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ON SOCIAL REGULATION IN NANNOTRIGONA (SCAPTOTRIGONA) POSTICA LATREILLE, WITH SPECIAL REFERENCE TO MALE PRODUCTION CYCLES (HYM., APIDAE, MELIPONINAE)

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RESUMO - A produção de machos em *Nannotrigona (Scaptotrigona) postica* e alguns aspectos correlacionados foram investigados e os resultados mostram que os fatores extrínsecos como temperatura e precipitação, através da sua importância na determinação das floradas e subsequente aumento na disponibilidade de mel e pólen, não são fatores determinantes imediatos da alta produtividade da colônia e da produção de machos, como consequência. Os dados também sugerem uma ausência de inibição da rainha no desenvolvimento ovariano das operárias e que a produção de machos ocorre independentemente da idade fisiológica da rainha. É possível que muitos outros fatores intrínsecos como alta densidade populacional da colônia (provavelmente consequente de condições favoráveis de estoque de pólen e mel) sejam mais importantes na determinação da produção de machos, uma vez que colônias que apresentam uma baixa densidade populacional não produzem machos, embora os ovários das operárias estejam sempre desenvolvidos. Além disso, a produção de machos parece estar ligada à oviposição das operárias, devido à relação existente entre ocorrência de machos e células de cria contendo mais do que um ovo por célula, numa mesma colônia.

ABSTRACT - The production of males in *Nannotrigona (Scaptotrigona) postica* and some related aspects were investigated, and the results show that the extrinsic factors, such as temperature and precipitation, through their importance in flowering and subsequent increase in the availability of honey and pollen do not immediately determine high colony productivity and production of males. The data also suggest an absence of queen inhibition in the ovarian development of the workers, and that male production occurs irrespective of the queen's physiological age. It is possible that many other intrinsic factors, such as high populational density

of the colony (probably a consequence of favorable conditions for storage of pollen and honey) are more important in determining male production, since colonies presenting a low populational density do not produce males, although the ovaries of the workers are always developed. Besides, the production of males seens to be related to workers oviposition, due to the relationship between the occurrence of males and brood cells containing more than one egg for each cell in the same colony

INTRODUCTION

The study of social regulation has been undertaken as an attempt to clarify many important aspects of the evolution of social behavior in insect societies.

Stingless bees have been studied from different standpoints; one of these which interests the author is connected with the production of males, its relationship with workers oviposition, and the main ethological factors involved in these processes.

It is known that in stingless bees, queens and workers are produced from fertilized eggs and males from unfertilized eggs. These latter are produced from workers' and queens' eggs (in most species) or only from queens' eggs (in a few species).

This fact is probably related to the ability of the workers to develop their ovaries and also some ethological mechanism inherent to each species in particular.

In *N.(S.) postica,* workers show two kinds of morphologically distinct eggs. One is the nutritive egg, large and roughly spherical, whose function is exclusively to feed the queen (Sakagami & Zucchi, 1963; Akahira, Sakagami & Zucchi, 1963; Dias, 1973; Bego, 1974, 1977) The other is the male-producing egg type, which produces males when laid inside the brood cells (Beig, 1972) Later, Dias (1973) observed that this latter type of egg, which was called functional egg, also was laid at the cells inner upper edge and immediately eaten by the queen or sometimes the workers. All these data together were important in starting our investigations on the controlling mechanism of male production and its implications in the oviposition process of the workers in *N. (S.) postica.*

MATERIAL AND METHODS

Seven colonies of *N.(S.) postica* were kept in hives based on those described by Nogueira-Neto (1970) They were from Batatais, and Ribeirão Preto, São Paulo State.

Over about two years, the rates of newly emerged workers and males were estimated by using samples of combs in pre-emergence phases. These combs were placed in an incubator until the emergence of the bees. After that, all samples of bees were returned to their respective colonies.

The observations were carried out at intervals of 10 days, so that there could be three samples per month. At the same time, when the colonies were opened, the internal conditions (diameter of the new combs, frequency of the bees working at the brood region, and the storage pots of food) were also observed.

According to these observations, the criterion used in classification of the colonies was: (+) weak, (++) regular, (+++) medium, (++++) strong.

The queen's age was also verified by using a code (colored paint on the thorax), and by the degree of wear to the queen's wings.

Once a month 20 ∽ 30 workers, whose preferential tasks were centered on the new brood combs ("nurse bees") were collected from the colonies. In general, these workers are preferably young, presenting a pale scutellum. However, older workers (intermediate age (I) and old (O)) were also sometimes collected, either because they were on the new combs working effectivelly, or exceptionally. This information was obtained through further observations carried out in observations hives.

The samples of workers were fixed in Dietrich for three days, then placed in 70% alcohol for observation. They were later dissected under a stereoscopic microscope and the ovarian development was estimated (fig. 3)

Finally, once a month, a small new brood comb was taken from each colony and the brood cells were opened under a stereoscopic microscope to record the frequency of cells containing one or more eggs.

The climatic data were taken at The Experimental Station of Ribeirão Preto (SP)

RESULTS

1. Male production cycles related to extrinsic factors

The results given in figs. 1 and 2 show that male production was asyncronic, i, e, the frequencies over about two years were variable from month to month, year to year and colony to colony.

In colonies 12 and 13 production of males followed successively, practically without interruption, whereas in colony 36 this fact did not occur. Male production was verified only in January, April, May and June at a very low frequency.

In colony 18, production of males took place at reasonable rates in November, December (1974), and May,June(1975). In April (1975) the frequencies were higher. In the succeeding periods the rates were insignificant or even, in some months, nil.

The rates found in colony 16, were more expressive during the following periods: April, May, July (1975), and February, March (1976)

In colony 24, male production was more expressive in April, May and June (1975). From July to January (1976) the rates were nil or practically nil; nevertheless, from Februa

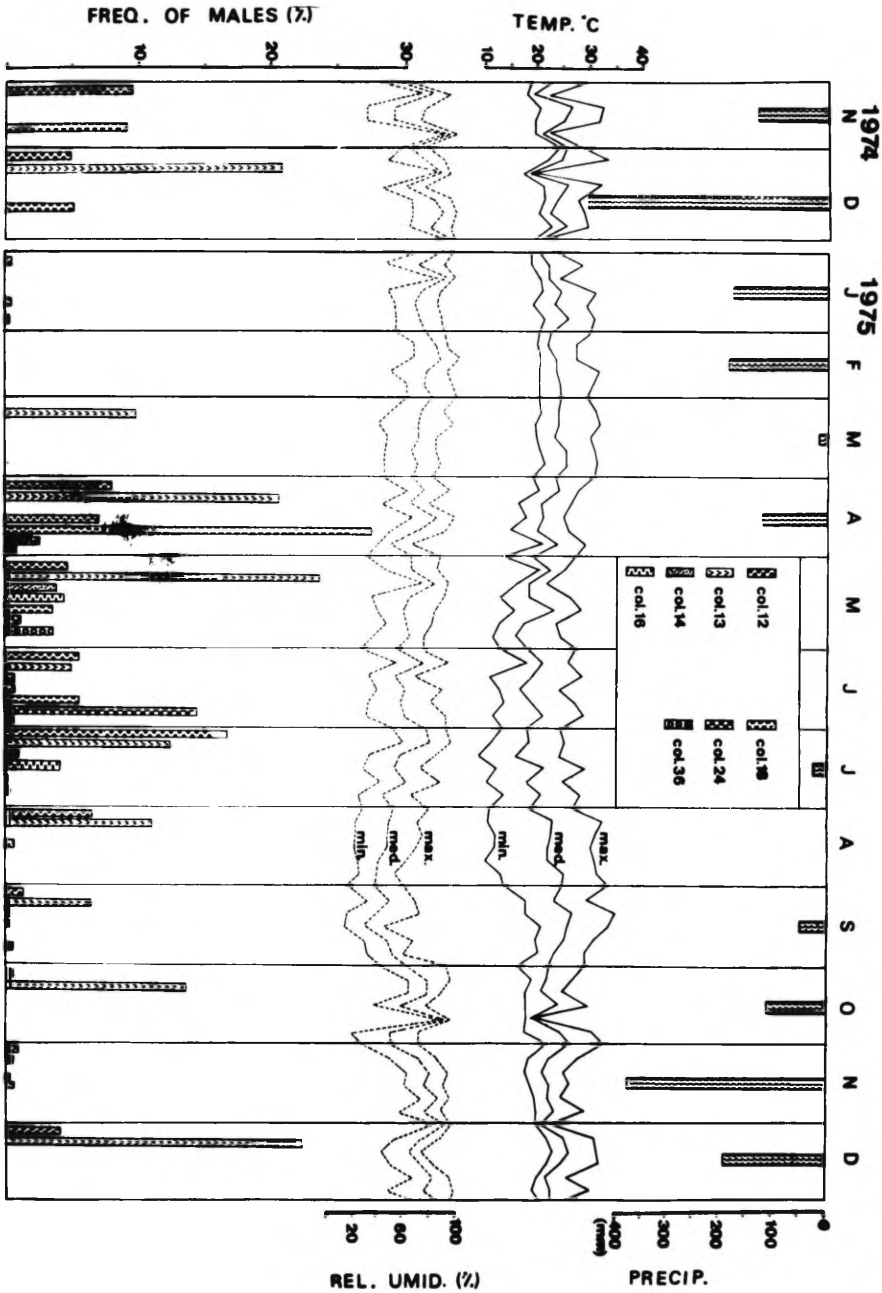

Figure 1 - Frequency of males related to climatic factors from November, 1974 to December, 1975.

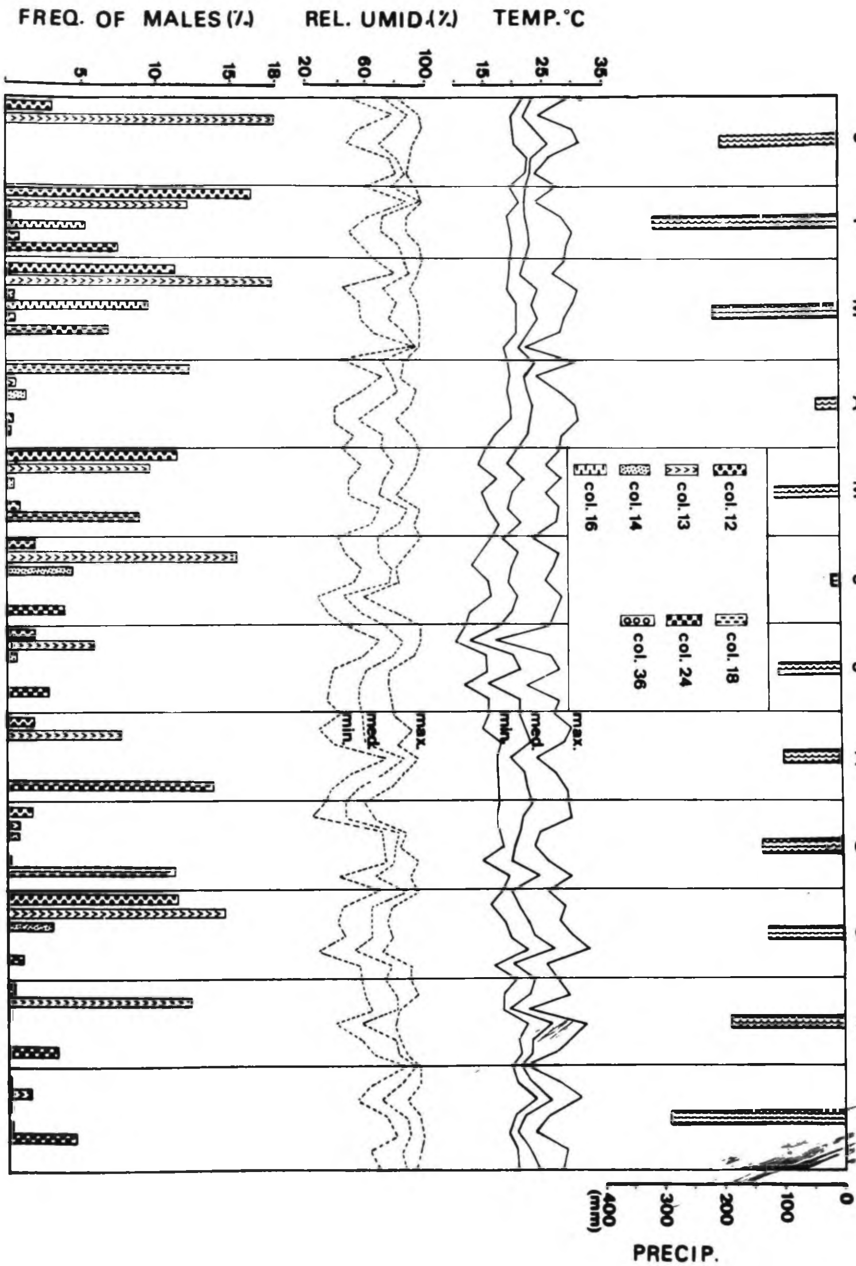

Figure 2 - Frequency of males related to climatic factors from January to December, 1976.

ry to December (1976), there were males in almost all the months, although the rates showed some variation.

Finally, in colony 14, the rates also did not have a fixed quantitative value. Males were produced in May, June, July and September (1975), and from February to December (1976), except in August. The values were always medium in some months and low in other cases (see the average frequencies in table 1)

Table 1 - Yearly average frequency (%) of males in *N. (S.) postica* colonies.

Number of colonies	1975	1976	Total average frequency (1975 and 1976)
12	3.97	6.10	5.04
13	10.42	9.67	10.04
14	0.50	0.94	0.72
16	1.33	1.21	1.27
18	3.10	0.26	1.68
24	1.55	5.31	3.43
36	0.50	0.00	0.25

2. Intrinsic factors of the colonies, related to male production.

From the results given in table 2 it can be seen that the strong colonies, which present high populational density (cols. 12 and 13) produce males at higher rates. This tendency is confirmed in colony 36 which, during almost all the time of observation, was in unfavorable conditions.

Although it has not been possible to quantify exactly all parameters (increase of the brood combs, and number of storage pots of food), we are sure that our qualitative criteria were important to control subsequent experiments,which were able to prove this fact (Bego, in preparation)

As to physiological age of the physogastric queens, it was possible to show that there was no relationship between this fact and production of males. In colony 12, males were produced before and after queen supersedure, and in colony 36 there were no males either before or after queen replacement.

Other types of combinations can be seen in table 2 for all colonies.

3. Worker oviposition related to male production.

The workers always develop their ovaries producing two types of eggs, nutritive (N) and functional (F), according to established stages (figs. 3, 4, 5 and tables 3, 4)

Table 2 - General conditions of the colonies (food, population and age of physogastric queens) in different periods of the year.

Periods	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
	1975									1976											
Number of the colonies																					
12	m ++++ OQ	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++ QR	m ++++ YQ	m ++++	m +++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	- ++++	- ++++
13	m ++++ YQ	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m +++	m ++++	m ++++	m +++	m ++++	m +++	m +++	m ++++	m +++	m ++++	- ++++	- +++
14	m ++++ YQ	m ++++	++++	m ++++	++++	++++	+++	++++	m +++	m ++++	m ++++	m ++++	m ++++	m ++++	++++	m ++++	m ++++	m ++++	m ++++	- ++++	- ++++
16	m ++++ YQ	m ++++	m ++++	m ++++	++++	m +++	++++	++++	m +++	m +++	+++ QR	++ YQ	++	++	++	+	+	+	+	- +	- +
18	m +++ QIA	+++	++	+	+	m +	+	+	m +	m ++	m +++	m +++	++ QR	++ YQ	++	m +	+	+	m +	- ++	- ++
24	m ++++ OQ	m +++	+++	m ++	++	+++ QR	+++ YQ	+++	m +++	m +++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	m ++++	- ++++	- ++++
36	m ++ OQ	++	++	+	++	+	+	++	+	++	+++ QR	+++ YQ	+++	++	++	+++	+++	+++	+++	- +++	- +++

QR - Queen replacement YQ - Young queen OQ - old queen QIA - Queen with intermediate age
+ weak ++ regular +++ medium ++++ strong m - males - no observed

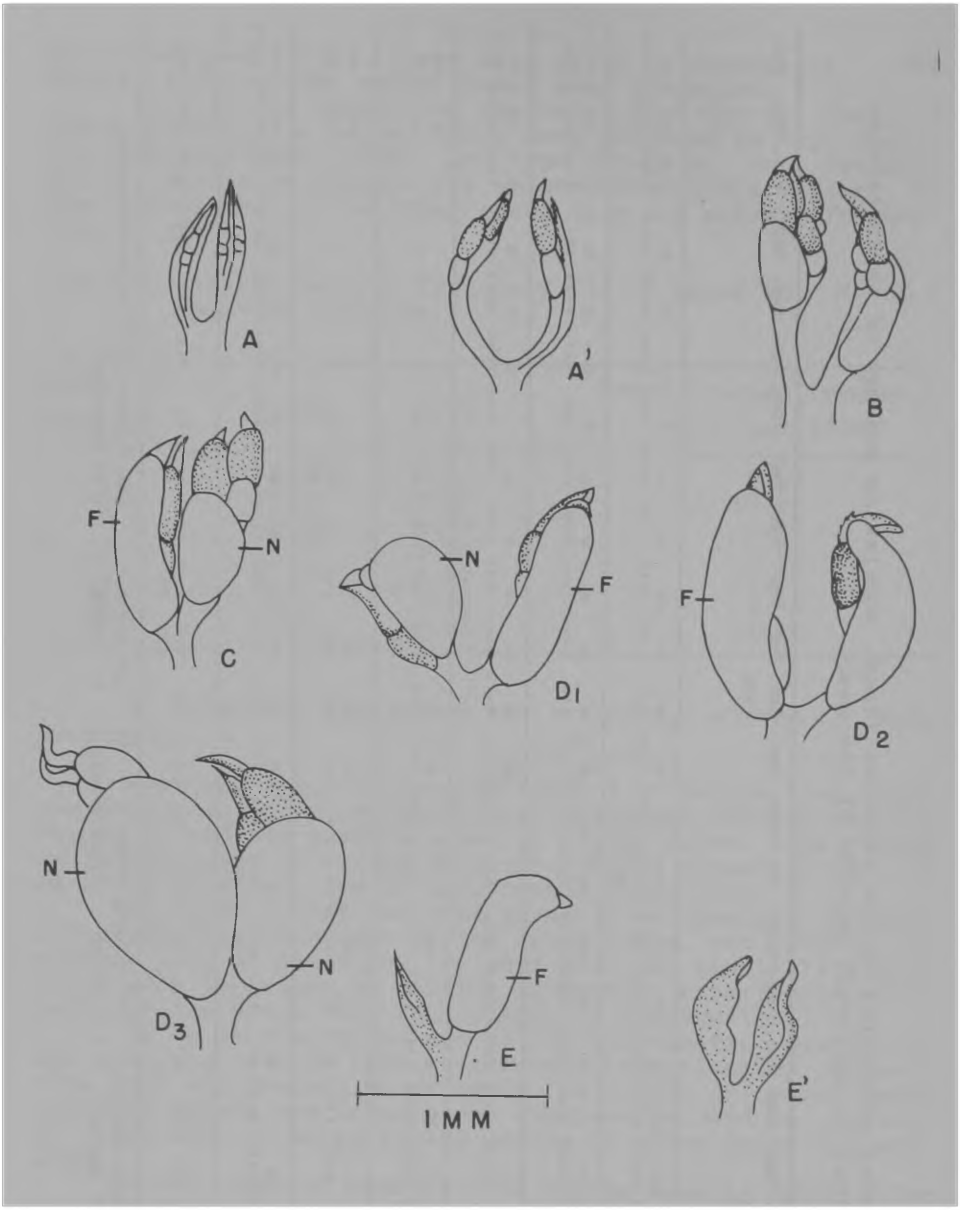

Figure 3 - Ovarian development stages in workers of *N. (S.) postica*. A,A' - undeveloped; B - beginning to develop; C - Developed; D_1, D_2, D_3 - fully developed; E - in degeneration; E' - degenerate eggs and oocytes N - Nutritive F - Functional.

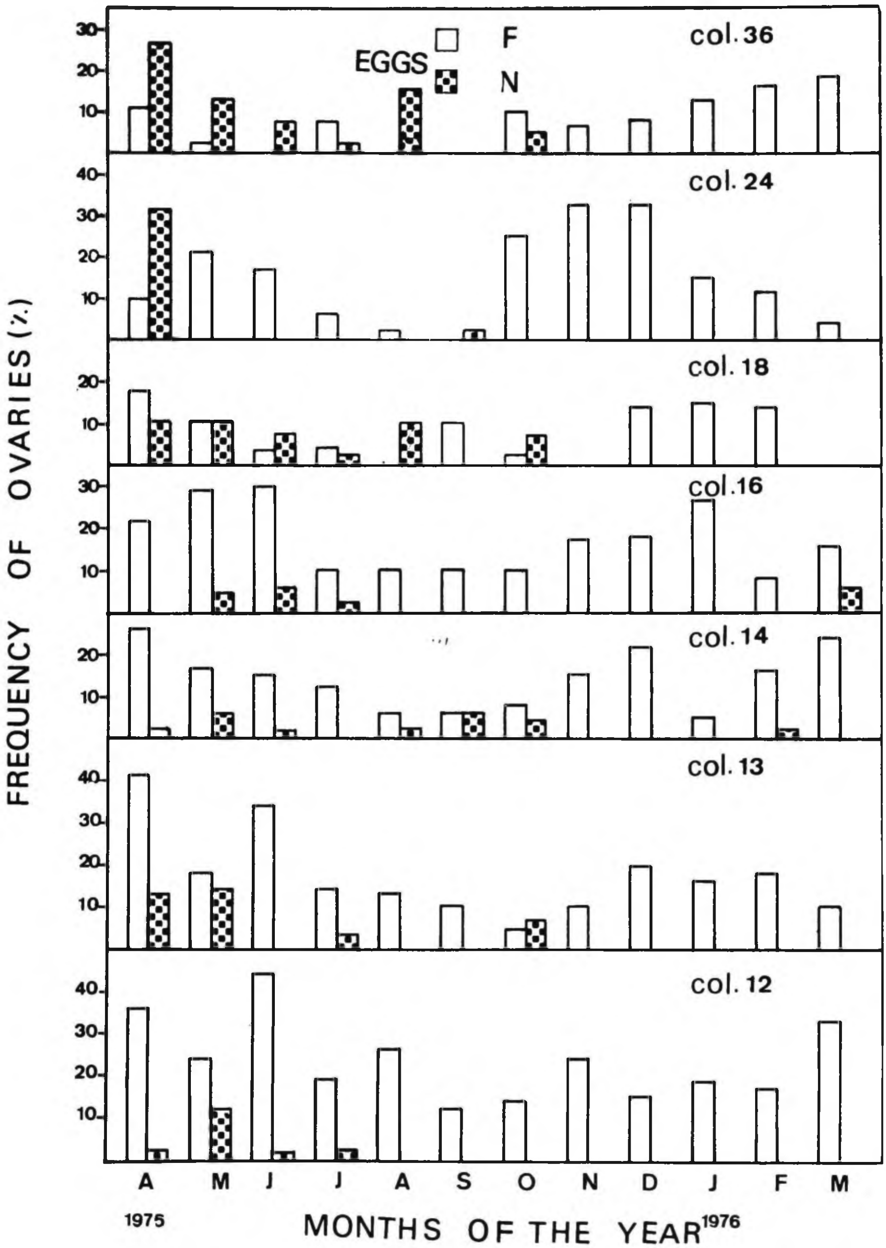

Figure 4 - Frequency of nutritive (N) and functional(F) eggs, in workers whose preferential tasks are centered in the new brood combs ("nurse bees")

Table 3 - Frequency of ovaries with nutritive (N) and functional (F) eggs related to relative age of the workers, whose preferential tasks are centered in the new brood combs ("nurse bees").

Number of the colonies	Frequency of ovaries with nutritive (N) and functional (F) eggs														Relative age of the workers
	12		13		14		16		18		24		36		
Periods	F	N	F	N	F	N	F	N	F	N	F	N	F	N	
April/75	21Y	1I	16Y	5 (3I/2Y)	16Y	1I	13Y	0	10Y	6I	3Y	9 (5Y/4I)	6Y	15 (13Y/2I)	the most part Y
May	10Y	5 (2I/3Y)	9Y	7 (3I/4Y)	9 (8Y/1I)	3Y	14Y	2Y	5Y	5Y	3Y	0	1Y	5I	the most part Y
June	24Y	1Y	19Y	0	9Y	1Y	11Y	2Y	1Y	2 (1Y/1I)	4Y	0	0	1Y	Y
July	9Y	1Y	5Y	1Y	6Y	0	5Y	1I	2Y	1I	3Y	0	3Y	1I	Y
August	13Y	0	7Y	0	3Y	1Y	5 (4Y/1I)	0	0	3θ	1Y	0	0	4θ	Y; θ for colonies 18,36
September	6Y	0	5Y	0	3Y	3Y	4Y	0	1Y	0	0	1Y	0	0	Y
October	7Y	0	2Y	3Y	4Y	2Y	4Y	0	1Y	3θ	10Y	0	4θ	2θ	Y; θ for colonies 18,36
November	10Y	0	4Y	0	6Y	0	7Y	0	0	0	13Y	0	3Y	0	Y
December	6Y	0	7Y	0	9Y	0	6Y	0	4Y	0	13Y	0	3Y	0	Y
January/76	7Y	0	6Y	0	2Y	0	11Y	0	6Y	0	6Y	0	5Y	0	Y
February	7Y	0	9Y	0	8Y	1I	4Y	0	7Y	0	6Y	0	8Y	0	Y
March	16Y	0	4Y	0	12Y	0	8Y	3Y	0	0	2Y	0	9Y	1Y	Y

Y - Young workers; I - workers of intermediate age; θ - old workers

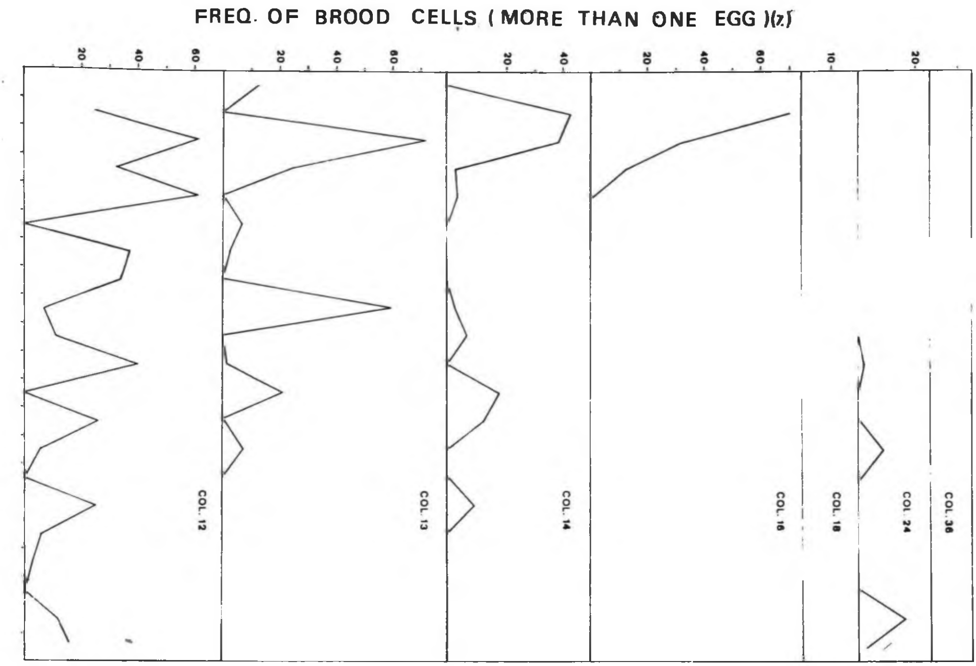

Figure 5 - Frequency of brood cells containing more than one egg for each cell, in different periods of the year.

During one year of observations it was possible to detect that the workers ("nurse bees") preferable develop functional eggs (F) The frequencies observed were variable in the same colony and from colony to colony throughout the year.

These workers have a pale scutellum and can easily be seen over the new brood cells in construction. The nutritive eggs (N) are also always produced, but, in low frequencies in this particular phase of the worker life cycle (fig. 4 and table 3). These latter are found at higher rates in older workers (intermediate (I) and old (O) relative age) These bees present a dark scutellum,and they do not usually work in the new brood cells in connection with construction and provisioning (Bego, 1981, in press)

As to workers' oviposition (functional eggs) it can be verified that there is a tendency to correlate phases of males production and number of cells containing more than one egg (fig. 5, 6 and table 4), thus strengthening the data of ··ig (1972).

Table 4 - Total frequency of functional eggs laid by the workers, inside of the brood cells

Number of Colonies	Number of eggs per brood cells	Number of cell with eggs	(%) of cells with eggs	Total number of opened brood cells
12	1	796	82.49	
	2	129	13.37	
	3	28	2.90	
	4	6	0.62	
	5	6	0.62	
				965
13	1	808	92.45	
	2	47	5.38	
	3	9	1.03	
	4	5	0.57	
	5	4	0.46	
	6	1	0.11	
				874
14	1	891	95.60	
	2	36	3.86	
	3	5	0.54	
				932
16	1	738	96.60	
	2	24	3.14	
	3	1	0.13	
	5	1	0.13	
				764
18	1	668	100.00	
				668
24	1	821	98.44	
	2	12	1.44	
	3	1	0.12	
				834
36	1	995	100.00	
				995

Table 5 - Observation about periods of male production

Species	Geographical region	Time of production	Author
Plebeia (P.) droryana	-	October	Nogueira-Neto (1954)
Plebeia (P.) droryana	Ribeirão Preto - S.P.	January∽April∽May; September∽December	Terada (1980)
Plebeia (F.) shrottkyi	-	March	Nogueira-Neto (1954)
Plebeia (F.) shrottkyi	Ribeirão Preto - S.P.	over whole year; one colony increased its frequency in June and July, before queen replacement.	Camillo-Atique(1977)
Nannotrigona (N.) testaceicornis	-	July	Nogueira-Neto (1954)
Melipona quadrifasciata anthidioides	-	August	Kerr (1969)
Melipona quadrifasciata anthidioides	Ribeirão Preto - S.P.	August and May	Silva (1973)
Melipona marginata	-	August and September	Kerr (1969)
Nannotrigona (S.) postica	Rio Claro S.P.	July∽August; September∽February	Beig (1972)
Bombus atratus	Ribeirão Preto - S.P.	varies from year to year and from colony to colony	Zucchi (1973)

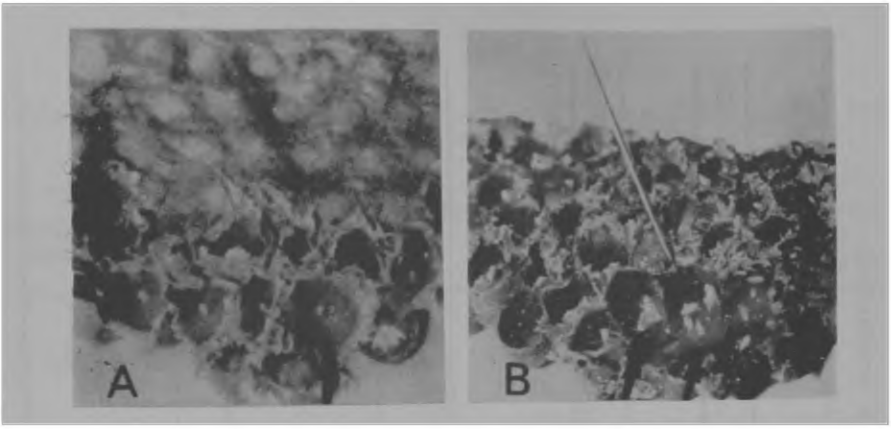

Figure 6 - Brood cells opened showing two (A) and more eggs (B) for each cell.

DISCUSSION

Several authors recorded periods of male production,in Meliponinae and *Bombus* in Brazil according to table 5.

Male production in Meliponinae as well as many other aspects must be connected with extrinsic and intrinsic fac - tors of the colonies.

It is known that, in addition to climatic factors which are determining in flowering and subsequent storage of food, there are internal mechanisms in the colonies which control the production of males. In some species of stingless bees under orphanage the workers develop their ovaries and lay eggs inside of the brood cells, and as a result the males are produced. This fact occurs in some species of *Plebeia* (Zucchi, 1973; Imperatriz-Fonseca & Oliveira, 1976; Camillo-Atigue, 1977; Terada, 1980), *Leurotrigona* (Terada, 1974),*Partamona (P.) testacea* (Sakagami,Beig & Akahira, 1964) and *Paratrigona subnuda* (Bego, unpublished)

In normal colonies, in some phases of the colony cycle, some of these species are even able to lay eggs and produce males. Zucchi (1973) suggests that in many of these cases there is partial inhibition by the physogastric queens.

In *Frieseomelitta* and probably *Duckeola,* even in queenless colonies the workers cannot develop their ovaries (Zucchi, 1973; Terada, 1974), and finally, in *Melipona* and *Nannotrigona (S.) postica* the workers always produce eggs (Silva, 1973; Zucchi, 1973; Sakagami & Zucchi, 1963; Beig, 1972; Dias, 1973; Bego, 1977, 1981). According to Silva (1973) in *Melipona* the males originate from queen and workers eggs, and in *N. (S.) postica,* most of them from workers (Beig, 1972)

Social regulation of male production in *N.(S.) postica* seens to be different from most species mentioned above.Good evidences to show absence of inhibition by the physogastric queens are summed up as follows:

1. The workers oogenesis and laying in the brood cells are

usual events;

2. Male production is not related to the physiological age of the queens;
3. Colonies under orphanage did not increase the rates of developed ovaries (Bego, 1974, 1977, 1981)

Our data suggest that the internal conditions of the colony such as populational density, amount of food and perhaps some ethological factors can be more directly responsible for male production. These last aspects will be intensively discussed later, in a subsequent paper.

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