

Original article

## Cell provisioning and oviposition in *Melipona beecheii* (Apidae, Meliponinae), with a note on caste determination

Johan Wilhelm VAN VEEN\*

Department of Social Insects, Ethology and Socio-ecology, Laboratory of Comparative Physiology, Utrecht University, P.O. Box 80.086, NL-3508 TB Utrecht, The Netherlands

(Received 19 January 1999; revised 27 August 1999; accepted 15 November 1999)

**Abstract** – We examined the food provisioning and oviposition process (POP) for worker- and gyne-producing cells of *Melipona beecheii*. POPs for both castes did not differ significantly in duration and number of trophic eggs oviposited by workers in the cells. The frequency of food discharges by workers in gyne-producing cells was significantly less than in worker-producing cells. This result is discussed in relation with caste determination. Seventy percent of all POPs took place at night. We hypothesized that foraging activities during the daytime reduced the number of workers available in the nest for provision of food to workers that participated in the POPs. It was found that the number of trophic eggs laid by workers during a POP was positively correlated to the preceding extra-oviposition period.

**stingless bee / caste / trophic worker egg / *Melipona beecheii***

### 1. INTRODUCTION

In stingless bees (Meliponinae), cells are mass-provisioned by workers before queen oviposition and subsequent cell closure, in a characteristic process [1, 12–14, 16] reviewed by Sakagami [11]. For *Melipona*, where only one cell is oviposited at a time, the food provisioning and oviposition process (POP) begins with the arrival of the queen at a completed ‘collared’ cell. The

queen stays near the cell, and a pre-discharge phase, lasting several minutes and characterized by excited workers, inserting their body in the cell, starts the process. Next, the queen violently taps the workers that insert their body in the cell with her antennae and forelegs, stimulating them to discharge larval food until the cell receives its full amount. During this discharge phase, the queen makes inspections of the cell, may consume some of the larval food, and eats

---

\* Present address: P.O. Box 475-3000, Heredia, Costa Rica  
E-mail: jvanveen@una.ac.cr

the trophic eggs laid by workers on top of the food. Once the cell is filled, the queen lays an egg vertically on the food, and then leaves the cell. Shortly after her oviposition, a worker inserts its abdomen in the cell and closes it, while rotating its body.

Caste determination in stingless bees is based on the amount of food a cell receives during the POP [15]. In the Trigonini, royal cells are larger and receive twice (or more) the amount of food as worker cells. In *Melipona*, gynes emerge from cells equal to worker cells. A model for caste polymorphism in *Melipona* based solely on genetic factors [5, 6] was shown to be incomplete; food quantity and quality were also found to be of importance in *M. beecheii* [2, 3]. Velthuis and Sommeijer [18] added a physiological component to Kerr's model in which only individuals heterozygous for two genes (each with two alleles) will develop into queens. In their model, genetic system A regulates development in the young larva, and is related to quantity or quality of food uptake. The heterozygous condition promotes queen development. Genetic system B regulates the level of juvenile hormone (JH) production at a later larval phase, and in a similar way promotes queen or worker development. Additional to these genetic factors, Velthuis and Sommeijer [18] assumed a sensor mechanism, which measures the amount or quality of the food ingested during larval development. The genetic programming for a lower level of JH is considered as an adaptation to inferior food conditions. In *M. quadrifasciata* [7], small amounts of food produced workers, whereas larger quantities of food produced both castes. Kerr [7] found that, when food was plentiful, the amount of provisions put in a cell was inversely related to the number of workers that discharged food in it. Pupae that weighed less than 72 mg did not become gynes. Up to 25% of pupae equal to or heavier than 72 mg developed into gynes. Larvae of *M. compressipes* in artificial cells provided with slightly more homogenized food (mixture of food taken

from many cells) than found in nature, developed in three workers to one queen [9]. When less optimal conditions are present, lower percentages of gynes are produced [7, 10, 17, 18]. It is believed that the quality of the food [4], through the quality or amount of an enzyme that it contains [18], is of importance for the determination of caste. In this paper, data are presented on physical and temporal elements of the typical cell provisioning and oviposition process in *M. beecheii* for worker- and gyne-producing cells.

## 2. MATERIALS AND METHODS

Observations were carried out on one colony of *M. beecheii* in Heredia, Costa Rica, from 13 to 20 May 1993. The colony was housed in a rectangular wooden observation box, which was placed in the laboratory inside a darkroom. The hive measured 30 (l) × 15 (w) × 15 cm (h), was made of 15-mm thick plywood, and was covered with a glass lid. An entrance tube 16 mm in diameter and about 20 cm long connected the colony with the outside. The colony consisted of about 1 000 adult bees, and had some honey and pollen stored in pots. Two days before the recordings started, the involucre covering the centrally-located brood nest was removed to allow for clear observation of the combs.

All POPs which occurred during the observation period were videotaped with the use of a red light. A total of 116 POPs was recorded between 13 May 18:00 h and 20 May 04:00 h. Subsequently all videotapes were analyzed, and the following data recorded: starting and ending time of POP, duration of POP, the number of food discharges by workers in the brood cell, the number of eggs laid by workers and the moment of oviposition, the behaviour of 'cell standing' performed by workers after a food discharge, the number of cell inspections by the queen, and the number of times the queen actually ate from the larval food

previously discharged in the cell. The moment the queen arrived at an empty cell designated the start of a POP, and the end of a POP the moment when the queen finished oviposition and withdrew from the cell. From these observations, the frequency distribution of POP events over the day was calculated, and a temporal distribution of the different elements of a POP was constructed.

Since cells which produce workers and gynes (and males) are indistinguishable and intermixed, all POPs were numbered and mapped on a drawn comb. When the first adult emerged (after 43 days), all cells were taken out of the colony and the sex of the developed pupae was determined. Through this method it was possible to compare typical POP elements for gyne and worker production, respectively.

### 3. RESULTS

#### 3.1. Provisioning and oviposition process

A total of 116 provisioning and oviposition processes were observed, of which 73 were worker- and 43 gyne-producing POPs. Data on the number of food discharges, cell standing behaviour of the workers, 'inspections' of the cell performed by the queen during the POPs and the eating of larval

food from the cell by the queen are given in Table I. POPs of cells that developed into workers had an average duration of 840 s (S.D. = 425 s,  $n = 73$ ), ranging from 338 to 2 441 s. POPs of cells that developed into gynes lasted 983 s on average (S.D. = 662 s,  $n = 43$ ), ranging from 312 to 3 693 s. These differences were not significant. The average number of discharges was smaller for gyne-producing cells than for worker-producing cells ( $t$ -test,  $df = 114$ ,  $Z = 2.23$ ,  $P = 0.028$ ). This result has important implications for caste determination (see Discussion). The eating of larval food from the cell by the queen was not significantly different in gyne- and worker-producing cells ( $t$ -test,  $df = 114$ ,  $Z = -1.7$ ,  $P = 0.09$ ).

No significant correlation was found between the number of discharges and eating of larval food (Spearman,  $r = 0.20$ ,  $P = 0.10$ ) from the cell by the queen, or with the number of queen inspections (Spearman,  $r = 0.17$ ,  $P = 0.15$ ). The frequency of queen inspections and eating of larval food were positively correlated in worker-producing cells ( $F = 2.8$ ,  $P < 0.025$ ), and in gyne-producing cells ( $F = 4.9$ ,  $P < 0.003$ ). No significant negative correlation (Spearman,  $r = 0.04$ ,  $P = 0.77$ ) between the eating of larval food by the queen and the number of trophic worker eggs (TWE) per POP was found. This is a clear indication that, whereas trophic worker eggs are the main source of nutrition for the queen, the eating of larval

**Table I.** Frequencies and range of some elements and behaviour of 116 worker- and gyne-producing POPs in *M. beecheii*; \* indicates a significant difference between the values obtained for worker- and gyne-producing POP.

	Worker-producing POP		Gyne-producing POP	
	$n = 73$		$n = 43$	
No. of discharges / cell*	12.2	(S.D = 2.3, range 6–19)	11.2	(S.D = 1.9, range 7–15)
No. of cell standings	9.6	(S.D = 8.1, range 0–52)	8.9	(S.D = 6.5, range 0–29)
No. of cell inspections	2.8	(S.D = 2.3, range 0–11)	3.0	(S.D = 2.5, range 0–10)
No. of larval food consumptions by queen	1.2	(S.D = 1.1, range 0–5)	1.6	(S.D = 1.3, range 0–7)

food from the cell during POP is mainly a mechanism by which the queen controls the quantity of food regurgitated in the cell.

The frequency of cell standing, a behaviour performed by workers after a food discharge or laying of a TWE, did not differ significantly between worker- and gyne-producing cells ( $t$ -test,  $df = 114$ ,  $Z = 0.55$ ,  $P = 0.59$ ).

### 3.2. Trophic worker-laid eggs

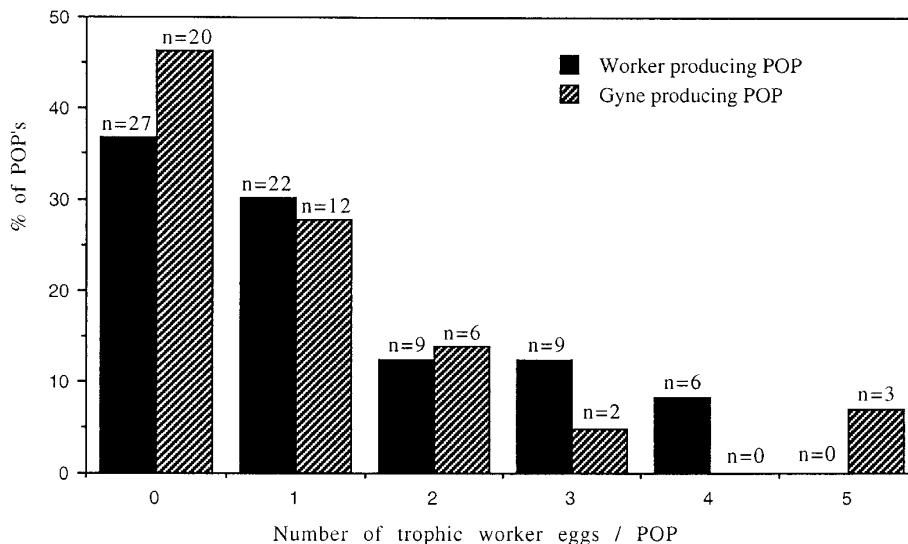
No differences were found in the average number of trophic eggs laid by workers in the cell before the queen oviposited between worker- and gyne-producing cells (Fig. 1). Gyne-producing cells had an average number of 1.0 (S.D. = 1.4) TWE, and worker-producing cells 1.2 (S.D. = 1.3) TWE.

The moment during a POP when a worker egg was laid in relation to the total number of worker eggs being laid is indicated in Figure 2. As can be seen, the moment of laying of the first worker egg is

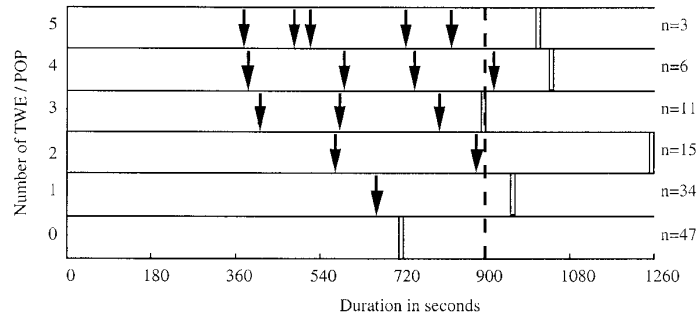
earlier if the total number of worker eggs being laid during a POP is higher (Spearman,  $r = -0.41$ ,  $P = 0.004$ ). The total duration of a POP was not correlated with the number of worker ovipositions (Spearman,  $r = 0.71$ ,  $P = 0.12$ ).

### 3.3. POP: day and night rhythm

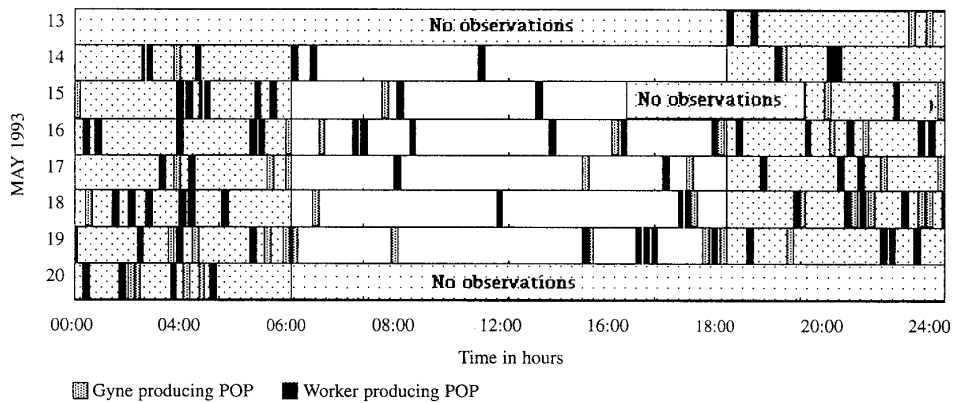
The distribution of POPs over the day for the whole observation period is given in Figure 3. The distribution of the POP events was not uniform over the day. During daylight hours, between 6:00 a.m. and 6:00 p.m., one POP took place on average every 122 min, while at night (between 6:00 p.m. and 6:00 a.m.), one POP took place every 59 min. This means that about 70% of all POPs were at night. It is interesting to note that at night only 15 (18%) out of 82 POPs were with two or more worker-laid eggs, whereas during the daytime 19 (56%) out of 34 POPs were with two or more TWEs ( $F = 17.5$ ,  $P < 0.022$ ). This results in 1.9 TWEs/POP during the



**Figure 1.** The number of trophic eggs laid by workers of *Melipona beecheii* during 73 worker and 43 gyne producing cell provisioning and oviposition processes. The absolute number of POP's is indicated above each bar.



**Figure 2.** The moment at which worker eggs were laid in relation to the total number of worker eggs laid during POP for *Melipona beecheii*. The arrows indicate the moment of laying and the vertical bars oviposition by the queen. The dashed line indicates the average duration of POP's (898 s). Data are presented for worker and gyne producing POP's together (*t*-test, *df* = 111, *Z* = -1.59, *P* = 0.12).



**Figure 3.** Worker and gyne producing POP's of *Melipona beecheii*. The shaded areas emphasizes the night time observations. As can be noted, less POP's happened during the day time (see text). The observations started 13 May at 18:00 h, and ended 20 May at 04:00 h. On 15 May, no recordings were made between 15:16 and 20:07 h.

day, and in 0.7 TWEs/POP at night. A highly significant regression line was calculated between the number of worker-laid eggs per POP and duration (in min) of the extra-oviposition period (EOP) preceding it ( $y = 6.4E - 3x + 0.683$ ,  $F = 21.04$ ,  $P < 0.0001$ ). This means that more TWEs are expected in a POP if the preceding EOP lasts longer. No significant differences were found between the eating of larval food by the queen during day and night.

#### 4. DISCUSSION

This study examined some behavioural recordings of the typical food provisioning and oviposition process in a stingless bee species with worker and gyne castes. The fact that gyne-producing cells received fewer larval food discharges than worker-producing cells may well indicate that they received more food. Kerr et al. [7] found that cells of *M. quadrifasciata* provided with a large

amount of food produced both castes, whereas cells with smaller amounts of food produced workers only. They found that the amount of provisions put in a cell was inversely related to the number of workers that discharged into it. The model as formulated by Velthuis and Sommeijer [18] and the observations of Darchen and Delage-Darchen [2, 3] on *M. beecheii* all stress the importance of food conditions for the determination of caste in *Melipona*. Since queens were found to take up larval food from both gyne- and worker-producing cells in apparently similar quantities (compare data presented in Tab. I), this behaviour is not very likely to influence the difference in food quantity between these cells.

The importance of both food and genetic factors in caste determination can be understood if environmental factors and colony reproductive aspects are taken into consideration. The two-locus, two-allele model [5, 6, 18] determines the percentage of individuals, generally accepted as 25% maximum [7, 10], with the potential of developing into gynes. Only under favourable trophic conditions does gene expression result in gyne production. Favourable food conditions are expected when florescence is abundant. In Costa Rica, foraging conditions are best between January and May [17]. A higher level of gyne production in this period is functional, because colony reproduction (swarming) can be expected at the beginning of this season, and supersede (replacement of the mother queen by a young vigorous queen) at the end of it. A lower level of gynes being produced all year round [17] is thought to be a mechanism by which the quality of the mother queen in the colony is tested constantly [8], and is, in a mass provisioning system, the only way for a colony to assure replacement of the mother queen in case she dies.

Apart from the different number of food discharges, gyne- and worker-producing POPs were found to be very similar in duration, and in the number of trophic worker

eggs, queen inspections, queen uptake of larval food and cell standing by workers (Tab. I; Fig. 1). While performing cell standing, a worker takes a position above the cell, which is similar to an oviposition pose. This makes it attractive to speculate that it might be a sort of ritualized egg-laying behaviour.

POPs with a high number of trophic worker eggs did not last longer than those with zero, one, or two eggs. In POPs with three or more worker eggs, the first egg was already released between six and seven min after the beginning, while in those with fewer TWEs this did not occur until after ten min (Fig. 2). The highly significant correlation between the duration of the extra-oviposition period preceding a POP and the number of worker eggs released is probably due to the continuous gradual maturation of these eggs in the ovaries of the workers. Sommeijer et al. [16] found that workers of *M. favosa* between the age of 10 to 27 days were the workers that had the most developed ovaries. In the daytime, especially when foraging conditions are good, a number of nestmates receive food from the older foragers and deposit it in the storage pots [15]. Since bees involved in the provisioning of a brood cell receive the food from other nestmates [15], it may well be that in the daytime fewer POPs can be performed than during the night when all the bees are in the hive, because of the high foraging activity. In a relatively small colony, not enough bees may be available to receive all the incoming food and at the same time maintain a high sequence of POPs.

**Résumé – Approvisionnement des cellules et ponte chez *Melipona beecheii* (Apidae, Meliponinae), avec une note sur le déterminisme des castes.** Le processus de ponte et d'approvisionnement des cellules en nourriture (POP) a été étudié au Costa-Rica sur 73 cellules de *Melipona beecheii* produisant des ouvrières et 43 cellules produisant des femelles sexuées. Chez les deux castes, les POP avaient la même durée et le

même nombre d'œufs trophiques pondus par les ouvrières dans les cellules. La reine a inspecté les deux types de cellules de la même manière, et aucune corrélation négative n'a été trouvée entre la consommation de nourriture larvaire dans les cellules par la reine et le nombre d'œufs trophiques pondus par les ouvrières au cours des POP. Ceci indique clairement que la consommation de nourriture larvaire dans la cellule est principalement un mécanisme par lequel la reine contrôle la quantité de nourriture régurgitée dans la cellule, tandis que les œufs trophiques d'ouvrières sont la principale source de nourriture pour celles-ci. Le nombre de déchargements de nourriture par les ouvrières dans les cellules qui produisent des femelles sexuées a été significativement plus faible que dans les cellules produisant des ouvrières. Ce résultat peut néanmoins indiquer que les cellules qui produisent des femelles sexuées reçoivent plus de nourriture [7]. Si tel est le cas, le modèle de déterminisme génétique de la caste deux gènes-deux allèles, formulé par Kerr [5, 6] et modifié par Velthuis et Sommeijer [18], qui insiste sur l'importance des conditions alimentaires, peut être valable si l'on prend en compte les facteurs du milieu et les aspects de la reproduction de la colonie. Ce n'est qu'en présence de conditions trophiques favorables, comme lors de floraisons abondantes, que l'expression génique aboutit à une production élevée de femelles sexuées, généralement évaluée à 25 % maximum. C'est aussi durant cette période que la colonie se reproduit et que la forte production de femelles sexuées est fonctionnelle.

Le rythme nyctéméral des POP a été analysé. 70 % des POP ont lieu la nuit entre 18.00 et 6.00 h. On émet l'hypothèse que, probablement à cause des activités de butinage durant la journée, il y a moins d'ouvrières disponibles dans le nid pour fournir la nourriture aux ouvrières qui prennent part aux POP. On a trouvé que le nombre d'œufs trophiques pondus par les ouvrières au cours d'un POP était positivement corrélé avec la période précédente en

dehors de la période de ponte. Les ouvrières ont pondus 1,9 œuf trophique en moyenne par POP durant la journée et seulement 0,7 durant la nuit.

#### **abeille sans aiguillon / caste / œuf trophique / *Melipona beecheii***

**Zusammenfassung – Zellversorgung und Eilage bei *Melipona beecheii* (Apidae, Meliponinae), mit einer Anmerkung zur Kastendetermination.** Der Prozess der Nahrungsversorgung und der Eilage (POP) wurde an 73 Arbeiterinnenzellen und an 43 geschlechtliche Weibchen produzierenden Zellen von *Melipona beecheii* untersucht. Bei beiden Kasten hatten die POPs die gleiche Dauer und Anzahl der von den Arbeiterinnen in die Zellen abgelegten trophischen Eier. Die Königinnen inspizierten beide Zelltypen in ähnlicher Weise, es wurde auch kein negativer Zusammenhang zwischen der Aufnahme von Larvennahrung durch die Königin aus den Zellen und der Anzahl der von den Arbeiterinnen während der POPs abgelegten trophischen Eier festgestellt. Dies ist ein deutlicher Hinweis, dass die Aufnahme der Larvennahrung aus der Zelle hauptsächlich ein Mechanismus zur Steuerung der in die Zellen regurgitierten Nahrungsmenge durch die Königin darstellt, wogegen die trophischen Arbeiterinneneier die Hauptnahrungsquelle der Königin darstellen. Die Anzahl von Nahrungsentladungen von Arbeiterinnen in die geschlechtliche Weibchen produzierenden Zellen war signifikant geringer als die in Arbeiterinnen produzierende Zellen. Dieses Ergebnis könnte allerdings anzeigen, dass weibchenproduzierende Zellen mehr Nahrung erhalten [7]. Falls dies zutrifft, kann das von Kerr [5, 6] formulierte und von Velthuis und Sommeijer [18] (die die Bedeutung der Ernährungsbedingungen hervorhoben) modifizierte zwei Gen-zwei Allel Modell genetischer Kastendetermination zutreffen, solange Umgebungsfaktoren und Faktoren der Koloniereproduktion mit in Betracht

gezogen werden. Nur falls die Bedingungen günstig sind (etwa wenn Blüten im Überfluss vorkommen) resultiert die Genexpression in einer hohen Erzeugung von geschlechtlichen Weibchen, diese wird generell als maximal 25 % angenommen. Dies ist die gleiche Zeit, in der auch die Vermehrung der Kolonien erfolgt und eine hohe Produktion von geschlechtlichen Weibchen funktional ist.

Der Tag-Nachtrhythmus der POPs wurde untersucht. Siebzig Prozent aller POPs fand bei Nacht statt, zwischen 18:00 und 6:00 h. Wir vermuten, dass dies auf die Sammelaktivität während des Tages zurückzuführen ist, durch die weniger Arbeiterinnen verfügbar sind, die die Nahrung für die an den POPs beteiligten Arbeiterinnen zur Verfügung stellen. Wir fanden, dass die Anzahl der von den Arbeiterinnen während der POPs gelegten trophischen Eier positiv mit der vorhergehenden Zeit ohne Eilagezeit korreliert war. Die Arbeiterinnen legten tagsüber durchschnittlich 1.9 trophische Eier pro POP und nur 0.7 während der Nacht.

#### Stachellose Biene / Kaste / trophisches Arbeiterinei / *Melipona beecheii*

#### ACKNOWLEDGMENTS

The author wishes to thank M. Camacho for videorecording part of the data. M.J. Sommeijer and two anonymous referees are thanked for their valuable comments on the manuscript. The staff of the Centre for Research on Tropical Beekeeping, National University of Costa Rica, is thanked for their cooperation. This study was undertaken as part of the research programme of the Regional Stingless Beekeeping Project (PRAM), which is financed by the Netherlands Organization for Cooperation in Higher Education (NUFFIC).

#### REFERENCES

- [1] Beig D., Sakagami S.F., Behavior studies of the stingless bees, with special reference to the oviposition process. II. *Melipona seminigra merrillae* Cookerell, Ann. Zool. Jpn. 37 (1964) 112–119.
- [2] Darchen R., Delage-Darchen B., Contribution à l'étude d'une abeille de Mexique *Melipona beecheii* B. (Hymenoptères : Apidés), Apidologie 6 (1975) 295–339.
- [3] Darchen R., Delage-Darchen B., Sur le déterminisme des castes chez les Meliponinés (Hymenoptères : Apidés), Bull. Biol. Fr. Belg. 111 (1977) 91–109.
- [4] Engels W., Reproduction and caste development in social bees, in: Eder J.P., Rembold C.M. (Eds.), Chemistry and Biology of Social Insects, J. Peperny Verlag, Munich, 1987, pp. 275–281.
- [5] Kerr W.E., Genetic determination of castes in the genus *Melipona*, Genetics 35 (1950) 143–152.
- [6] Kerr W.E., Sex determination in bees. III. Caste determination and genetic control in *Melipona*, Insectes Soc. 21 (1974) 357–368.
- [7] Kerr W.E., Stort A.C., Montenegro M.J., Importância de alguns fatores ambientais na determinação das castas de gênero *Melipona*, Ann. Acad. Bras. Cienc. 38 (1966) 149–168.
- [8] Koedam D., Aguilar Monge I., Sommeijer M.J., Social interactions of gynes and their longevity in queenright colonies of *Melipona favosa* (Apidae, Meliponinae), Neth. J. Zool. 45 (1996) 480–494.
- [9] Maciel-Silva V.L., Kerr W.E., Sex determination in bees. XXVII. Castes obtained from larvae fed homogenized food in *Melipona compressipes* (Hymenoptera, Apidae), Apidologie 22 (1991) 15–19.
- [10] Michener C.D., The Social Behaviour of the Bees, Harvard Univ. Press, Cambridge, MA, USA, 1974.
- [11] Sakagami S.F., Stingless bees, in: Hermann H.R. (Ed.), Social Insects, Academic Press, New York, Vol. 3, 1982, pp. 361–423.
- [12] Sakagami S.F., Oniki Y., Behavior studies of the stingless bees, with special reference to the oviposition process. I. *Melipona compressipes manaosensis* Schwarz, J. Fac. Sci., Hokkaido Univ. Ser. VI, Zool. 16 (1963) 300–318.
- [13] Sakagami S.F., Zucchi R., Portugal Araújo de V., Oviposition behavior of an aberrant African stingless bee *Meliponula bocandei*, with notes on the mechanism and evolution of oviposition behavior in stingless bees, J. Fac. Sci., Hokkaido Univ. Ser. VI, Zool. 20 (1977) 647–690.
- [14] Sommeijer M.J., de Bruijn L.L.M., Social behavior of stingless bees: 'bee-dances' by workers of the royal court and the rhythmicity of brood cell provisioning and oviposition behavior, Behaviour 89 (1984) 229–315.



- [15] Sommeijer M.J., de Bruijn L.L.M., Intranidal feeding, trophallaxis, and sociality in stingless bees, in: Hunt J.H., Nalepa C.A. (Eds.), *Nourishment and Evolution in Insect Societies*, Westview Press, Oxford, 1994, pp. 391–418.
- [16] Sommeijer M.J., Houtekamer J.L., Bos W., Cell construction and egg-laying in *Trigona nigra paupera* with a note on the adaptive significance of oviposition behaviour in stingless bees, *Insectes Soc.* 31 (1984) 199–217.
- [17] van Veen J.W., Arce H., Sommeijer M.J., Brood production of *Melipona beecheii* in relation to dry season foraging, in: Billen J. (Ed.), *Biology and Evolution of Social Insects*, Leuven Univ. Press, Leuven, Belgium, 1992, pp. 81–87.
- [18] Velthuis H.H.W., Sommeijer M.J., Roles of morphogenetic hormones in caste polymorphism in stingless bees, in: Gupta A.P. (Ed.), *Morphogenetic Hormones of Arthropods*, Rutgers Univ. Press, New Brunswick, USA, 1991, pp. 346–383.