Behaviour of males, gynes and workers at drone congregation sites of the stingless bee *Melipona favosa* (Apidae: Meliponini)

The behaviour of drones, gynes and workers was studied at four drone congregation sites (DCS's) of *Melipona favosa* Fabricius. Drone congregations are situated at breezy places and may exist for several weeks. Males can visit the congregation for at least six successive days. Males resting on the substrate of the site typically perform intensive food solicitations. They also rhythmically expel and inhale the crop contents between their mouth parts. Males regularly depart from the congregation and some visit flowers during their departures. Several gynes may visit the drone congregation on a single day. Workers play a role in the establishment of a DCS. They fight among them at incipient drone congregations and at that stage they deposit mud and odoriferous plant materials on the substrate of the site. Experiments with caged workers and caged males and with the controlled release of gynes near grouped drones indicated the importance of chemical communication at the congregation site. Males, particularly when they are disturbed, are strongly attractive to gynes. Workers which are stressed because of mutual fighting are strongly attractive to males.

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M.J. Sommeijer, L.L.M. de Bruijn & F.J.A.J. Meeuwsen

Utrecht University, Social Insects Department
P.O. Box 80086
3508 TB Utrecht
The Netherlands
m.j.sommeijer@bio.uu.nl

Introduction

Little is known about the mating biology of stingless bees (Apidae: Meliponini) and very few papers are published about the behaviour of *Melipona* sexuals outside the nest. Drones of *Melipona* departing from the nest behave very differently from drones of the honeybee *Apis mellifera* Linaeus. *Melipona favosa* males leave the colony when about eighteen days of age and do not return to the mother nest (Van Veen *et al.* 1997). At *Melipona* drone congregations, drones group in large numbers, most likely for contact (and mating) with virgin queens. Despite their conspicuousness, observations of *Melipona* drone congregations are rare. This is probably due to the infrequent occurrence of this behaviour. W. Engels and E. Engels reported that drone congregations are very common in the trigonid *Scaptotrigona postica* (Latreille) (E Engels & W Engels 1984, E Engels *et al.* 1993, W Engels *et al.* 1997).

The first description of a *Melipona* drone congregation was by Michener (1946). This author observed congregations of 25-70 *M. favosa* males in March and in December in Panama. The congregations were observed to occur at the same site for several days. Van Veen *et al.* (1997) described the behaviour of males of *M. costaricensis* Friese (previously *M. fasciata* Lepeletier) congregating in front of a nest in Costa Rica. For a period of about three weeks drones assembled each morning. By marking drones these authors found that individual drones participate for several days. Marked drones occasionally left the congregation for periods of on average 30 minutes. In our previous description of a single *M. favosa* (F.) drone congregation we reported about the attractiveness of this congregation for gynes (Sommeijer & De Bruijn 1995).

Figure 1. Position of DCS1 on a vertical concrete wall. Photo: M.J. Sommeijer
Positie van een darrenverzamelplaats (DCS1) op een betonnen muur.

As from 1993, we were able to study four different *M. favosa* drone congregations in Trinidad and Tobago W.I. In this article we focus on the behaviour of males, gynes and workers at these sites.

Material and methods

In this study we observed four drone congregations of *M. favosa* in Trinidad and Tobago W.I. These were the only drone congregations of this species that we could find during a period of 20 years (with on average more than one visit per year to this country during which we always searched for drone congregations). The drone congregation sites and our observations were in general terms characterised as follows:

Site 1 1993 - on a concrete retaining wall, no nest observed in near neighbourhood, one day of intensive observations.
Site 2 1998 - on a garden wall of concrete blocks, one weak nest at a distance of 3 m., observed for some weeks.
Site 3 1998 - on the wall of a destroyed house that was built of hollow building blocks, remnant of a nest at a distance of about 1 m., observations over five days.
Site 4 1998 - on the wall of a wooden shed in which eight hived colonies were installed, about three hours observations until disappearance of the congregation.

At Site 1 preliminary experiments were carried out (Sommeijer & De Bruijn 1995). At Site 3 we were able to carry out more experiments with respect to olfactorial stimuli between workers, males and gynes. For this we brought gynes and workers from colonies at a distance of ten kilometres to the site. The males used in these experiments were shortly before collected from the congregation. The experiments concerned the caging of workers and males in cages, individually or in small groups. We used flexible nylon mesh cages that could be squeezed slightly to stress the bees in it. New cages were used for every test. Caged bees were either placed at the end of a stick that could be brought near to the grouped drones, or were placed on the ground beneath the congregation. Individually marked gynes were released about three metres away from the congregation. Marking was done by applying water-based paint marks on the thorax. The schedule of observations and experimental operations at Site 3 was:

9 August, discovery of the site and first observations on behaviour of drones for 45 minutes;
10 August, further recordings of undisturbed behaviour for 120 minutes;
11 August, recordings of undisturbed behaviour;
12 August, after 60 minutes recording of undisturbed behaviour some drones are marked;
13 August, experimental manipulations and releases of gynes.

Figure 2. Detail of congregating drones at DCS1. Photo: M.J. Sommeijer.
Detail van de verzamelde darren op DCS1.

Figure 3. Fighting workers on the ground below the incipient DCS4. Left a killed worker. Photo: L.L.M. de Bruijn
Vechtende werksters op de grond onder de zich vormende DCS4. Links een gedode werkster.

Results

At all four drone congregations grouped males occurred at a fixed site on a vertical substrate (figures 1-2). Since this is different from the airborne 'Drone Congregation Area, DCA' of the honeybee, we now define the area of a *Melipona* male congregation as a Drone Congregation Site (DCS). Different from our first observation of a DCS (Sommeijer & de Bruijn 1995) and from the description by Michener (1946), some recently observed DCS's were at a distance of less than two meters from a nest exit. The DCS's were situated at least 1.5 metres above ground level.

Worker behaviour at a DCS

At all drone congregations we also found workers. At DCS1 and DCS2 there were dead workers on the ground below the site. Obviously, these workers had been fighting, as most specimens were still entangled in fighting positions. Deterio-

rated remains of workers indicated that some had been under the site for days. At DCS3 we observed two workers actually fighting in the periphery. At DCS4 we observed the grouping of about 30 workers. Many food exchanges took place among these workers and aggressive interactions between workers occurred. Some aggressively locked workers fell on the ground and kept on fighting there (figure 3). At the end of the morning fifteen dead workers were found on this spot. During this observation, males gradually arrived at this site. These males kept aside from the workers. Workers also initiated trophallactic contacts with males. Males were also attacked by workers but were not killed.

At all sites we found deposits of plant resin. We actually observed the deposition of plant material by workers at the incipient DCS4 when still very few males were present. Workers deposited plant resin and small parts of white flower petals on the substrate (figure 4). Beside these plant materials we also found mud ridges at the substrate of DCS1. The elaborate mud ridges resembled the typical mud ridges at the entrances of *M. favosa* nests. We did not observe the actual construction of these ridges but we conclude that they were made by workers since we have never observed drones working at such constructions.

Figure 4. Workers interacting heavily at DCS4. Above a droplet of resin deposited by these workers with on top of this a part of a flower petal. Photo: L.L.M. de Bruijn
Drukke interactie tussen werksters op DCS4. Bovenaan is door deze werksters een druppeltje plantenhars afgezet met daarop een stukje van een bloemblad.

Male behaviour at a DCS

The congregations consisted of numerous drones. At DCS1 we recorded a maximum number of about 400 drones present at the same time. The number of drones at DCS2 was about 300-350; at DCS3 we counted maximally 200 drones, and at DCS4, which was considered to be incipient (see above), we finally counted up to ten drones beside 30 workers. The presence of males at a DCS has a diurnal pattern. In the period 16-21 July 1998 the first drones arrived at DCS2 at

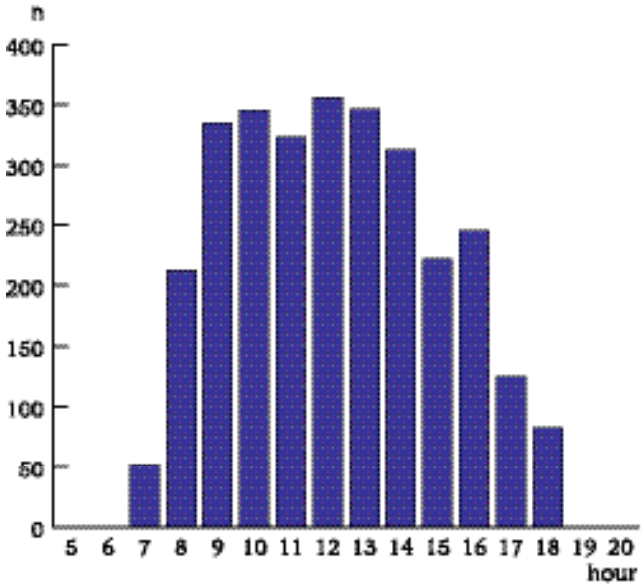

Figure 5. An example of the typical daily pattern of the presence of males at DCS2. The number of males at this site is given at different hours of a day.
Voorbeeld van het kenmerkende dagelijkse verloop van de aanwezigheid van darren op de darrenverzamelplaats DCS2. Het aantal aanwezige darren is gegeven voor verschillende tijdstippen van de dag.

about 06.10 hrs. The last ones to leave departed from the site at about 18.15 hrs. During showers the drones departed, to return again after the shower. An example of the typical pattern of male presence over the day at this DCS is given in figure 5. DCS's can exist for several weeks. By marking individual drones at DCS2 we observed that males may visit a DCS for at least six successive days (figure 6).

At the site most males sat still. About 10% of the males walked around, making intensive contact with many other males. There were always some males hovering in front of the site. The proportion of hovering males was low in the morning and highest around noon. When landing, males typically landed on top of other males resting at the site, even when empty space was available.

Male behaviour at the site was further characterised by typical food-related behaviour. Most conspicuous were the intensive solicitations for food between males, particularly directly upon landing. These solicitations are vigorous: males jump on top of each other, firmly grab each other and may subsequently fall in locked positions to the ground (figures 6-7). Certain individuals play a pronounced role in soliciting. They walk from one male to another, soliciting from many individuals. Actual food exchanges between males took place but was rare. Males also frequently expelled droplets of liquid, brought these between the mouth parts and wiped their *glossa* through this droplet while gradually drawing the liquid in (figure 8). This repeated behaviour is similar to the dehydrating behaviour of workers inside a nest. Some males had swollen abdomens while those of others were less inflated. In one observation where the abdominal size of a male increased clearly within a few seconds, there was no relation to food behaviour.

Individually marked males left the site regularly to return within a few minutes. The occasional returning of males covered with pollen indicated that they left to visit flowers.

Now and then the whole group of males took off suddenly, without any perceivable reason. They sometimes remained hovering in a large cone-shaped group in front of, and orientated towards, the site. At other occasions the males disappeared from the site, to return again after a few minutes. Males also took off upon disturbance. The congregated males always flew up as soon as we collected or touched one of the drones for marking.

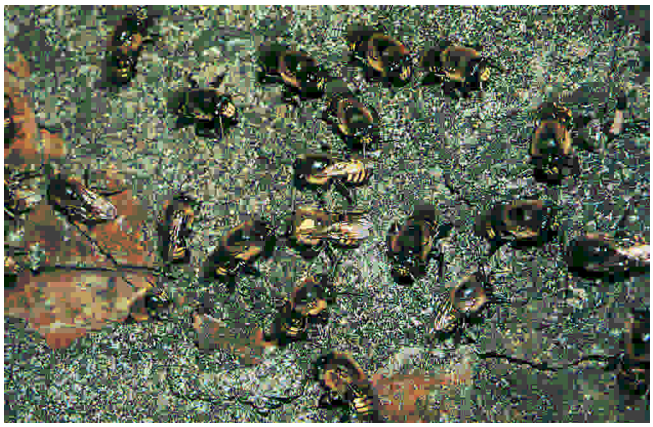

Figure 6. Marked drone soliciting for food from another drone. Photo: L.L.M. de Bruijn
Een gemerkte dar bedelt om voedsel bij een andere dar.

Gyne behaviour at a DCS

During the observations on DCS1, DCS2 and DCS3 we recorded the arrival of various gynes within a few hours. Gynes arrived from down-wind. At DCS1 we collected ten gynes on a single day between 11.15 hrs and 13.30 hrs. These gynes landed directly on the substrate. They continuously moved around, making intensive contact with the substrate, rubbing their abdomen over it and trying to enter crevices. The gynes did not initiate contact with males. Gynes arriving at the DCS showed various stages of abdominal inflation. The largest gynes had an abdominal sizes about 250% of those of the smallest gynes. While gynes were walking around over the substrate, the inflation of their abdomen could change rapidly. When one of the larger gynes was collected her abdomen rapidly decreased.

Interactions between males and gynes

Every time a gyne arrived at DCS1, almost all males abruptly took flight. When the males landed again, they generally did not make physical contact with the gynes. However, at DCS2 we observed three times the landing of a male on top of a gyne walking on the site substrate. Here we also observed twice that a male grabbed a gyne in flight. The male and the gyne clung together and fell down in this position. We could not confirm if this concerned actual mating.

Observations and experiments concerning olfactory stimuli

The following observations and experiments were done to further analyse the importance of olfaction in interactions at a DCS.

a. Workers attracting males When a cage containing five workers which were slightly pressed was placed at ground level at a distance of two meters from the DCS, males were immediately attracted to this cage. Before this, we never observed males flying that low. In the next experiment, we brought worker-containing cages near to the grouping males. Males immediately flew towards the cage when it was within 1-0.5 metre. They followed the cage for up to about two metres when it was slowly moved away. In all cases males flew at the down-wind side of the cages. Empty cages had no effect, cages containing only one worker and cages with unsqueezed workers had less effect.

b. Males attracting gynes

When congregating drones were undisturbed, visits of gynes were not very frequent. We observed DCS3 without disturbance for a total time of 5.5 hours over three days before the first gyne arrived. At DCS1, after one hour of undisturbed observation during which no gynes were seen, gynes arrived immediately after catching some of the drones for marking. The newly arrived gynes also came to an insect net which had contained males, situated at a distance of ten metres from the male congregation. We tested whether male scent was involved in attracting gynes by leaving a few males in this net. The net was now placed at a different spot, also ten metres from the male congregation. Again it attracted gynes. They landed on the net and on the bag in which the handle of the insect net rested. The gynes tried to enter the dark interior and the folds of the bag. Gynes also landed on our hands immediately after we had manipulated males. The results of the experiments with caged males at DCS3 confirmed these observations: within minutes after placing five slightly pressed males together in a cage, one or two gynes were circling around it. The immediate arrival of gynes after handling males was observed at all studied DCS's. Clearly, the smell of disturbed males is important in attracting gynes.

c. Males influencing male behaviour

Bringing a cage with slightly pressed males near to congregating males resulted in the sudden flying of the congregation away from the cage. After three to five minutes the males returned. The repelling effect of molested males also appeared when a male was taken from a group of resting males.

Figure 7. Aggressive food solicitation, in which the drone in the back solicits from the drone in the front. Left a drone with extended *glossa* and raised antennae. Photo: L.L.M. de Bruijn
Agressief bedelen tussen darren (de achterste dar bedelt bij de voorste). Links een dar met uitgestoken tong en opgestoken antennen.

Discussion

In contrast to the airborne drone congregations of the honeybee, drones of *Melipona* congregate on a substrate. All DCS's were situated at vertical sites well exposed at breezy places. This is in accordance with our observations concerning the importance of olfactory stimuli. The rapid changes in size of the abdomens of drones and gynes also point to actively using pheromones from abdominal glands. The drone congregations of *S. postica*, described in detail by E Engels & W Engels (1984), occur in front of nests where virgin queens are to be accepted and also at strong nests with laying queens which are not being replaced.

We consider the behaviour of workers essential for the establishment of a DCS in *M. favosa*. Fighting workers were found at the four DCS's which we were able to study. As we have seen at the incipient site and as was confirmed by our experiments, fighting workers are very attractive to males. This must be based on chemicals emitted by these workers. Further attraction results also from worker behaviour through the deposits of odoriferous resin and flower parts and from food exchanges between workers.

The alternative hypothesis for the attraction of males by means of an odour flag from gynes within a nearby nest can probably be rejected. In three out of four cases there was no strong nest containing gynes nearby. In two cases only a very weak nest was situated within a distance of two metres (upon opening these appeared to contain only some remains of brood combs with few cells and very few workers). Only in near DCS4 a number of nests was present, which indicates that a DCS can develop in the vicinity of strong nests as well. This was also the case in the DCS of *M. fasciata* described by Van Veen *et al.* (1997). Engels *et al.* (1997) documented that in *S. postica* drones are attracted to a colony containing a virgin queen. However, in this stingless bee species queen production is fundamentally different and virgin queens are not permanently produced, and never in large numbers, as in *Melipona*.

In an established DCS of *M. favosa* food exchanges between males are frequent. The overt aggression among males may be related to the fact that males from whom food is solicited do not show any crouching behaviour. Crouching is an appeasing behaviour by bees refusing to donate after being solicited (Sakagami 1982, Sommeijer *et al.* 1985). The abundant food exchanges combined with the frequent dehydration by males may produce an odour flag which probably attracts other males as well as gynes. Food odour is probably also a stimulus for many other insects to visit the DCS; DCS's are frequently visited by honeybee workers, wasps and flies, among which phorid flies. The attractiveness of aggregated males of stingless bees for parasitic phorid flies has been recorded by Brown (1997). E Engels & W Engels (1984) state that aggregation sites of *S. postica* are scent-marked by pheromones. This is also possible in *Melipona*.

Lightly squeezed males in the cages of our experiments may simulate stressed males like those aggressively approached for trophallaxis. The chemicals emitted by molested males have a different effect compared to those emitted by molested workers. Scent released by stressed males attracts gynes, whereas worker scent attracts males. The prompt attraction of gynes to disturbed males illustrates the effectiveness of these male pheromones. The quick appearance of gynes also indicates that gynes are commonly present in the field. In previous papers we reported about the nest-departing behaviour of *Melipona* gynes (Sommeijer & de Bruijn 2003, Sommeijer *et al.* 2003b, c)

Figure 8. Drone dehydrating a droplet of honey stomach contents, expelled between his mandibles. This is a frequent behaviour of males at the DCS. Photo: M.J. Sommeijer

Dar met een grote druppel nectar de kaken. Het op deze wijze indikken van nectar uit de honingmaag komt veel voor bij darren op een DCS.

The behaviour of workers and males on a DCS poses questions about origin and relatedness of the acting drones, workers and gynes. It has been recently established that workers are the major producers of drones in *M. favosa*. Nearby colonies produce, asynchronously, large numbers of drones in periods of just a few weeks (Chinh *et al.* 2003, Sommeijer *et al.* 2003a). This, combined with our present observations, leads us to hypothesize that workers from a certain nest help their sons to establish a mating site by producing an odour flag through engaging in fighting among themselves. Aggression may in this way improve the inclusive fitness of workers. The relatedness of the drones and the workers at the DCS should urgently be studied by the use of modern genetic methods.

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Samenvatting

Gedrag van darren, koninginnen en werksters van *Melipona favosa* (Apidae: Meliponini) op een darrenverzamelplaats

Angelloze bijen (Apidae: Meliponini) komen voor in alle tropische gebieden. Er is nog niet veel bekend over hun paringsbiologie. Voor de soorten van het geslacht *Melipona* is het gedrag van darren en koninginnen buiten het nest nog vrijwel onbekend. Wij onderzochten het gedrag van darren, werksters en jonge koninginnen op een viertal darrenverzamelplaatsen, 'drone congregation sites (DCS's)'. Darrenverzamelplaatsen van *Melipona* worden weinig waargenomen: er zijn slechts twee andere publicaties over (Michener 1946, Van Veen *et al*. 1997).

Darrenverzamelplaatsen komen voor op tamelijk winderige plaatsen en kunnen wekenlang in stand blijven. De verzameling vormt zich iedere ochtend opnieuw en verdwijnt als het donker wordt. Individuele darren kunnen de verzamelplaats minstens zes opeenvolgende dagen bezoeken. Darren die op het substraat zitten vertonen onderling intensief voedselbedelgedrag. Ze dampen ook nectar in, waarbij ze bij herhaling vanuit hun honingmaag een druppel tussen de kaken brengen en langzaam weer opzuigen. Darren verlaten de verzamelplaats af en toe. Sommige darren bezoeken ook bloemen, omdat ze met stuifmeel bestoven zijn. Per dag bezoeken verschillende jonge koninginnen (gynes) de darrenverzamelplaats.

Werksters blijken een belangrijke rol te spelen bij de vorming van een darrenverzamelplaats. Werksters vechten onderling op de verzamelplaats, die hierdoor aantrekkelijk wordt voor darren. Veel werksters overleven deze gevechten niet en onder alle darrenverzamelplaatsen troffen we dode exemplaren aan. Werksters zetten op het substraat van de darrenverzamelplaats ook modder en geurende plantenmaterialen af. Een darrenverzamelplaats is ook aantrekkelijk voor andere insecten, zoals honingbijen, wespen en vliegen. Experimenten waarbij werksters en darren in kooitjes werden aangeboden aan de verzamelde darren toonden aan dat chemische communicatie erg belangrijk is. Darren worden sterk aangetrokken door de geur van werksters (vooral wanneer deze gestresst zijn); jonge koninginnen worden zeer sterk aangetrokken door de geur van darren. De maagdelijke koninginnen worden vooral aangetrokken als de darren verstoord worden. Dit kan het gevolg zijn van het zeer intensieve en agressieve voedselbedelen, maar bijvoorbeeld in onze experimenten ook door het wegvangen van darren.