Review article

Division of labor during brood production in stingless bees with special reference to individual participation

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Abstract – The focus of this paper is the process for brood production known as the Provisioning and Oviposition Process (POP), and particularly the individual behavior observed in the facultatively polygynous stingless bee *Melipona bicolor*. Following individually marked bees revealed that ovarian development is correlated with individual behavior differences. While most of the eggs laid by workers are consumed by the queen (trophic eggs), workers contribute significantly in male production with reproductive eggs, illustrating the reproductive conflict at the individual level. From an evolutionary outlook, "benefactor" behaviors may evolve if workers conserve the "hope" of reproduction. This indicates that an important function of trophic eggs is to keep the ovaries active. It is also possible that ovary development represents an internal factor promoting division of labor: reproductive workers are specialized or elite bees with low response thresholds and high activity levels that restrain the participation of other workers.

stingless bees / division of labor / POP / *Melipona bicolor* / reproductive competition / Apidae

1. DIVISION OF LABOR IN STINGLESS BEES

It has long been acknowledged that division of labor among hymenopteran workers presents age-correlated patterns of task performance (temporal polyethism) that spatially follow a centrifugal sequence (Wilson, 1985). In stingless bees, very young bees produce wax and work in the brood nest where they were born. They move further away from it as they age until they leave the nest to become foragers. Although the age ranges during which particular tasks are executed vary across species, bees generally pass through four stages: callow, nurse bee, housekeeper and forager. The tasks associated with these stages are: (1) incubation and repairs of the brood chamber; (2) construction and provisioning of cells, cleaning of the nest, and feeding young adults and the queen; (3) further cleaning of the nest, reconstruction of the involucrum, reception and ripening of nectar,

and guard duty at the entrance of the nest; (4) foraging for pollen, nectar, propolis and other materials (Wille, 1983). Meliponine workers exhibit considerable flexibility in task allocation: tasks are not rigidly established but depend on the conditions of the colony. For example it is possible to force a colony consisting only of old workers to rear brood by partially re-activating the hypopharyngeal glands (Sakagami, 1982).

2. DIVERSE APPROACHES TO THE STUDY OF DIVISION OF LABOR

Classic works representing purely ethological descriptions on division of labor in meliponines date back to 1955. Since then, a multitude of studies have addressed general aspects of division of labor, the provisioning and oviposition process (POP), conflict between colony members, evolution of behavior, taxonomical comparisons, and systematics versus behavior (Online supplementary list

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of references[1]). However, few focused on specialization and individual behavior.

Although in many of the studies bees were individually marked, results were presented for age groups providing a general pattern and thus lacking information on individual differences in behavior. Individual differentiation has been studied in other Hymenoptera, e.g. in ants (Gordon, 1999), wasps (O'Donnell and Jeanne, 1990) and in *Apis* bees (Visscher and Camazine, 1999). In stingless bees, intranidal individual behavior has been studied in *Melipona favosa* (Sommeijer et al., 1982; Kolmes and Sommeijer, 1992), *M. bicolor* (Bego, 1983), *Trigona* (*Tetragonula*) *minangkabau* (Inoue et al., 1996), and *M. subnitida* (Koedam et al., 1999). As for extranidal tasks, some works that addressed individual differentiation related to food and resin collection has been described in *M. beecheii* (Biesmeijer and Tóth, 1998), and concerning task partitioning in nectar collection, there is information compiled for five species of stingless bees (Hart and Ratnieks, 2002).

Recent models of colony organization are interested in how mechanisms at the individual level generate organization and behavior at the colonial level. It is now recognized that the genotype of a colony is distributed among hundreds or thousand of genetically diverse individuals. In computer simulations, division of labor emerges by introducing minimal variances in the responses of individuals to a task; such results may indicate that organization in a colony emerges as a self-organized system derived from the independent actions of workers (Ronacher and Wehner, 1999). In order to clarify the mechanisms that generate colonial organization and behavior, models based on proximal mechanisms that incorporate both internal and external factors have been developed (Beshers and Fewell, 2001). The response threshold model refers to internal individual limits that regulate individual responses to diverse tasks. Such a model assumes that response thresholds are specific for each task and that each individual can have a different threshold for each task (Beshers et al., 1999; Bonabeau and Theraulaz, 1999).

This concept generates a simple but powerful feedback system that distributes the appropriate number of workers for each task, in a self-organized manner. For social insects there exist empirical evidences supporting such models (Robinson, 1992; Bonabeau and Theraulaz, 1999; Beshers and Fewell, 2001). Thresholds can be fixed when they do not change with age, may be reinforced by repetition, or may be "forgotten" if not performed (Bonabeau et al., 1996). Literature also presents empirical evidence in favor of the existence of reinforcement thresholds in social insects (Theraulaz et al., 1991; O'Donnell, 1998; Robson and Traniello, 1999). In stingless bees, indirect evidence for response thresholds in *M. bicolor* is found in the pioneering work of Bego (1983). She perceived that each individual carries out different percentages of the tasks and never the total repertoire of the colony. She verified that, on average, an individual carries out 50% to 70% of the total number of tasks. This research demonstrates worker heterogeneity in stingless bees.

Among a group of workers available for the execution of a task "x," some individuals may present a high preference for such task and dedicate themselves more frequently when compared with the average task performance frequency of other workers of the same age. These workers are called specialists or elite (Robson and Traniello, 1999). There is empirical evidence in favor of the existence of specialization in Hymenoptera (Rissing, 1981; Calderone and Page, 1988; O'Donnell and Jeanne, 1990). Kolmes and Sommeijer (1992), working with the stingless bee *M. favosa*, found that the construction of brood cells is a task performed by an elite group of workers. Inoue et al. (1996) presented evidence on individual variation between workers for the bee *Trigona* (*Tetragonula*) *minangkabau*. They verified that the population of a nest can be divided into two main groups: nurse bees that can spend all their lives taking care of the brood, and foragers that hardly or never work on the comb. Here we see evidence for strong and very limited response thresholds leading to extreme specializations.

Finally, individual behavior must be integrated with colonial organization. The model

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[1] Available at: http://www.edpsciences.org

of social inhibition explains that temporary polyethism is the result of the interaction of an intrinsic process of behavioral development with an inhibiting effect produced by other workers (Huang and Robinson, 1999). This model combines the physiological state in relation to the task together with the external influence produced by the interactions between workers (Beshers and Fewell, 2001).

3. THE PROVISIONING AND OVIPOSITION PROCESS IN STINGLESS BEES

Brood production resembles that of solitary bees and follows a general pattern in all species of stingless bees. Workers construct and mass provision the cells; the queen then lays her eggs on top of this liquid provision, and, finally, the workers seal the cells. This sequence of behaviors has been termed the provisioning and oviposition process (POP). Within each cell, the new individual develops without any further interference from its kin. The first POP in meliponine was observed by Drory in 1872 in *M. scutellaris* and since POP has been recognized as a highly elaborate process with complex social interactions. The most outstanding fact commonly observed in this process is the laying of two types of eggs by the workers: trophic eggs (TEs) that are consumed by the queen, and reproductive eggs (REs) that develop into males (Sakagami, 1982). During the period when bees are most engaged in brood care, it is noticeable that their wax production is at its peak, and dissection reveals developed ovaries, while the crop contains large amounts of pollen. Such factors are not found when bees end their participation in the POP (Sakagami et al., 1963; Bego, 1990; Simoes and Bego, 1991).

The POP has been studied in at least 50 species of stingless bees. Classic works on POP observed characteristics such as age of the bees involved, number of provisions per cell, time interval between ovipositions, daily oviposition rate, duration of pre-provisioning, provisioning and post-provisioning phases and general behavioral interactions between the queen and the discharging workers. The process was divided in phases and definitions for the diverse repertories were established (Sakagami and Zucchi, 1966). Comparisons of the POP in different species have been used to establish relations between systematics and behavior (Sakagami and Zucchi, 1966; Drummond et al., 2000) and phylogenetic relationships (Zucchi et al., 1999).

For all meliponine, Sakagami (1982) recognizes four patterns of cell construction and provisioning:

- Successive: provisioning of cells occur one by one followed by the oviposition of the queen as happens in *M. bicolor*. In this species, cells are at different stages of construction at any given time so there are several provision and oviposition cycles per day.
- Synchronous: several cells receive provisions at about the same time. The queen can oviposite all at once, or in batches.
- Semisynchronous: successive cell construction is followed by synchronous provisioning.
- Composite: in a given comb there is primarily synchronous provisioning, but some cells are provisioned in succession.

In meliponine bees, cells can be constructed in clusters or in horizontally or spirally organized combs. Only in *Dactylurina* are the combs vertical and double layered, which is a remarkable parallel to the cell arrangement used by *Apis*. Cells can be spherical- or columnar-like; they are made of pure wax or cerumen (Wille, 1983; Nogueira-Neto, 1997). Each cell is built by several young wax-producing workers. Complete cells have a rim of wax, called a collar, which is used for sealing it after oviposition. The number of cells produced per day varies from about 10 in *Melipona* colonies up to several hundred in *Trigona* species. In *Melipona* species, all bees are born from identical cells, while in most other genera, queen cells are larger than those that produce workers and males (Sakagami, 1982). Sommeijer et al. (1982) followed individuals of the stingless bee *M. favosa* and demonstrated that groups of workers build larval cells and subsequently provision them as teams. However, inactivity is often recorded as one of the most common behavioral states

of workers in the nest. Later this same author (Sommeijer et al., 1984) states that the court of workers that surround the queen during her resting periods, termed the extra-oviposition periods, consists of the same individuals that were mostly engaged in cell construction. The authors therefore conclude that the function of such extra-oviposition courts is principally to convey information to the queen and encourage her to set out towards the completed cells to lay.

Provisioning and oviposition begin once the queen appears and positions herself near an empty cell. The queen must be near a cell to encourage regurgitation. A court of bees forms around the queen and the finished cell(s), and the workers sequentially insert their head into the cells and regurgitate food until the cell is about two thirds full. The first regurgitation may or may not be stimulated by the queen, depending on the species (Sakagami, 1982). In *M. favosa,* the workers engaged in provisioning seek food from different workers apparently specialized in carrying provisions from the storage pots to bees participating in the POP (Sommeijer et al., 1985).

Once the appropriate food level is reached, workers may lay TEs. The place where the TE is laid varies among species: it could be placed on top of the larval food (as in *M. bicolor),* at the rim of the cell collar, or just on top of the comb. In *Cephalotrigona*, TE-layers present their egg directly to the queen by flipping their abdomen, while in *Lestrimelitta,* the worker eats her own egg in the presence of the queen (Michener, 1974; Sakagami, 1982). *Trigonisca*, *Duckeola, Frieseomelitta* and *Tetragonula* are the only meliponine that do not produce trophic eggs (Sakagami and Zucchi, 1968, 1974; Terada, 1970; Sakagami and Inoue, 1990). The behavior of these infertile workers during the POP is considered simple and is characterized by the absence of the typical excitement and the elaborate queen-worker interactions found in all other species where workers do lay eggs (Sakagami and Zucchi, 1968, 1974; Sakagami and Inoue, 1990). The simplicity of the POP in these sterile bees suggests that the ritualized interactions between workers and queens are associated with the conflict over the production of males;

thus, ritualization partially resolves the conflicts and produces cooperation (Crespi, 1992). TEs are considered specialized eggs for queen consumption (Sakagami, 1982; Koedam et al., 2001; Velthuis et al., 2003a) because of their high protein content (Velthuis et al., 2001, 2003b). However, it could very well be that TEs serve another very important function, as will be discussed.

To finalize the POP, a worker seals the cell by folding the rim collar inward. This is done using their mandibles and working around the cell or most commonly by inserting the tip of the abdomen and folding the collar in a rotating motion (as in *M. bicolor)*. The seal is completed with final mandibular work (Sakagami, 1982). Occasionally, a sealing worker may stop rotating and lay a reproductive egg (RE); it could also happen that, after a cell is sealed, a "sneak" worker reopens the cell and lays. REs are very different from TEs because REs develop into males. The reproductive worker may or may not consume the egg laid by the queen; so, in some cases, there can be two eggs per cell (Beig, 1972; Contel and Kerr, 1976; Machado et al., 1984; Bego, 1990; Koedam et al., 1999; Sommeijer et al., 1999; Koedam et al., 2001). Beig (1972) states that, in *Scaptotrigona,* worker REs develop faster so that these larvae hatch and consume the fertilized eggs laid by the queen; however, in *M. bicolor*, 50% of cells that contain eggs from both queen and reproductive worker produce a m6027SP female, demonstrating that there is also the possibility for competition among the larvae (Koedam et al., unpublished data). The production of eggs in queenright conditions represents one great distinction between most meliponine and *Apis* bees: in stingless bees, many workers present ovarian development (Sakagami et al., 1963), providing evidence for reproductive competition. In several meliponine species, workers contribute substantially in the production of males (Beig, 1972; Contel and Kerr, 1976; Koedam et al., 1999; Sommeijer et al., 1999; Tóth et al., 2002). Sneak RE-laying workers present extremely different behaviors when compared to TE-laying workers and to non-laying workers. During the POP, workers laying REs act agitated and when they succeed in laying, they

engage in an extremely prolonged sealing activity. This behavior has been considered to be a mechanism to avoid the consumption of their eggs by nest-mates (Koedam et al., 2001).

There are two hypotheses about the nature of TEs. Koedam et al. (2001) protected TEs from being consumed by the queen; they report that some TEs in *M. bicolor* can produce weak larvae when compared to other larvae of the same age. Such information indicates that TEs could be degenerate reproductive eggs. In a recent article, Velthuis et al. (2003b) assert that, due to their high content in proteins, TEs are produced specifically to feed the queen. However, some of the proteins found in TEs could actually be enzymes in the process of denaturing the egg. Besides the lack of a typically patterned chorion, TEs are also bigger and rounder than REs (Koedam et al., 1996; Koedam et al., 2001); workers may perceive this morphological difference in size, or may time the egg from the moment it is ready to be laid and thus, modify their laying behavior. The nature of TEs and REs would be an interesting factor to clarify. If TEs are proved to be degenerating REs, such information would give more support to the hypothesis on the hierarchies of participation that workers present in the POP where reproductive workers are closest to the ancestral type while the non-laying or sterile workers are the modified type. The present study lends support to the idea that the order of involvement in POP should be greatest for the reproductive workers that contribute more to POP than for TE-laying workers and least for bees that never lay any type of egg.

4. *MELIPONA BICOLOR*: A FACULTATIVE POLYGYNOUS BEE

In stingless bees, several species have been reported to have provisional polygyny when there is queen replacement (Silva et al., 1972); but this is a small and specific time-period within the colony cycle far from real polygyny. In *Melipona*, a nest of *M. subnitida* may have 5 queens, and *M. marginata* presented two queens in laboratory conditions (Bego,

1989; Imperatriz-Fonseca, unpublished data). In contrast, only *M. bicolor* presents a truly facultative polygyny (Kerr, 1949; Nogueira Neto, 1997; Velthuis et al., 2001) and has been object of intense investigation (Velthuis, 2006, this issue).

In polygynous *M. bicolor* colonies, conflicts potentially arise among queens over whether and how to divide reproduction, since cell provisioning occurs cell-by-cell. Indeed considerable reproductive skew has been observed among queens (Velthuis et al., 2001; Velthuis, 2006, this issue). However, encounters among queens at a given cell are placid and interactions are ritualized (Bego, 1989). At the end of this paper, I will describe and comment on an interesting behavioral difference that may arise among individual queens in polygynous conditions.

5. VARIOUS IMPLICATIONS OF OVARIAN DEVELOPMENT AND ITS RELATION TO WORKER ACTIVITY LEVELS: A CASE STUDY IN *MELIPONA BICOLOR*

In *M. bicolor*, individual weight and extent of ovarian development was correlated with the levels of activity presented by individual workers during POP. These results that will be further presented, may indicate that ovarian development is necessary for workers to effectively assist in brood production, explaining the so called idiosyncratic inclination to attend the brood (Oster and Wilson, 1978). Behavioral differences in *M. bicolor* divide workers into three groups: non-layers, layers of TEs and layers of REs. Egg-layers, regardless of the type of egg, participate most in POPs both in terms of presence (constancy) and their significant contributions during each POP (assiduity). The correlations may be indicating that ovarian development plays an important role in task partition in the colony by influencing the degree of involvement of each worker.

To obtain insight into individual variation in activity during POP, the behavior of individual bees was tracked (495 continuous filming hours per colony) in one polygynous (Col1) and one monogynous (Col2) colony of
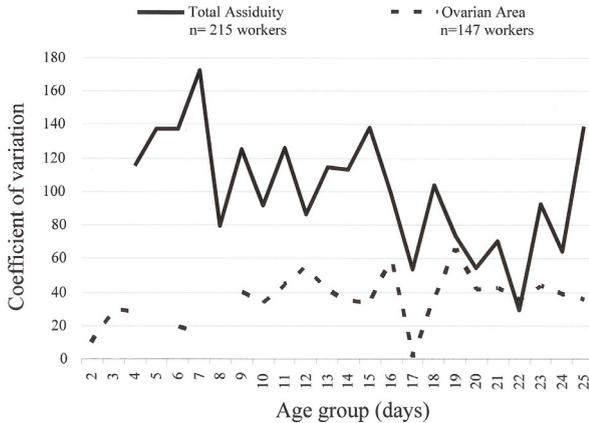
Figure 1. Coefficient of variation for the ovarian area and behavioral assiduity between age groups in *M. bicolor*. (No ovarian measurements were made for colony 1. Lack of CV means there was data of only one or no specimens).

M. bicolor. Research methods include standard procedures for colony maintenance and worker marking (Sakagami, 1966; Koedam et al., 1999; for details see Cepeda-Aponte, 2003). The occurrence of the behaviors of body insertion (partial or total), larval food discharge, and egg laying (trophic or reproductive) were noted for each worker of known age participating in a POP. For each individual I calculated:

Assiduity: total frequency of each basic behavior monitored;

Constancy: total number of POP in which an individual participated.

At the end of the monitoring period, all marked workers in Col2 were sacrificed, individually weighted, and dissected. Ovaries were fixed and photographed; ovarian area was measured (for details see Cepeda-Aponte, 2003).

In Figure 1 the coefficient of variation calculated for each age group revealed that individual workers of the same age group were very different morphologically (ovary size) as well as behaviorally. The coefficient of variation of the ovary area was found to have a wide range of values, varying from 0.98 to 65.4. The coefficient of variation for the total participation or assiduity per age group also presented great variations that fluctuated between 29.2 and 172.6. Spearman correlation of these coefficients presented no significance when the data was treated as a whole. Correlations appeared only once the sample was separated into types of workers: non egg-layers versus egg-layers.

5.1. Behavioral differences between egg layers and non egg layers

Only Col1 presented eleven reproductive individuals that laid from one to three REs. Figures 2 and 3 depict their participation (assiduity and constancy respectively) in all behaviors, demonstrating their extraordinary performance: reproductive workers were involved 7 times more often in POPs (Fig. 3) than TE-layers, and were extremely active (Fig. 2). When the performance of the TE-layers was analyzed in Col1 and Col2, TE-layers of Col2 were significantly more active than TE-layers of Col1 that presented RE-layers. Behavioral data of the non-laying bees presented no statistical differences between the colonies. All TE-layers differed significantly from non-layers. (Kruskall-Wallis and U test post hoc $P <$ 0.0125). TE-layers presented a two-fold higher POP activity than did non-laying bees (Figs. 2, 3). So, it appears that the hierarchy of presence and participation in POP is RE-layers > TE-layers > non layers.

Another way to perceive the great contribution of egg layers in the POP is by calculating an average participation per bee as shown in Table I. In Col1 the extraordinary efforts of only eleven reproductive workers (3% of a total of 353 bees) are evident as they participated in almost 1/4 of the total number of
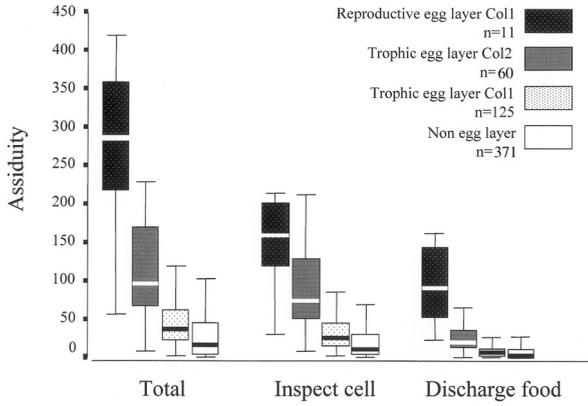
Figure 2. Comparison of behavioral assiduity for the three types of workers participating in POP in *M. bicolor*. (Significant difference (>0.0125) between and among all groups).
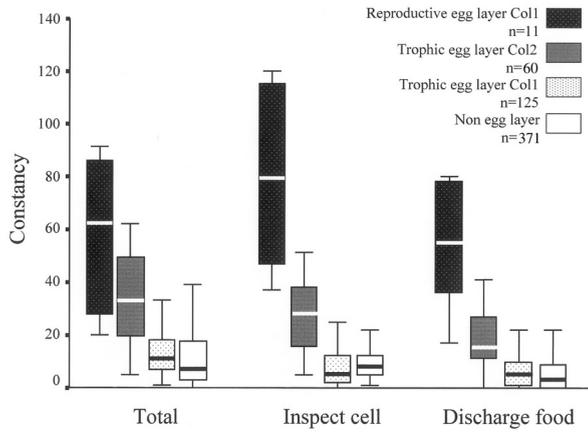
Figure 3. Comparison of behavioral constancy of the three types of workers participating in POP in *M. bicolor*. (Significant difference (>0.0125) between and among all groups).

**Table I.** Average participation in POP per individual in *M. bicolor*.

	Colony 1 workers n = 353	Total %	Colony 2 Workers n = 214	Total %
Total number of events in 480 consecutive hours	13 689		15 380	
Non egg layers	Each of 217 workers participated in average in 24 events	38	Each of 154 workers participated in average in 50 events	50
Trophic egg layers	Each of 125 workers participated in average in 44 events	40	Each of 60 workers participated in average in 128 events	50
Reproductive egg layers	Each of 11 workers participated in average in 271 events	22	No reproductive workers	

recorded events. In this same Col1, TE-layers, represented about 1/3 of the workers (n = 125) and participated in 40% of the total events. In Col2, where there were no reproductive workers, a small number of TE-layers (n = 60) worked 2.5 times more than non-laying workers (n = 154) and participated in 50% of the events. As was mentioned earlier, there was a significant difference in activity between the TE-layers of both colonies, and such a difference is also perceptible here. From this analysis it may be inferred that, even though there were only eleven reproductive workers, their high levels of performance competitively reduced the participation of other workers of Col1 (social inhibition model).

Workers in both colonies presented an evident variability in individual behavior within each age group. By separating workers into groups characterized by the type of egg laid or its lack, significant differences appeared between these three types of workers for all variables measured. These analyses illustrate the great "interest" laying workers appear to have in the POP. Sommeijer (1984) reports that in *M. favosa,* laying workers are not the principal provisioners of the cell they lay in. This does not necessarily mean that these egg layers do not participate in POPs: they may have been active provisioners of other cells. That is, laying bees are more involved in POP and participate frequently, but they do not necessarily secure or monopolize a specific cell.

5.2. Morphological differences between egg layers and non egg layers

According to the present results, laying workers are behaviorally very different from non-laying ones because laying workers (both TE- or RE-layers) are more constant and more assiduous in the POP. Now let us compare these behavioral characteristics against morphological factors (ovarian area and body weight) and maximum age reached. Weight was considered to be an indirect indicator of nutritional influence. It was found that bees have a tendency to loose weight as they age. There is great variability of weight within each age group but two clusters may be seen: a first
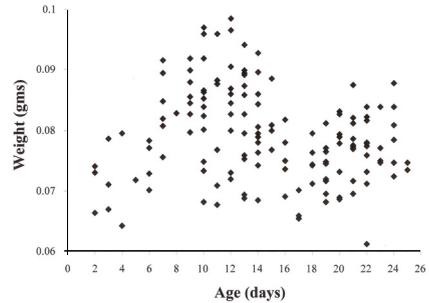
Figure 4. Weight changes per age group of workers participating in POP in *M. bicolor*.

heavier group of young bees up to 14 days old, and a second, lighter group (Fig. 4).

When analyzing the TE-layers, there was no correlation between ovarian area and behavioral variables (Tab. II). This result was due to age differences within the complete group of egg layers: when sacrificed, bees were between 13 and 25 days old, which means that, in young bees, ovarian development should have been at its peak while ovaries had degenerated in older bees. However, in the same group the heaviest laying workers (n = 48) were more constant and assiduous in the POP, inspecting the cells, and laying their eggs; these same behaviors were found to be correlated with age in TE-layers. As for the non-egg-laying group, it could contain bees that were either too young or about to lay, plus old bees that effectively had never laid. So, they were divided and analyzed in two subgroups: bees between 4 and 12 days of age, the prime time and maximum age for egg production (Cepeda-Aponte, unpublished data), and older bees between 13 and 23 day old. For the young non-layers, it was seen that, as they age, grow heavier and their ovaries mature, their apparent involvement in the POP increases, and they become more engaged in inspecting cells and discharging larval food. However, since correlation coefficients were never higher than 0.49, an intricate feedback relation between nutritional input, ovarian development, hormonal levels, social interactions and even learning capacity might exist, besides the effect introduced by those young bees that would never lay. For the old non-layers, there was a negative

Table II. Significant correlation coefficients (Spearman) between physical and behavioral variables in *M. bicolor*.
TE = trophic eggs

			TE layers 13–25 days old	n	P	Non egg layers n = 51 4–12 days old	P	Non egg layers n = 40 13–23 days old	P
AGE vs.		Body weight							
		Ovarian Area				0.42	<0.005	−0.34	<0.05
	TOTAL	Assiduity				0.40	<0.005	0.49	<0.005
		Constancy	0.33	36	<0.05	0.43	<0.005	0.46	<0.005
	INSERT	Assiduity	0.45	36	<0.05	0.40	<0.005	0.49	<0.005
		Constancy				0.44	<0.005	0.51	<0.005
	DISCHARGE FOOD	Assiduity				0.39	<0.005	0.33	<0.05
		Constancy				0.40	<0.005	0.37	<0.05
	LAY TE	Constancy	0.35	36	<0.05				
	SEAL CELL	Constancy							
OVARIAN AREA vs.		Body weight							
		Age						−0.34	<0.05
	TOTAL	Assiduity				0.44	<0.005		
		Constancy				0.48	<0.005		
	INSERT	Assiduity				0.39	<0.05		
		Constancy				0.44	<0.005		
	DISCHARGE FOOD	Assiduity				0.48	<0.005		
		Constancy				0.49	<0.005		
	LAY TE	Constancy							
	SEAL CELL	Constancy							
BODY WEIGHT vs.		Ovarian Area							
		Age							
	TOTAL	Assiduity	0.34	48	<0.05	0.36	<0.05		
		Constancy	0.32	48	<0.05	0.37	<0.05		
	INSERT	Assiduity	0.39	48	<0.05	0.37	<0.05		
		Constancy	0.36	48	<0.05	0.38	<0.05		
	DISCHARGE FOOD	Assiduity				0.34	<0.005		
		Constancy				0.33	<0.005		
	LAY TE	Constancy	0.34	48	<0.05				
	SEAL CELL	Constancy							

correlation between age and ovarian area, as anticipated. However, the expected negative correlations of the level of POP participation with ovarian area or weight were not found, and instead, behavior was positively correlated with age. These last results demonstrate the difficulty for the analysis due to the variability within each group plus the already mentioned interweaving of internal and external factors. Larger sample size taken from more colonies would be necessary to test the validity of these preliminary but interesting observations.

5.3. Egg laying: proximate and ultimate mechanisms

Egg layers in *M. bicolor* are so involved in the POP that they could be called elite, exceptional or specialized individuals (Robson and Traniello, 1999; Velthuis, unpublished data). In this study, egg layers represented between 28% and 39% of the total marked workers. This seems to indicate that differences in ovarian development might influence response thresholds leading to the diverse levels

of participation in the POP. Individuals drawn to the POP could improve the execution of a task through repetition or auto-reinforcement. When non-layers with high response thresholds, and therefore low competitiveness, approach a POP, the high competitiveness of egg-layers would drive them away and the lack of "practice" would make them "forget" such tasks and cause them to forage for work elsewhere. The behavior observed in *M. bicolor* nicely fits models on response threshold, auto-reinforcement and social inhibition (Beshers and Fewell, 2001). It will be necessary to pay more attention to egg-layers since they may represent key individuals in the POP and, hence, in colony growth (Robson and Traniello, 1999).

Egg-laying has been related to division of labor. In several social hymenopteran species, behavioral changes of aging workers mirror with changes in the development of their ovaries: young individuals that work in the nest present developed ovaries, while older ones forage and present degenerate ovaries (Wilson, 1985). From an ultimate perspective, it is possible that the generation of ovaries of diverse sizes made division of labor possible, so, less fertile females gave up reproduction to help probably related and more fertile females (Bourke, 1988). Another interpretation could be that laying workers would prefer to remain in the nest, close to their reproductive interests, while not exposing themselves to predators (Franks and Scovell, 1983). A modified type is presented by Inoue et al. (1996) who found that, in *Trigona* (*Tetragonula*) *minangkabau*, the population of a nest could be divided into workers dedicating all their lives to care for the brood, and foragers that hardly remained with the brood and yet, all these worker bees are totally sterile.

It is also feasible to discuss possible ultimate mechanisms. Why have workers retained ovarian development? What is seen in *M. bicolor* is females trying to reproduce: in these eusocial bees the ancient conflict over individual reproduction appears to persist. Even in *Apis* bees, where queen and larvae strongly inhibit ovarian development in workers, some may escape control and lay eggs (Bourke, 1988; Ratnieks and Visscher, 1989).

And when honeybee workers of the same age were isolated, they presented individual differences in ovarian development (Velthuis, 1970). Furthermore, in genera of stingless bees that present totally sterile workers, their behavior during POP lacks the typical excited traits observed in species where workers do produce eggs (Sakagami and Zucchi, 1968, 1974). Lin and Michener (1972) argue that a benefactor behavior may develop even where no altruism is involved, provided there is a significant contribution to male production by workers. Whenever a female conserves the "hope" of reproduction, her participation in colony tasks may be considered as an investment in her future reproduction. In many meliponine species exists a percentage of workers that lay reproductive eggs and contribute significantly to male production. Furthermore, the same individual worker may produce both trophic and reproductive eggs (Koedam et al., 1999; Cepeda-Aponte, unpublished data). This evidence may indicate that there is selection to maintain the production of TEs in an individual that can produce both types of eggs (Kukuk, 1992); it could also indicate that sons of reproductive workers have a high reproductive success. TEs may have the function of deceiving the queen, but West-Eberhard (1981) proposes a more interesting hypothesis. This author states that the function of TEs is to maintain ovary activity. This would indicate that their potential role as the principal source of nutrients for the queen (Sakagami, 1982) may be a byproduct for the cooperative interaction (and competition) between worker and queen.

6. THE BEHAVIORAL "DILEMMAS" OF A QUEEN

While the focus of my research was on worker behavior in *M. bicolor*, the time extensive observation also yielded interesting, novel information on queen behavior as well. By studying and comparing POPs in the polygynous and in the monogynous colony, it was possible to perceive the great pressure that multiple queens place on each other. Nutrition is a determinant factor in queen behavior
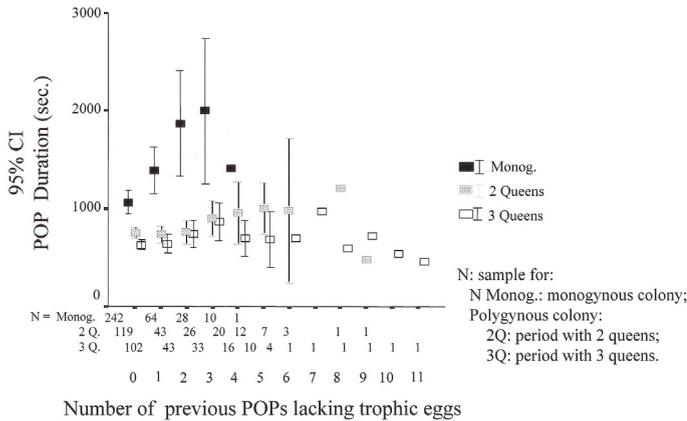
Figure 5. Comparing average and confidence intervals on the duration of POPs depending on number of queens, for the monogynous (Col1) and polygynous (Col2) colonies of *M. bicolor*. Data grouped by ranks based on the presence of TEs in previous POP.

and her performance as an egg-layer (Wheeler, 1996; Velthuis et al., 2001; Velthuis, 2006, this issue). Queens have three ways of feeding: by trophallaxis with workers; or during POP by ingesting larval food directly from the provisioned cell, and/or consuming trophic eggs (Sakagami, 1982). TEs are laid during the post-provisioning phase: the full cell seems to serve as an incentive for the workers to lay, but, at the same time, the close presence of the queen restrains the worker from doing so. As previously stated, in the POP, the ovipositions of both queen and workers involve stereotyped rituals in their behavior. Sakagami et al. (1973) explain that at the core of this process lies the conflict of the worker that fears the queen but feels compelled to defend the finished cell to lay her own egg. In polygynous conditions, queens are constantly inspecting the cell and, with their proximity to the cell, intimidate the workers. Workers consequently should reduce the number of TEs they lay (Velthuis et al., 2001; Velthuis, 2006, this issue).

In this case study, a total of 393 continuous POPs for the monogynous colony and a total of 508 continuous POPs for the polygynous colony were filmed for the same amount of time. Information for this latter colony was divided into period I and period II; the first with 241 POPs with three queens laying, while the second period presented 267 POPs, but had only two laying queens. In the polygynous colony, the three queens were similar in various aspects. Egg laying and consumption of trophic eggs presented no statistical differ-

ences or worker discrimination, confirming a previous report (Velthuis et al., 2001), so each queen had an equal chance to lay her eggs and feed. In addition, the queens were of similar age: when observations began, queen A was 176 days old, queen B was 165 days while queen C was 222 days. These differences were not considered significant since queens in laboratory conditions have been reported to live up to two years (Imperatriz-Fonseca, unpublished data). Since *M. bicolor* is a species that presents no direct aggression (Bego, 1989), the following results may reveal yet another way in which competition is manifested in these polygynous bees.

In both monogynous and polygynous colonies, I ranked each POP depending on how many preceding POP lacked TE presentation: that is, if the preceding POP presented TE, the rank of the following POP was "0", rank "1" if only the anterior POP lacked TE, etc. An additive effect was considered when there was more than 1 TE per cell. In the monogynous colony, the duration of the POP was related to the amount and frequency of TEs previously ingested: the queen was apparently capable of modulating the duration of each POP by postponing her oviposition and waiting for a worker to lay a TE. As seen in Figure 5, the average duration of a POP increased as more POPs without TE accumulated resulting in extremely long POPs lasting more than 30 min; however most POPs (n = 242) lasted on average 15 min. In the polygynous colony, when the colony had only two queens, a subtle but

similar pattern was still perceivable but this changed when the colony had three queens: there was no such pattern and instead most POPs were rather short (n = 102, mean = 12 min), the longest POP lasting not more than 16 min. These POPs of short duration could reflect the urge each queen had in laying her own egg in what seems to be a competitive situation, where queens prefer to lay rather than to wait and feed on TEs. From Figure 5 it is also clear that in the monogynous colony there were hardly more than three successive POPs without TE (n = 10 of 393 POP), while in the polygynous colony, up to 11 consecutive POPs can occur without release of TEs. Other studies in polygynous colonies with few queens, revealed that each queen had a higher probability of presiding alone over a POP (Velthuis et al., 2001), than in polygynous colonies with numerous queens, where the probability of an encounter with other queens at the same cell increased. This factor therefore interferes with the possibility of modulating the duration of the POP and of waiting to receive a TE. In consequence the lack of such nutrition may lead to cessation of egg-laying for some queens and to the development of a reproductive skew.

7. CONCLUSION

In *M. bicolor*, the best-fed, heavy workers keep their ovaries activated probably in "hope of reproduction", and they participate effectively in as many POPs as possible. This observation places trophic eggs into a new perspective: is it a specialized nutritional package for the queen (Velthuis et al., 2003b), or is it a byproduct of worker ovarian activity, that is, is it an over-mature reproductive egg?

As for the queens, we see that when she is alone, a queen can modulate the duration of the post-provisioning phase in order to receive trophic eggs. In a polygynous condition, the priority of the queens is to place their eggs as fast as possible, and this could result in a disadvantageous nutritional condition that could explain the establishment of reproductive hierarchies or skews (Velthuis et al., 2001) and to the abandonment of reproductive functions.

Résumé – **Division du travail pendant la production de couvain chez les abeilles sans aiguillon, en particulier du point de vue de la participation individuelle.** La division du travail est une marque de socialité. Des études récentes ont révélé que des individus, qui avaient été considérés comme des entités désintéressées, faisaient preuve de plasticité. Le but de cet article est de fournir une information sur les approches adoptées pour étudier le comportement des insectes sociaux, en se focalisant principalement sur les modèles récents qui concernent plus le comportement individuel et montrent comment celui-ci s'intègre dans la division du travail au sein de la colonie. L'article présente un cas d'étude réalisé sur l'abeille sans aiguillon *Melipona bicolor* qui, comme la plupart des abeilles sans aiguillon, possède des ouvrières capables de pondre deux types d'œufs. Certains, les œufs reproducteurs, se développent en mâles et contribuent à la production des mâles de la colonie. D'autres, les œufs trophiques, constituent la principale source de nourriture des reines. Des ouvrières ont été marquées et filmées individuellement durant 20 j consécutifs, de façon à suivre leur comportement dans le processus de production du couvain appelé Processus d'Approvisionnement et de Ponte (POP). A la fin de la période d'observation, les individus ont été pesés et la surface de leurs ovaires mesurée. Le poids constituait une mesure indirecte de la consommation de nourriture. Les ouvrières d'un même groupe d'âge ont présenté une grande variation dans leurs caractéristiques morphologiques et comportementales (Fig. 1). L'analyse a en outre montré que les ouvrières qui pondaient des œufs étaient plus impliquées dans le processus de production de couvain que celles qui n'avaient pondu aucun type d'œuf (Figs. 2, 3 ; Tab. I). Les ouvrières avaient aussi tendance à perdre du poids avec l'âge (Fig. 4), mais les ouvrières les plus lourdes qui pondaient des œufs participaient plus au POP (Tab. II). Le groupe des non-pondeuses comprend les jeunes abeilles et il n'a pas été possible de dire si celles-ci auraient pu devenir ou non des pondeuses. On a pourtant noté que la participation des jeunes abeilles au POP augmentait parallèlement à leur poids et au

développement de leurs ovaires (Tab. II). On en a déduit que les abeilles les mieux nourries et possédant des ovaires actifs pouvaient représenter la condition ancestrale de la compétition pour la reproduction entre les femelles et que les œufs pondus par ces ouvrières auraient la fonction principale de maintenir leurs ovaires actifs afin de contribuer au pool génétique par la production de mâles. *M. bicolor* a aussi la particularité d'être la seule abeille eusociale pour laquelle la polygynie facultative a été prouvée. En comparant la durée du POP dans une colonie mono-reine et une colonie à trois reines, on a remarqué qu'une reine toute seule pouvait prolonger la durée du POP jusqu'à ce qu'elle obtienne des œufs trophiques pour s'en nourrir. Par contre dans une colonie à plusieurs reines, la compétition pour la ponte est représentée par des POPs plus courts au cours desquels les reines « préfèrent » pondre plutôt que se nourrir (Fig. 5). Ainsi, dans une colonie à plusieurs reines, la priorité des reines est de placer leurs œufs le plus vite possible ; cela peut déboucher sur une mauvaise nutrition qui pourrait expliquer la mise en place de hiérarchies de reproduction et l'abandon des fonctions de reproduction par des reines individuelles.

abeille sans aiguillon / division du travail / POP / compétition pour la reproduction / *Melipona bicolor* **/ Apidae**

Zusammenfassung – Arbeitsteilung in der Brutaufzucht bei Stachellosen Bienen, mit besonderer Berücksichtigung der individuellen Beteiligung. Ziel dieser Arbeit ist es, über Untersuchungsansätze zum Studium von Verhalten bei sozialen Insekten zu informieren, wobei besonders neuere Modelle in Betracht gezogen werden, die sich auf das individuelle Verhalten und dessen Integration in die Arbeitsteilung innerhalb der Kolonie beziehen. In dieser Fallstudie an der Stachellosen Biene *Melipona bicolor* geht es um die Eiablage von Arbeiterinnen, die wie bei den meisten Stachellosen Biene zwei Typen von Eiern produzieren können. Zum einen sind dies reproduktive Eier, aus denen Männchen entstehen können und die damit einen Anteil an der Männchenproduktion der Kolonie darstellen. Zum anderen produzieren sie Nähreier, die die Hauptnahrungsquelle für die Königin darstellen. Arbeiterinnen bekannten Alters wurden individuell markiert, und über einen Zeitraum von 20 Tagen wurde ihr Verhalten im Brutproduktionsprozess kontinuierlich gefilmt. Dieser Prozess wird als Verproviantierungs- und Eiablageprozess (Provisioning and Oviposition Process, POP) bezeichnet. Am Ende der Beobachtungsperiode wurden die Bienen gewogen und eine Flächenmessung der Ovarien vorgenommen. Das Gewicht stellt hierbei ein indirektes Mass für die Nahrungsaufnahme dar. Arbeiterinnen der gleichen Altersklasse zeig-

ten grosse Variabilität hinsichtlich morphologischer Merkmale und im Verhalten (Abb. 1). Die weitere Analyse zeigte, dass Arbeiterinnen, die Eier legten, sich stärker im Brutproduktionsprozess engagierten als Arbeiterinnen, die niemals Eier legten (Abb. 2, 3; Tab. I). Auch bei einem tendenziellen Gewichtsverlust mit zunehmendem Alter (Abb. 4) waren es stets die schwereren Arbeiterinnen, die Eier legten und in den POP eingebunden waren (Tab. II). Innerhalb der nichtlegenden Arbeiterinnen befand sich auch eine Gruppe junger Arbeiterinnen, für die es nicht möglich war zu entscheiden, ob sie später Eier legen würden oder nicht. Jedoch auch bei diesen jungen Arbeiterinnen waren es die schwereren, die grössere Ovarien hatten und in stärkerem Mass am POP teilnahmen (Tab. II).

Daraus kann geschlossen werden, dass die am besten gefütterten Bienen mit den aktiven Ovarien die anzestralen Bedingungen der Reproduktionskonkurrenz zwischen Weibchen repräsentieren und dass die Produktion von Nähreiern bei diesen Arbeiterinnen vor allem dazu dienen könnte, die Ovarien in einem aktiven Zustand zu halten, um so auch gegebenenfalls Männchen zu produzieren und damit zum Genpool beitragen zu können.

M. bicolor ist zudem von speziellem Interesse, weil es die einzige eusoziale Bienenart ist, für die eine fakultative Polygynie nachgewiesen ist. Beim Vergleich der POP-Dauer in einer Kolonie mit nur einer Könign mit einer Kolonie mit drei Königinnen zeigte sich, dass eine Königin, solange sie alleine ist, die POP-Dauer hinauszögern kann, bis sie ein Nährei erhält. In einer Kolonie mit mehreren Königinnen waren die POPs wesentlich kürzer, was auf eine Konkurrenz zur Eiablage hindeutet, d.h. die Königinnen ziehen es vor, zu legen statt auf ein Nährei zu warten (Abb. 5). Diese verstärkte Legetendenz in Kolonien mit mehreren Königinnen könnte bei diesen zu einer unvorteilhaften Ernährungssituation führen und damit die Etablierung von Reproduktionshierarchien fördern, bzw. bei einzelnen Königinnen ganz zur Aufgabe der reproduktiven Funktionen führen.

Stachellose Bienen / Arbeitsteilung / POP / *Melipona bicolor* **/ Verhalten**

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