DRONE CONGREGATIONS IN MELIPONINI: WHAT DO THEY TELL US?

O QUE NOS INFORMAM AS AGREGAÇÕES DE MACHOS EM MELIPONINI ?

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ABSTRACT: At some point during their life, Meliponini males leave their nests and form congregations which can be made up of dozens up to several hundreds of individuals. There, males, which individually may frequent the same agglomeration for subsequent days, are seen to dehydrate nectar. Analysis in four species of *Melipona* show that the sugar concentration of the nectars from congregated males is positively correlated with ambient temperature, and that the volume and percentage of sugars is always lower than nectars sampled from returning foragers. Except for what they can carry off from their home colony, other sources from which congregated males may obtain their nectar are still unknown.

KEYWORDS: Stingless bees. Drone congregation. Nectar loads. Sugar concentration.

INTRODUCTION

The life cycle of males of the Meliponini starts off with a stay of two to three weeks within the nest during which they show little activity. However, at the end of this intranidal period, they start to receive nectar through trophallaxis with returning foragers and dehydrate it. Dehydration behaviour is a well-defined task and workers perform it in order to preserve and store nectar for future consumption. There is no evidence that the dehydrating activity of males contributes in any way to the advantage of the colony like workers do. Males rather seem to use nectar for their own benefit before they leave the nest permanently. Little is known about the males' extranidal activities (CORTOPASSI-LAURINO, 1979, VAN VEEN et al., 1997) but as they do never return to the nest, it is believed that they live a lifestyle like solitary male bees do.

Alcock et al. (1978), suggest that male bees patrol and/or wait at certain places where there is a chance to encounter females. The energy they need to perform these activities should come either from nectar they transport out of their nest of origin or from nectar which they actively harvest at flowers. For example, males of *Melitoma segmentaria* (Anthophoridae) not only overnight inside flowers of *Ipomoea* cf. *purpurea*, as they also collect their nectar and dehydrate it (CORTOPASSI-LAURINO, pers. comm.). Different from males of *Xylocopa frontalis* which feed on flowers, males of the facultative social bees, like *Xylocopa nigrocinta*, continue as an integrated part of their family groups and are fed by females which can be sisters as well as their mother (WITTMANN; SCHOLZ, 1989). Dehydrate nectar from males of *Bombus lucorum* is already known for some time (BERTSCH, 1984).

In the Meliponini, the origin of nectar carried by males active outside the nest is still doubtful also because they have hardly been observed on or collected from flowers (KERR, 1951 apud NOGUEIRA-NETO, 1997).

The males of the Meliponini form congregations that can be observed along the year, nearby active nests. These congregations often appear near colonies that have a young bee queen inside. Dozens up to hundreds of individuals may participate in these agglomerations, and in these cases their behavior is characterized by remaining still for most of the time, sometimes interrupted by bouts of flying activity. When keeping still, they maintain a typical body posture in which their antennas are directed forwards. Meanwhile, they may clean their abdomen and antennas from time to time although it is more common to see them dehydrating nectar (VAN VEEN et al., 1997).

In *Melipona fasciata*, young queens were attracted by congregation of males that occurred at a distance of 12 meters from a nest, although mating has never been observed (SOMMEIJER; BRUIJN, 1995).

Environmental conditions influence the formation of male groupings: males of *Plebeia droryana* leave their nest when the outside temperature is between 19-26ºC, and in December this happens earlier than in October. Male congregation of *Scaptotrigona pectoralis* only occurs with temperatures over 24ºC, and the highest numbers of individuals occurs when it is more than 30ºC (CORTOPASSI-LAURINO, 1979, MAY-ITZA, 2003).

Paxton (2000) and Cameron et al. (2004), using micro satellite techniques, determined that congregated males from *Scaptotrigona postica* and *Trigona collina* from Thailand originated from

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many, in some cases up to over a hundred different nests.

Although the extranidal dehydration of nectar seems typical for males of Meliponini, workers of *Oxytrigona tataira* and *Oxytrigona obscura* can also perform this extranidal activity. This was verified when these workers were seen collecting secretions of treehoppers, nectar of banana flowers (*Musa* sp) and syrup used as artificial food for colonies. (CORTOPASSI-LAURINO, 1979, CORTOPASSI-LAURINO, pers. comm.)

The dehydration of sugary liquids is an activity associated with both solitary and social female bees, but in the case of males, it seems to be linked to many Meliponini and a few solitary species. It is believed that through this process, as far as males are concerned, weight is reduced and the concentration of sugar is enhanced which together allow for a better performance in finding a mate (BERTSCH, 1984, WITTMANN; SCHOLZ, 1989).

The objectives of this study were to register a possible seasonality of male congregations in Meliponini and to give an account of the behaviour of these males especially when nectar dehydration is concerned. In addition, throughout a day the variation in the energetic value of these nectars, reflected by the nectars' quantities of sugars, was determined, and a quantitative comparison between male-dehydrated nectars and those brought inside the nest by workers was made.

MATERIALS AND METHODS

The species of bees involved in the analysis of samples of male-dehydrated nectar as well as samples of nectar carried by foragers were *Melipona scutellaris, M. crinita, M. flavolineata* and *M. eburnea fuscopilosa*. *M. scutellaris* is a native species from the Mata Atlântica region of northeastern Brazil, whereas the others are from the Amazon region. In total 89 workers and 85 males were sampled. Some part of the data referring to the nectars of foragers from Amazon species was already published in Cortopassi-Laurino et al. (2007).

Male congregations occurrences from these species were observed in January and October in Xapuri and January in Igarassu. From these males, a nectar sample was obtained by gently pressing the individual's abdomen in a dorso-ventral direction. The regurgitated liquid was collected by placing a glass capillar between the bee's mandibles. After this procedure, the bee was immediately released. Next, the volume of the capillary liquid was measured in a refractometer (Atago) to determine the percentage of the nectar's sugars (grams of sugar/grams of liquid). Then, this percentage was converted into a value expressing the amount of sugars or sugar concentration (mg/ml) in the nectar regurgitated by the bee. To express the sugar concentration in terms of energy (J), concentrations were multiplied by 16.7 (DAFNI, 1992). Given that these samples have a non-normal distribution, we used the non-paremetric correlation of Spearman.

The thorax temperature of males in aggregations was measured with a non contact infrared thermometer Radioshack.

RESULTS

Male congregations occurrence is demonstrated in Table1. It shows that such groups were present mostly during the second half of the year. One nest of *Trigona spinipes* had male congregations during the same months of June or July, for four consecutive years.

Table. 1 Male Congregations occurrence in different regions of Brazil

Year	Month	Stingless bee
Acre State		
2004	Jan	Mc, Mflav, Mfusc
2000	Oct	Mc, Mflav.
Pernambuco State		
2006	Jan	Msc
São Paulo State		
2007	Oct	Ta, Pl
	Sep	Ta
	Jun	Ts, Scpt 1, 2
2006	Agos	Nt
	Jul	Ts 1, 2
	Jun	Scpt

2005	Agos	Scpt
	Jul	Ts 1, 2
	May	Scpt
2004	Nov	Pr
	Sep	Ta
	Jul	Ts
	Jun	Ts
2003	Sep	Scpt, Ts
	Jun	Ts
2002	Nov	Nt
	Oct	Ps

Mc = *Melipona crinita*, Mfusc = *Melipona eburnea fuscopilosa*, Mflav = *Melipona flavolineata,* Msc = *Melipona scutellaris*, Nt = *Nannotrigona testaceicornis,* Pl = *Plebeia* sp, Pr = *Plebeia remota*, Ps = *Paratrigona subnuda*; Scpt = *Scaptotrigona* sp, Ta = *Tetragonisca angustula*, Ts = *Trigona spinipes*.

The volume of nectar loads and the percentage of their sugars, regurgitated by males of *M. scutellaris* (n=40), represented in Figure1, showed that nectar becomes more concentrated over the day (3-48%), but that its total amount varied, revealing a maximum of 12μL. Sugar concentration correlated positively with temperature (Rho=0.686, *p*=0.01).

Related to the volume, (Table 2), the smallest bee of the four species studied, *M. flavolineata*, had the smallest nectar load, 16.8 μL for foragers and 6.6 μL for males. The other species, with similar body sizes, had maximum nectar volumes between 26.7- 40 μL.

When nectar regurgitated by males was compared with nectar regurgitated by returning foragers from the same species, as can be seen in Tab.2, the average nectar loads as well as the sugar concentration, were always higher in foragers. The nectar load for foragers was 1.6-3.9 times higher than in males. At the same time, the sugar concentration was 1.0-2.4 times higher for foragers. Comparing the maximum values of sugar concentration in nectar, only for *M.crinita* it was similar for both sexes (69% and 68%).

Measurements of the thorax temperature of males in aggregations, (Table 3), are positively correlated with ambient temperature (Rho=0.856, *p*=0.01, n=76).

Table 2. Volume and sugar concentration from nectar loads of *Melipona* spp(M= males, W= workers)

		n	vol.(μL)	sugar conc.(%)	mean:mg sug/ μL	max.:mg sug/ μL	vol.W/ vol.M	%sug.W/ %sug.M
	M	40	1.6-12.0	3-48	1.6	6.1		
M.scutellaris			x = 5.4	x =27.3	26.8 J		3.9 x >	1.3
	W	18	3.2-31.2	12-51	8.78	19.7		
			x = 21.1	x =36.1	146.6 J			
	M	33	2.7-29,3	6-69	1.7	26.6		
M. crinita			x =13.4	x =11.4	28.0 J		1.6 x >	2.4
	W	45	23.8-40	6-68	6.34	36.3		
			x =21.3	x =27.02	105.9 J			
	M	8	2.1-7.2	5-33	1.1	2.7		
M.e.fuscopilosa			x = 4.5	x =22.4	18.7 J		3.2 x >	1.6
	W	18	14.7-26.7	11-62	5.9	21.4		
			x = 14.7	x =34.7	98.6 J			
	M	4	4.0-6.6	8-26	1.0	1.9		
M. flavolineata			x = 5.3	x =18	16.7 J		2.7 x >	1.0
	W	8	8.7-16.8	11-31	2.7	9.8		
			x = 14.2	x =18.3	45.0 J			

DISCUSSION

Seasonal occurrence of male agglomerations

The production of males has been described as a short-lasting event and occurring in at least twelve species of Meliponini (VELTHUIS et al., 2006). In the State of São Paulo, where many observations were carried out, the highest frequency of male congregations occurring in the second half of the year corresponds to a common practice of regional stingless beekeepers to split colonies preferably at this time of the year, an activity that can hardly continue up to February. Other species for which male groupings have been observed are: *Frieseomelitta doederleini, Melipona subnitida, Trigona fulviventris* and *Plebeia* spp., which suggests that this phenomenon of male aggregations is universal among stingless bees.

Male groups can form early in the morning and persist all day, but the number of individuals participating in these groups increases progressively. In *P. droryana*, the time frame during which males may leave the nest is much wider (8:20-14:30h) than the time frame during which queens start their nuptial flight (13:00-13:10). (CORTOPASSI-LAURINO, 1979, CORTOPASSI-LAURINO, pers. comm.). A virgin queen of *M. crinita* was spotted outside her nest at the time the size of the male agglomeration was already large: 10:20h (37ºC and 53%UR).

The span of male groupings is variable but males of a *Plebeia* species, marked with a colored dust, were identified for at least seven consecutive days in the vicinity of a nest. The frequency of male groupings in *T. spinipes* and their seasonal occurrence was remarkable because they appeared during São Paulo's warmest winter days. It is possible that large *T. spinipes* colonies are less dependent on environmental conditions, which allows them to have more homogeneous cycles of supersedure and or production of queens.

It is known that when males leave their colony, they carry with them some nectar which would be enough to subsist the first few extranidal days, but whether this is sufficient for a longer period of time is yet unclear. With almost no literature citations on extranidal males visiting flowers, it is supposed that they either are forced to live on the colony-derived nectar or that they should receive additional nectar from worker bees by means of trophallaxis which, in the case of most species, would have to take place somewhere away from congregations. In *M. subnitida*, an endemic species of the caatinga, the males can form agglomerations like in the other species. However, they were also seen to form such type of groups together with workers at one of the lateral sides of a large stingless beekeepers' stable, a behavioral situation locally named "situar"; at such occasions, trophallaxis was observed between the two sexes. At the same time, both workers and virgin queens entered empty bee hives. The whole scenario suggests that males can receive food from workers when outside their nest, but if this is a frequent event and in which species it would occur, remains to be investigated.

Occasionally, certain wasps participated in these agglomerations by trying to obtain nectar from dehydrating males or from males having trophallaxis with other bees.

There can be no doubt that male congregations attract predators. Males in *Plebeia* sp, *Tetragonisca angustula* and *Trigona spinipes* have been observed to be attacked by wasps. In *Scaptotrigona postica*, Koedam et al. (2007), observed how the wasp *Trachypus boharti* (Rubio) predates exclusively on males which they select from clouds of flying bees in front of the bees' nest entrance. Chemical analyses have shown that the cuticular hydrocarbon profile of males and workers is significantly different (Koedam, pers. comm.). In *T. spinipes*, grouped males that are attacked seek cover by moving to the inferior part of leaves. Other predatory insects like reduviid bugs also have been observed to feed on males, for example on males of *M. scutellaris* grouped in bushes.

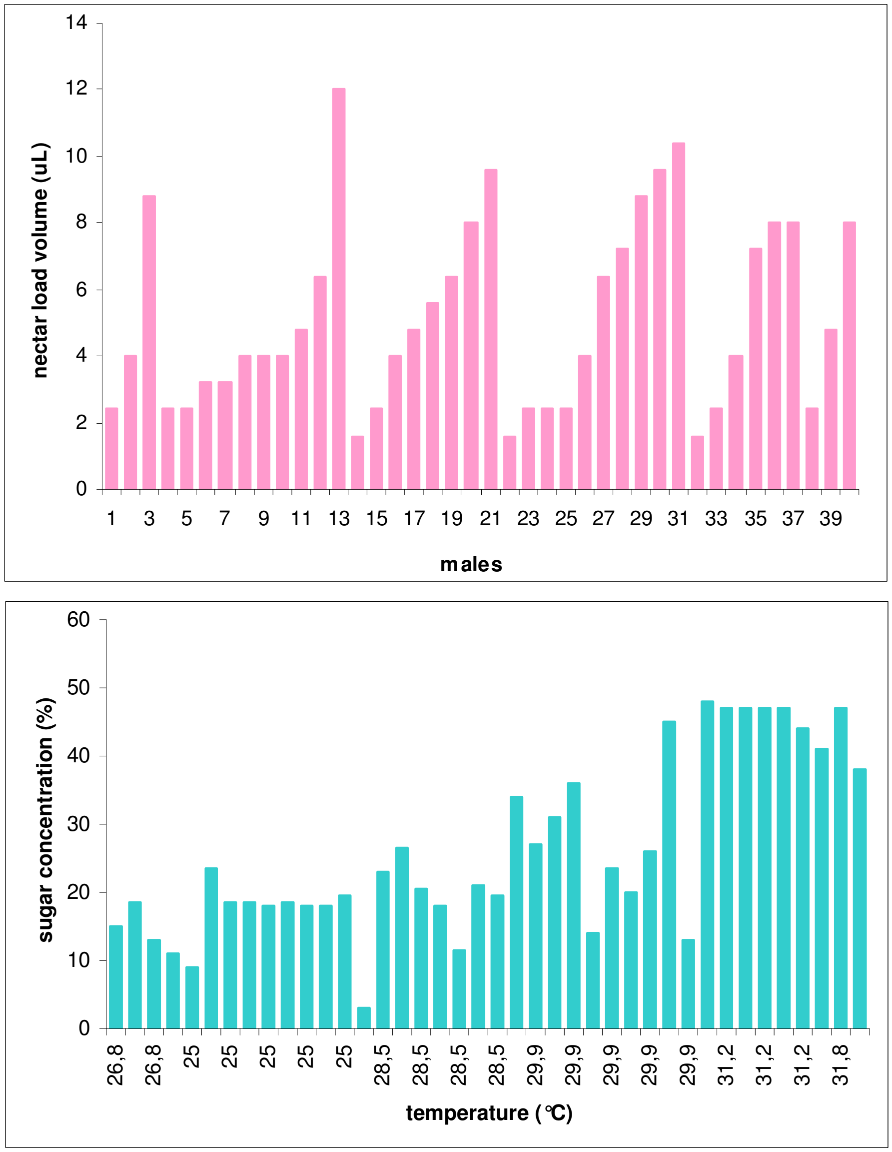

Figure 1. Volume and sugar concentration of the nectar loads from aggregated males of *Melipona scutellaris* in Igarassu-PE

Volume of regurgitated nectar

A bee is able to evaporate water from the nectar that it carries by alternately ingesting and regurgitating small quantities of it, and this process is visible as the constant disappearance and recurrence of a droplet between the bees's opened mandibles and extended tongue. Nectar load in worker bees may be associated with the availability of flowers and its morphological characteristics, and for males it may be with the level of nectar concentration during the process of dehydration.

We found that workers of the larger *Melipona* species carry out larger amounts of nectar and these results confirm earlier work on some of the smallest and largest stingless bees *M. marginata and M. fuliginosa* from Panama. For these species values of respectively 12.5 e 65.8μL were measured (ROUBIK; BUCHMANN, 1984). So far, related to volume, perhaps there is a relation between body size and nectar load, but more data should be analyzed to confirm this suggestion.

Sugar percentage of regurgitated nectar

The concentrations of sugars can be associated with different factors like morphological and physiological characteristics of flowers, the size and color of the bee, environmental conditions like temperature and humidity, and even competition between species (BIESMEIJER et al., 1999). The higher values of the temperature in the thorax of grouped males than that of the surrounding environment (Table3) may also facilitate the dehydration of nectar.

Table 3. Thorax temperatures of males in aggregations

	Thorax temperatures of males			
Local temperature	Media	Range	SD	N
27.5	27.5	25.4-31.0	1.7	11
29.5	32.8	28.4-37.8	2.8	53
31.5	32.8	31.2-39.8	2.4	12

In our research we found maximum sugar concentrations in nectar of workers to be between 31-68%, a value range which is smaller than what has already been found for Central American *Melipona* bees: 63-72.4% (ROUBIK; BUCHMANN, 1984, BIESMEIJER et al., 1999). In the African genera *Meliponula* and *Hypotrigona* the sugar concentration was between 41-43% (KAJOBE, 2007), and for Meliponini of Costa Rica this value was between 49-66% (ROUBIK et al., 1995). For bees of the genus *Trigona* from Borneo the average values lie between 24.9-48.6% (LEONHARDT et al., 2007). The nectar load data were all obtained from bees which exclusively carried only nectar which shows that the nectars foragers bring to their nest are generally concentrated.

The unexpectedly low concentration of nectars found in *M. flavolineata* foragers may be explained by the fact that this species nests exclusively high up in trees and therefore suffers from strong insolation. It may be that it therefore needs more aqueous nectar to help lower the temperature of its nest, when this reaches a critical level, by means of water evaporation. This hypothesis gets support from the fact that the honey of this species has on average the same sugar concentration as honeys from other species which nest at ground level like for instance *M. fuliginosa*, (nest that we observed and transferred to a box), indicating that in-nest nectar dehydration in *M. flavolineata* must be stronger. The yellowish body color of these workers may also be an important factor in the dispersion of heat.

Comparison between nectars carried by foragers and by males.

Our first conclusion is that males, like foragers, in general carry more aqueous nectars during the first hours of the day, a fact that is in accordance with Roubik and Buchmann (1984), for foragers of *Melipona* spp. Before leaving the nest, between 8:20-14:30h, males of *P. droryana*, receive and dehydrate nectars which are collected by returning foragers. This fact suggests that most of the flowers visited by foragers produce more aqueous nectar in the morning and that, along the day, these concentrations augments because of evaporation or because of a higher production of sugars. *Tithonia diversifolia* (Asteraceae), where *M. scutellaris* workers collected nectar is an example of flower producing aqueous nectar in the morning.

It is interesting to notice that males always carried the smaller nectar volumes, even considering that their body size are similar to those of the workers. This may be related to the fact that males manipulate nectar for their own purpose instead of collecting nectar from flowers and flying it to the nest to be shared among colony members. In our opinion, the nectar load of workers being two to four times the size of that of males stresses the possible difference in altruism among the sexes as far as the manipulation and use of colony-derived nectar is concerned.

The concentration of sugars was found to be up to 2.5 times higher in nectars of workers than of these males of the same species. Inside the colony, these nectars are dehydrated by workers up to a point where they consist of only 25-30% of water which marks their transformation into pure honey (CORTOPASSI-LAURINO et al., 2007). For workers, the higher the concentration of sugars in nectars harvested in the field, the less labour the nest-bees have to condense the nectars to the required level. However, it seems that for certain species the evaporation of the nectars' water is also needed to reduce heat inside the colony or to control humidity. A single observation on *M. crinita* illustrates this fact when we observed workers vibrating their wings and at the same time dehydrating nectar at the entrance of their nest which had received strong insulation over the whole afternoon.

The energy in Joules calculated from the nectars demonstrates that these values are similar for the four species of males studied, but clearly lower than the values based on nectars derived from worker bees (45.0-146.6 J). This, once more, suggests that males use nectar mainly for themselves and not to feed other bees.

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RESUMO: No ciclo de vida dos machos de Meliponini, depois que deixam os ninhos, eles podem se agrupar formando aglomerados com dezenas a centenas de indivíduos. Nestes locais, onde vários machos retornam durante dias consecutivos, eles desidratam néctar. Análises deste néctar transportado indicam que a concentração apresenta correlação positiva com a temperatura, e que o volume e a porcentagem de açúcares são sempre menores do que os das operárias das quatro espécies de *Melipona* estudadas. Outras fontes de origem deste néctar, além do ninho de origem dos machos são desconhecidas.

PALAVRAS-CHAVE: Abelhas sem ferrão. Agregados de machos. Cargas de néctar. Concentração de açúcares

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