Such inter-MEP periods had an average duration of 31.8 days (± 10.6 SD).

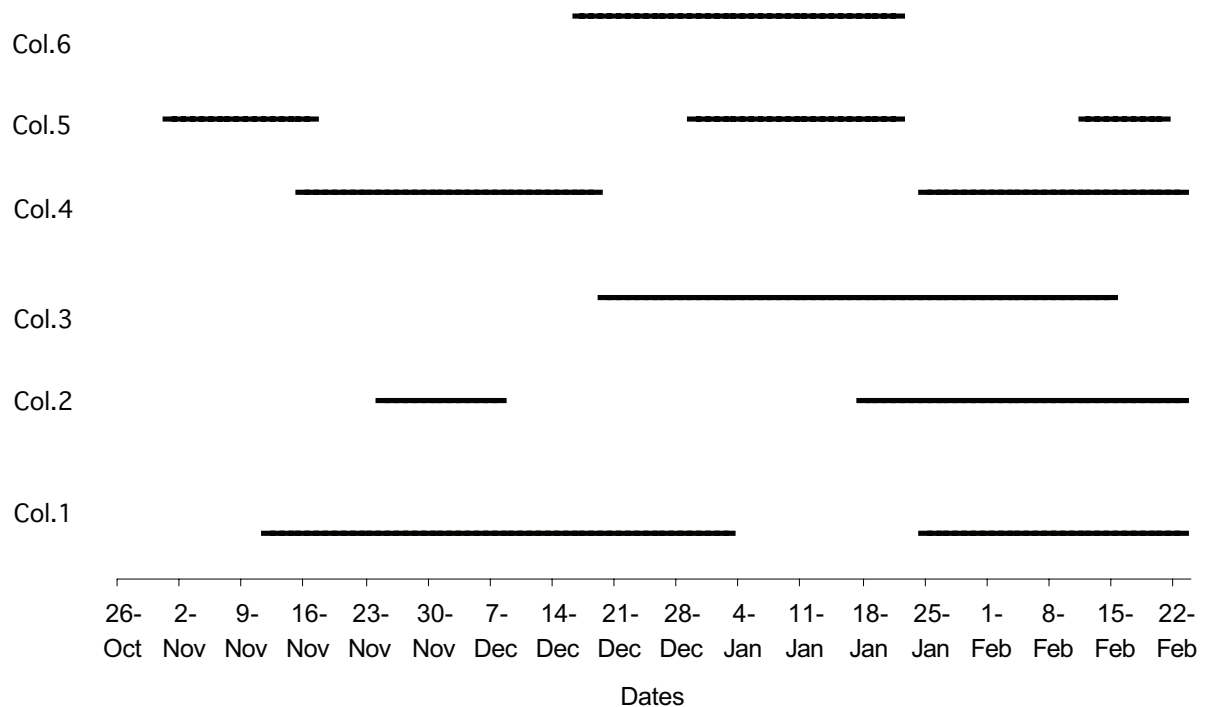


Figure 2. Male Emerging Periods as they occurred in the different colonies observed in the field (black bars: MEPs; illustration from Chinh *et al.* 2003)

Table 1. Sex of offspring produced by inseminated queens and by reproductive egg-laying workers, summed for four colonies of *Melipona favosa* (data from Sommeijer *et al.* 1999)

Sex	Egg layer:	Inseminated queens		Reproductive workers		Total
		number	% of sexes	number	% of sex	
Female		1174	100	0	0	1174
% of offspring		97.2		0.0		66.0
Male		33	5.5	571	94.5	604
% of offspring		2.7		100.0		34.0
Total		1207	67.9	571	32.1	1778

It was further remarkable that the pattern of occurrence of MEPs for different colonies, placed at the same site, was distinctly off phase. While certain colonies were in the peak of male production, neighbouring colonies were not producing males at all (Fig. 2).

An important question concerns the maternity of these drones since both queen and workers can produce haploid eggs. That male production by workers is possible, has been reported for many stingless bee species. We tracked the origin of the emerging males in *M. favosa*, and it was clear that most males emerging in a MEP resulted from worker ovipositions (Sommeijer *et al.* 1999). Of the total production of males, only 5.5% were produced by the queen (Table 1). As has been earlier reported (Sommeijer & Van Buren 1992), laying workers of *M. favosa* can show two markedly different behavioural patterns during the provisioning and oviposition process: workers laying trophic eggs (that serve as food for the queen) immediately retreat after releasing their eggs, whereas the workers laying reproductive eggs remain on the cell and close it directly after oviposition.

CONCLUSION

Gynes can only be produced by the inseminated laying queen. Since the queen hardly oviposits during a 'Male Producing Period (MPP)', there will be hardly any gynes emerging during a MEP. This implies that not only the production of drones is batched, but that also the production of gynes is rhythmically fluctuating. Thus within a single colony, the production of gynes will never coincide with the production of males.

However, the asynchronous production of drones in neighbouring colonies will lead to the simultaneous occurrence of both sexes from different colonies. From this we hypothesise that the function of these typical patterns for the production of the sexuals of *M. favosa* is to contribute to genetic variation within the population. Further studies are urgently needed to establish whether our hypothesised mechanism for promoting out breeding also occurs in other species of the genus *Melipona*.

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