Apidologie 37 (2006) 222–239
© INRA/DIB-AGIB/ EDP Sciences, 2006
DOI: 10.1051/apido:2006025

Original article

The polygyny of *Melipona bicolor*: scramble competition among queens

Hayo H.W. VELTHUIS[a], Han DE VRIES[b], Vera L. IMPERATRIZ–FONSECA[c]

[a] Klemit 1, 5325 KG Wellseind, The Netherlands
[b] Department of Behavioural Biology, Utrecht University, The Netherlands
[c] Departamento de Ecologia, Instituto de Biologia, Universidade de São Paulo, Brazil

Received 23 December 2005 – Revised 14 February 2006 – Accepted 15 February 2006

Abstract – The stingless bee *Melipona bicolor* is facultatively polygynous, a unique character among the bees. Polygynous colonies were not more productive than monogynous colonies. During the process of provisioning and oviposition of cells (POP) a queen may be either alone or together with one or two other queens. If together, each queen has on average the same chance to lay the egg, indicating that there is no dominance mechanism involved. During the POP, a queen may ingest some of the larval food and a trophic egg laid by a worker. Worker egg laying is less frequent in multiple queen POPs. The most active queen has proportionally more single-queen POPs and more trophic eggs. Such nutritional advantage and the resulting output of eggs could depend on chance, but a lasting qualitative difference among queens probably exists as well. Though we could outline the mechanisms behind the outcome of this scramble competition for egg laying, the adaptive significance of this polygyny remains largely mysterious.

polygyny / stingless bee / *Melipona bicolor* / scramble competition / trophallaxis / trophic eggs / Apidae / Meliponini

1. INTRODUCTION

Nearly all species of social bees are characterised by colonies that are headed by a single queen. Polygyny, the presence of several laying queens, whenever it occurs in bees, is only temporary. A well-known example is the replacement of an old or damaged queen in the honeybee colony by a young one. The two queens may live together for a limited period of time, sometimes both laying eggs. Similar transient conditions have been found in some species of stingless bees (Apidae, Meliponini) (da Silva, 1972; Witter and Wittmann, 1997). *Melipona bicolor*, a stingless bee species from Southern Brazil, however, is a facultative polygynous species, where the cohabitation of laying queens is not a transient situation. It often has several laying queens in the colony, living together for considerable periods of time.

Polygyny is not uncommon in ants, and has been studied relatively well in a number of species. Two different ontogenies exist in the ants. The first form of polygyny is cooperation among queens upon founding a colony (pleometrosis), a cooperation that enhances the success in the early period. When the colony grows, its polygyny develops into a functionally monogynous situation, either with a dominant queen that lays the eggs and a queue of other inseminated queens on a waiting list or in a truly monogynous condition. In the second form there is secondary polygyny, where monogynous colonies accept invading new queens and where subsequently more than one queen is involved in the production of sexuals. In both situations the degree of relatedness among the queens plays a

---

Corresponding author: H.H.W. Velthuis,
hhwv@xs4all.nl

role in our understanding of the evolutionary origin of polygyny.

Hölldobler and Wilson (1990) and Keller (1993) reviewed the adaptive significance of and the ecological conditions that favour polygyny in these ants. In a fragmented habitat, where the population of an ant species is subdivided into small and isolated local populations, such mini-populations would benefit from the increased effective population size (increased genetic variation) that goes along with polygyny. In contrast, if genetic variability in small isolated populations was small, altruistic cooperation would be favoured. Indeed, the intuitive assumption that the queens in polygynous colonies are related often holds but is not universally supported. Apart from selection at the levels of direct and indirect fitness of the individuals, colony-level selection and population-level selection appear to be involved.

Among the ecological conditions that may favour polygyny in ants we find: (a) the difficulty in founding a colony for some species because of nest-site limitation and predation on gynes, a factor that would favour the selection for polygyny in *Formica* (Rosengren et al., 1993); (b) the abundance of food (in *Myrmica*, Herbers, 1993), where territorial defence breaks down under abundant food supply, with the consequence that colonies become polygynous and the relatedness of workers with the sexuals that are produced in their colony drops. However, as Herbers (1993) already concluded, the role of ecological factors as the prime cause for the emergence and maintenance of polygyny in ants is still poorly understood.

Would this information on polygyny among the ants help us understand the polygyny in *M. bicolor*? So far, the answer seems to be negative. The stingless bees form a diverse group of several hundred mainly tropical species, belonging to about 25 genera (Michener, 2000). The nests are in pre-existing cavities such as in the trunks and main branches of trees, in abandoned arboreal termite nests, or deep underground in abandoned ant and rodent nests. In most species, the colonies are long-lived, persisting perhaps for decades, while individual queens may live for 1–3 years. In many of the species, colony founding, which is by swarming, might be difficult due to a shortage of vacant nesting sites. Consequently, in many species swarming is rather infrequent. This is particularly true for most *Melipona,* a neotropical genus that contains about 40 species, of which 35 are found in Brazil (Silveira et al., 2002). It is only in *M. bicolor* that we find polygyny. What is it that distinguishes *M. bicolor* from its congeneric relatives? What conditions could explain the occurrence of polygyny exclusively in this species and why do polygynous and monogynous colonies cooccur? Before addressing questions like these, we should definitely know more about the basic biology of the species and about proximate mechanisms that regulate social processes within the colony.

All *Melipona* species have well-protected nests inside a pre-existing cavity, which can be entered only through a long, narrow and strong entrance tube penetrating deep into the nest. In the nest cavity itself two parts can be distinguished, a part containing the several layers of horizontally arranged combs enclosed in a well-developed involucrum and, outside the involucrum, a number of food pots in which large amounts of food may be stored in separate honey and pollen pots. Because of the large amounts of food they produce, a number of these species are kept in managed hives, a factor that in turn has promoted the study of their biology.

The brood combs of *Melipona* are constructed sequentially and are removed once the brood has emerged from the cells. New cells are added at the margin of the youngest comb(s). Their construction, and especially the mass provisioning, egg laying and cell closure is a process involving a number of individuals that engage in intense social interactions. This process, known as POP (Provision and Oviposition Process), has been described for many species (for reviews, see Michener, 1974; Sakagami, 1982; Zucchi, 1993; Zucchi et al., 1999).

Two subspecies of *M. bicolor*, *M. b. bicolor* Lepeletier and *M. b. nigra* Lepeletier have been distinguished. For the latter the synonym *M. b. schencki* Gribodo exists. They differ in coloration and in the geographic area of their distribution. In the older literature these three names were used for separate species. *M. bicolor* is a bee from forested areas, where it nests in tree cavities near the soil, usually at damp places near a river or rivulet (Nogueira-Neto, 1997). The occurrence of polygyny in this species was first noted by Kerr (1949), but this aspect has not been previously studied. In fact, only a few studies have been published on this species. Bego (1983) reported on several bionomic aspects and da Silva et al. (1972) experimented with artificial insemination of its queens. According to the latter authors, the species can be kept in the laboratory without great difficulty, provided the colonies succeed in maintaining good control of humidity in the nest.

In this paper we investigate the cohabitation of queens. Do queens cooperate in creating egg laying opportunities? Alternatively, and perhaps also additionally, are they competitors? What governs the partitioning of egg laying among them? Is there a dominance order among the queens and by what means is this dominance maintained? Are polygynous colonies larger than monogynous colonies, and what could be the advantage to the colony to be polygynous?

2. MATERIALS AND METHODS

The colonies of *M. bicolor* originated from Cunha, São Paulo State, and were brought to the Bee Laboratory of the Ecology Department, Institute for Biosciences, University of São Paulo. Upon arrival they were housed in observation hives of the type described by Sakagami (1966). The temperature in the outer boxes was maintained at approximately 28 °C. The queen(s) in the colonies were marked with a paint dot on the thorax, each one with a different colour. These colour codes are abbreviated in the text and tables, but are explained in Table I. When needed, the colonies were fed sugar solution and provided with pollen from other stingless bee or honey bee colonies.

To get an answer to our questions, we studied the behaviours of and interactions between a queen and the workers, and among queens, both during the time periods in which POPs occurred and in the periods in between. The latter are usually characterised by much lower activity levels and we describe these behavioural aspects in a qualitative sense. Quantitative data were collected on the participation of each of the queens of a polygynous colony in these POPs, the frequencies with which they were involved in egg laying and their consumption of the different types of food.

The observations were made during the months March–April and October–November. Altogether, five polygynous colonies were observed intensively. During these observations the cover glass of the outer box had to be removed to enable a better view; as a consequence, the temperature control was interrupted. Fortunately, in *M. bicolor* new combs are always constructed on top of the pile of already existing combs; when at the bottom of the pile the bees have emerged from the lowest comb, the entire pile slowly sinks. Therefore, in this species it is unnecessary to dismember the pile of combs to obtain access to the cell construction region. To get a good view of the upper comb, the top part of the involucrum was removed, which represented a minor disturbance. However as incidental light can disturb progress in cell construction and eventually cause the interruption of an ongoing POP, dim illumination with cold red light was used during the observation periods, a measure that largely prevented disturbance.

The following colonies were subject of study: in 1995 a colony containing two egg laying queens was chosen for observations. In the beginning, for a period of 10 days, observations were made during daytime only. However, when it was discovered that at night the rate of cell production was distinctly higher than during daytime, we decided to observe the colony 24 h/day. This was done for a period of 4 days. An additional advantage of permanent surveillance was that diurnal activity cycles of queens no longer had an impact on our estimations of their activity.

In 1998, a colony having three laying queens was selected. The observations were made during 18 days for 24 h a day. All the events during this time were videotaped to correct any error or resolve uncertainty in the direct observations. Later that year this colony, still with the same three queens, was used again for experiments. During the latter period of 12 days, a number of POPs were recorded that are included in the present paper. These two periods are indicated as 1998-A and 1998-B, respectively.

In March 1999, the colonies of *M. bicolor* in the laboratory had ceased brood production, probably as a response to a period of over two months of excessive rainfall. When the weather conditions improved, it was possible to study the onset of brood production. For this purpose, a colony having three marked queens was selected. The observations again covered 24 h a day and lasted 10.5 days. Data collection was supported by video recording. In the analysis of oviposition rates the data are separated into a first period (1999-I, 6 days) during which brood cell production slowly increased, and a second period (1999-II, 4.5 days) when this production had attained normal values for a colony of its size.

Finally, in the second half of 2000, we studied a colony with three queens that was made by uniting three monogynous colonies, two of them being weaker than the third. Each of these monogynous colonies had a young queen. Three months later, the observations reported here started. They were of 24 h/day, supported by video registration, and lasted 20 days. Again, all POPs occurring during this period were registered. At the onset of the observations the ages of the queens were 165, 176 and 222 days, respectively. Because one of the queens stopped laying eggs, the observation period was divided in a first part (2000-I, 10.5 days) with three active queens, and a second part (2000-II, 9.5 days) with only two.

In the analysis of these five sets of data, not all information was available for each POP. Consequently the number of POPs for a given set may vary, depending upon the particular question addressed.

3. RESULTS

In the first two parts of this section a generalised and qualitative description will be given of the behaviour of the queen and workers of *M. bicolor*. Here just those elements and sequences will be mentioned that appear useful for the understanding of the mechanisms involved in the maintenance of polygyny in this species. In the following parts of this section quantitative data relevant to our theme will be presented.

3.1. Queen and worker behaviour and their interactions

On inspection of a brood nest a queen may either be visible to us on the smaller upper comb or on the larger one below it, or she may be invisible, on the bottom of the nest, where she is thought to be at rest. This interpretation is supported by the observation that sometimes a queen can be found on the involucrum, where she hardly moves. Usually at such times she is surrounded by a court of worker bees, also rather immobile. Due to her heavy abdomen she is typically unable to walk in the narrow space between combs.

A period of time spent resting is followed by a period of activity, during which the queen patrols over the comb where cells are being constructed at its margin. Most of the time she walks along this margin, sometimes stopping at an empty cell completely or nearly completely constructed. This is the case when the tubular cell protrudes above the comb, as if having a “collar”. The queen actively interacts with workers by antennating them. If the worker is active in cell construction, the queen is simply ignored. However, if the worker stands or walks on the comb and is antennated frontally by the queen she usually retreats. In some workers, antennation may also result in bending the head down, whereupon the queen may beat with her antennae the occiput region of the worker’s head. Withdrawal and head bending may co-occur. The beating by the queen, in some cases of higher intensity than in others, may result in a withdrawal of the worker by quickly moving backward. She either escapes the attention of the queen, or the queen follows. In the latter case the queen may request trophallaxis by extending the tongue, an action that usually remains without effect. Trophallaxis may occur, in which case the worker must lift her head, allowing the queen to position the tongue in between the mandibles of the worker. A worker that offers food has the mandibles spread open, the same position as used in threat display. If a worker retreats but is followed by the queen, once the worker has reached the comb margin and tends to descend in a backward movement, she suddenly finds herself in a position where trophallaxis can be forced by the queen: the position of her head, relative to the head of the queen, approaches the proper position for a trophallaxis. This conformity often allows the queen to put the tongue in between the worker’s mandibles, which might lead to an actual food exchange, sometimes through a transient moment of biting the tongue of the queen. After a trophallaxis of variable duration, the worker may escape to lower levels and disappear from sight.

Often, during periods in between POPs, there are but few workers present on the comb. If the queen remains on the comb, perhaps because she found cells ready to be provisioned, her incessant approaches to workers causes them to become more active. The queen may position herself for some period of time near such a cell. From that position she then solicits trophallaxis, eventually abandoning that cell if unsuccessful. The undirected movements on the comb and the positioning near the selected cells have been called "patrolling", "arousal" and "cell fixation" (Sakagami, 1982; see also Zucchi et al., 1999). During this cell fixation (the preprovisioning stage of the POP) a worker, usually from a vertical position at the outer wall of the empty cell or its neighbouring and already sealed cells, no longer retreats when approached by the queen. She resists the solicitations, may dart towards the queen from the other side of the cell, but such interactions ultimately end either in feeding the queen or by inserting the head and thorax into the empty cell. The latter behaviour may be repeated several times, and is usually performed by a sequence of several workers. In this position the worker might respond to beatings by the queen on the abdominal tip of the worker; the queen beats with her front legs and antennae and one of the possible responses by the worker is to regurgitate food into the cell. This response demarcates the transition from the preprovisioning stage into the provisioning stage of the POP. After this first food deposition, the respective worker quickly departs from the cell to lower levels in the nest, but she may return later on and may deposit another portion of food into the same cell.

The duration of the provisioning stage depends very much on the availability of food circulating among the workers. A number of workers will come to the cell, and either deposit food or just insert their head and thorax into the cell, an action we called deep cell insertion. Some of those inserting workers disappear from the scene and may return later to deposit; others will stay, and insert later on again. In the early stages of provisioning the depositing workers adopt a vertical position and insert their fore-bodies into the cell. With increasingly higher food levels in the cell they stand on the comb and remain in a horizontal position. Food deposition is visible due to the strong abdominal contractions of such workers and from examinations of the cell contents. Increase in the food levels and movements of the surface of the liquid food confirm the indirectly obtained information. At the end of the provisioning stage, a worker's deposition of food appears to be inhibited by the food level in the cell, and the abdominal contractions, though visible, remain small.

Meanwhile the queen participates in the process. During preprovisioning she is close to the cell, often having her mandibles just above the cell margin, while after the first food deposition she withdraws and usually stays at one cell diameter distance. Her stimulation of workers through beatings, which may lead to regurgitation, usually ceases after the first depositions of food. Even when she walks away for a brief period of time, further provisioning by the workers is not interrupted. While standing at 1–2 cell diameters distance she regularly appears to collect information by outstretching her antennae, or she may approach the cell and insert her head, only to withdraw again.

The end of the provisioning stage can only be identified afterwards: there are no further depositions, only insertions, and a worker may lay an egg. However, the queen indicates the onset of the postprovisioning stage. After she has inserted her head she moves still further away from the cell, up till 3–5 cell diameters. From that position she may regularly come closer to the cell, putting the very tip of the antennae just above the cell and then retreating again, or inserting the head and subsequently returning to her distant position. Both approaches are considered inspections of the situation. It may occur that the queen consumes food from the cell, an act that may stimulate a worker to replenish the food quantity by regurgitation. If a worker positions herself onto the cell and produces a trophic egg, an act that takes about 5 seconds on average, the queen usually notices this and approaches the cell while the worker is still laying the egg. Typically, she beats the worker's thorax and abdomen from the side and, as soon as the worker departs, starts to consume the egg, upon which she retreats again. Occasionally she does not detect the laying worker, and the worker will depart after her oviposition. In such a case it may take some time before the queen comes to the cell and finds the egg. In the meantime, several workers may have inserted the head into the cell. It appears as if, for the queen, the laying worker is recognisable from a distance whereas a trophic egg she produced is far less discernible.

At a certain moment in the postprovisioning stage, the queen moves towards the cell, eats from the food therein and lays an egg. She then leaves the cell, which remains open until a worker comes, sits on the cell and closes it by folding the collar inward in a rotational movement. These are the oviposition period and the cell closure period, respectively. Often the queen remains active, leading to a sequence of several POPs, after which she disappears from sight for a long period of rest.

3.2. Interactions among queens

In a polygynous colony there are interactions among the queens. While resting, they may be together, having a common court, but when active they may respond to each other upon encounter. During the patrolling phase a queen may try to touch the abdomen of the other one, which then turns her abdomen away. This may lead to two queens circling, each trying to get at the opponent's abdomen, while simultaneously withdrawing her own abdomen. Such circling queens are more common during the provisioning phase, once they have met each other at the cell. However, the cell appears to be more important, because the circling almost always ends in the two queens standing side by side, their heads directed towards the cell, in turns moving forward for an inspection or insertion. However, while a solitary queen often remains relatively motionless, a couple or triplet of queens is always moving: sideways, perhaps to manoeuvre away the competitor, and for- and backwards. Especially in case of a triplet of queens the anthropomorphic interpretation is one of increased nervousness and tension between them.

For an individual queen the frequency of inspection and insertion is not elevated when compared to a cell attended by a single queen. However, for the workers approaching the cell, two queens may constitute a larger barrier than a single one would. The attainability of the cell is certainly affected, which might find its expression not only in the temporal aspects of the POP, but also in the type and frequency of worker behaviours performed.

The acts of consuming trophic worker eggs or larval food and of egg laying by one of the queens does not appear to be different for one of a pair of queens, compared to POPs with a solitary queen. The other queen usually does not respond when a queen approaches the cell or eats from its contents. When there is a laying worker, the two may approach her simultaneously and it depends on the position that each of them has relative to the laying worker which queen will get the egg; it is the one near the worker's abdomen, because the worker departs by moving forward. When a queen positions herself on the cell in preparation for oviposition, the other queen comes forward, antennates the abdomen of the laying queen near the cell rim, and usually departs already before oviposition is completed. In conclusion, when more than one queen participates in a POP, there is no mutual egg destruction or mutilation, only interference in the competition for opportunities to oviposit.

3.3. Repartition of egg laying opportunities among queens

In the colonies with two or three egg laying queens, just a single queen was present during some POPs. In other cases one or two queens joined the first one or, in still other instances, two queens jointly searched for a cell ready for provisioning, which led to their joint presence during the entire POP. Because a queen almost never withdrew from the cell during the POP, this had the consequence that at the onset of the postprovisioning stage some POPs had a single queen, while in other POPs

Table I. Basic data on the frequencies of presence of a single or multiple queens at cells during POPs. Abbreviations for queen identity are: sm: no marking; av: yellow/green; B1: blue; Gr: green; W: white; R: red; P: pink; U: unmarked.

Colony	Queen	Frequency of queen presence					Total queen presence
		Single	Both				
1995	sm	74	37				111
	av	45	37				82 +
n. of POPs: 156 =		119	+ 37				193
			In combinations				
		Single	Bl+Gr	Bl+W	Gr+W	All 3	
1998-A	Bl	118	102	53	—	64	337
	Gr	58	102	—	36	64	260
	W	45	—	53	36	64	198 +
n. of POPs: 476 =		221	+ 102	+ 53	+ 36	+ 64	795
			In combinations				
		Single	Bl+Gr	Bl+W	Gr+W	All 3	
1998-B	Bl	36	1	6	—	0	43
	Gr	14	1	—	2	0	17
	W	9	—	6	2	0	17 +
n. of POPs: 68 =		59	+ 1	+ 6	+ 2	+ 0	77
			In combinations				
		Single	R+Gr	R+W	Gr+W	All 3	
1999	R	48	21	17	—	8	94
	Gr	20	21	—	6	8	55
	W	16	—	17	6	8	47 +
n. of POPs: 136 =		84	+ 21	+ 17	+ 6	+ 8	196
			In combinations				
		Single	P+W	P+U	W+U	All 3	
2000-I	P	44	16	64	—	43	167
	W	5	16	—	29	43	93
	U	39	—	64	29	43	175 +
n. of POPs: 240 =		88	+ 16	+ 64	+ 29	+ 43	435
		Single	Both				
2000-II	P	62	93				155
	U	105	93				198 +
n. of POPs: 260 =		167	+ 93				353

two or three queens were present. We observed which queen would lay the egg, and whether the number of queens present at the cell had an impact on the characteristics of the postprovisioning stage. The moment of arrival at the cell hardly had an impact on the chance of laying the egg, only a late arrival of a second queen could make the first queen oviposit immediately. Table I gives the number of times that each of the queens was present at a cell, for each of the groups in which either the queen was alone or queens were together. Obviously,

Table II. Characteristics of the colonies: total egg production, egg laying rates (POPs/day), consumption of trophic eggs (TE), and level of competition among queens. *: Values for colonies with 2 queens, all other colonies had 3 egg laying queens.

					Most productive queen	
Colony	Number of POPs	Number of POPs/day	Number of TEs/POP	Proportion of POPs with > 1 queen	Share in egg laying	Share in TE consumption
1995	156	19.0	0.67	0.24	0.61*	0.65*
1998-A	476	26.4	0.32	0.54	0.46	0.59
1998-B	68	24.0	0.76	0.13	0.61	0.57
1999-I	40	6.7	0.13	0.23	0.50	0.20
1999-II	98	21.8	0.21	0.43	0.46	0.67
2000-I	240	22.8	0.49	0.63	0.42	0.51
2000-II	260	27.4	0.48	0.36	0.61*	0.68*

in multiple queen POPs, presence does not mean laying the egg. The proportion of cells that had a single queen differed among the colonies observed, and varied from 0.37–0.87. By means of χ^2 tests (see Appendix available at http://www.edpsciences.org/apido) we investigated whether queens were attracted to or avoided each other during POPs, or whether their encounters were at random. Randomness was indicated for the data of 1998-A (χ^2 = 3.92, d.f. = 3, P = 0.27), 1998-B (χ^2 = 5.51, d.f. = 3, P = 0.14) and 1999 (χ^2 = 0.17, d.f. = 3, P = 0.98), while for sample 2000-I randomness is rejected (χ^2 = 9.25, d.f. = 3, P = 0.026). Of course, such an analysis for colonies with only two queens is not possible (degrees of freedom = 0).

During the postprovisioning stage of a POP we often recorded one or several workers laying a trophic egg, and mostly when there was just a single queen present at the cell. In POPs attended by two or three queens, trophic eggs were less frequently laid, and again less so when three compared to two queens were present. These trophic eggs were always eaten by a queen, but not necessarily by the one that laid the fertile egg later on. It never occurred that more than one fertile egg was laid in a cell.

In Table II, third column, the colonies are compared with regard to the daily rates of cell production. Except for the first period of colony 1999, characterised by low production rates, production ranged from 19.0–27.4 cells per day, which is typical for a good colony of this species. In November 1998 production rates were also determined in three monogynous colonies, and were found to be 17.6, 22.0 and 29.9 cells/day, respectively. These averages were obtained over periods of 36, 18 and 20 days, respectively.

In addition, Table II also presents the data on the occurrence of trophic eggs. Among colonies, the average number of trophic eggs per POP varied from 0.13–0.76. There is no correlation (Kendall rank correlation) with the production rates of cells. Also the proportion of POPs with more than a single queen in attendance (varying from 0.13–0.63) is not correlated with either the cell production rate or the frequencies of trophic eggs in the cells. These features appear to be regulated independently.

Finally, Table II compares the egg laying and egg consumption rates by the most productive queen of each of the colonies. The share of the most productive queen in egg laying fluctuated from 0.42–0.61 in colonies with three queens, and was 0.61 in the two colonies which had only two queens. Except for the data on 1999-I (in which only very few trophic eggs were laid), her share in the consumption of trophic eggs was almost equal or higher than in the production of eggs. Necessarily, the less productive queens had a less favourable ratio

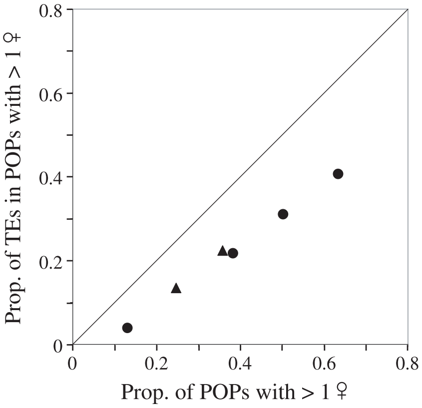

Figure 1. The proportion of trophic eggs (TEs) in the POPs with more than one queen as a function of the relative frequencies of such POPs. Colonies and periods with three queens are represented by dots, while triangles show the values found when only two physogastric queens were present in the colony. If the probability for the appearance of a trophic worker egg is independent from the number of queens attending the provisioned cell, data points should lie on the diagonal. If the laying of a trophic egg is hampered when more than one queen is attending, due to reduced duration of the postprovisioning stage or accessibility of the cell, then a curved line below the diagonal is expected, having points of intersection with the diagonal at 0.0 and 1.0.

of eggs consumed versus eggs laid. Indirectly, we arrive at the conclusion that there is a link between the rates of egg production and egg consumption in these queens.

In Figure 1 the proportion of the cells attended by more than one queen is related to the proportion of trophic eggs laid in these multiple queen POPs. These two characteristics of each set of data are compared with two models. The first model assumes that trophic egg laying is independent from the number of queens attending the cell. In that case, the data points should lie on the diagonal in the figure. Except for 1999, all points deviate significantly from the diagonal (χ^2 tests, all $P < 0.01$). The other model supposes that the queen determines how long the postprovisioning stage will take: either being of short or of long duration. If two queens are present at the cell, the postprovisioning stage will be of long duration only when both queens decide to wait, which is the condition that promotes the appearance of trophic eggs. As a consequence, trophic eggs will appear less frequently when there is more than one queen at the cell. The data points contrast with the first, but conform to this second model.

3.4. Food acquisition and egg laying rates of queens

In the course of a POP, three sources of food become available to a queen: (1) she may obtain some food by means of trophallaxis during the preprovisioning stage; (2) she almost always consumes from the provisioning of the cell immediately before ovipositing but, in addition, may already have eaten this kind of food earlier during the postprovisioning stage, and (3) she obtains food by consuming trophic eggs. Trophallaxis as well as food uptake from a brood cell concerns the mixture of sugars and pollen grains in the food for larvae, while trophic eggs are qualitatively different, containing the proteins that workers obtained and synthesised after digesting pollen.

The different conditions in which we encountered queens in the various colonies, allowed us to investigate the relative importance of these three food sources for the egg laying rates of queens.

The colony of 1999 provided data to illustrate the role of trophallaxis (Tab. III). During the first period of six days of observation, an extremely high frequency of requests for food was found. On day 3 this amounted to over 10 times/hour, while over the second period of 4.5 days the average frequency was considerably lower. In the first period, the rate of success of begging for food increased across days (Kendall rank correlation = 0.73, n = 6, two-tailed $P = 0.04$). Among the queens this success rate did not differ.

Part C of Table III specifies the average food intake by queens per egg laid. While intake through trophallaxis changed considerably over the time period of the observations, larval food intake from the cell provisionings was relatively constant. The same holds for the consumption of trophic eggs, which was, in

Table III. Food acquisition and egg laying in colony 1999. The values obtained for the first 6 days, during which egg laying was re-initiated, are compared with the daily averages from the second period, in which productivity attained a normal value (see Tab. II). All trophallaxis requests in the first period are included, for the second period only those requests that occurred during the preprovisioning stage.

	First period						Second period
Day	1	2	3	4	5	6	average/day
A – Trophallaxis							
n. times asking	106	135	256	127	114	88	20.4
success rate	0.28	0.33	0.36	0.50	0.51	0.42	0.71
B – Food uptake and egg laying							
duration of larval food intake (s)	–	–	73	104	87	94	158
n. trophic eggs eaten	1	0	0	2	1	1	4.7
n. eggs laid	3	2	8	8	9	10	21.8
C – Food consumption per egg laid							
n. times trophallaxis	10	22	11.5	8	6.5	3.7	0.65
duration of larval food intake (s)	–	–	9	13	9.5	9.5	6.7
n. trophic eggs eaten	0.3	0.0	0.0	0.25	0.1	0.1	0.2

fact, extremely low in this colony. In contrast to the consumption of larval food, a behaviour the queen can perform independently from the workers and which we may expect to be conform to her nutritional requirements, both trophallaxis and the consumption of trophic eggs depend on what workers offer.

In this colony, during the first days of observations, the success rate of food requests fluctuated significantly, depending on whether it occurred during the preprovisioning stage of a POP or in between POPs. In between POPs the queen(s) appeared on the comb regularly and could stay for considerable time (h). Success rates of food requests ranged from 0.10–0.16. During the preprovisioning stage however, when queens stayed near the selected cell, their success rates ranged from 0.33–0.86 (Tab. IV). The high rates of food requests decreased beginning on the fourth day, but success rates in the preprovisioning stage remained high, 0.71.

Table IV. Trophallactic activity during the first days of re-initiation of egg laying in colony 1999.

Observation period in- / outside POP	Day	Number of trophallaxis requests by queen(s)	Success rate
In	1	41	0.46
Out	2	51	0.10
Out	2	51	0.14
In	3	71	0.52
Out	3	36	0.16
In	4	71	0.44
In	4	45	0.33
Out	4	19	0.11
In	4	7	0.86
Out	4	10	0.10
In	4	17	0.70

The conclusion is that one function of asking for trophallaxis is the activation of workers in the preparation of a POP. In this colony, reactivating after a period without any brood production, this arousal of workers by the queen was very prominently present; trophallaxis was more frequent than in colonies with a steady brood production. Perhaps, in this unusual situation, the queen may have received some food by means of trophallaxis. Under

Table V. (a) The relation between food consumption and egg laying at the individual level in colony 1999-II, days 7 to 11. Queen R consumed less food from the cell and more trophic eggs per egg laid compared to the other queens. (b) Conditions for and frequencies of egg laying and trophic egg consumption by the queens. Queen R obtained her high productivity by being the most active of the three queens. She was most often alone in a POP, the condition that allowed her to ingest a large number of trophic eggs.

(a)

	Trophallaxis					Ratios per egg laid	
Queen	Requests (n)	Success rate	Food from cells (sec)	Trophic eggs eaten	Number of eggs laid by queen	Food from cells (sec)	Trophic eggs
R	26	0.62	278	14	45	6.2	0.31
Gr	38	0.63	166	2	21	7.9	0.09
W	29	0.90	210	5	32	6.6	0.16

(b)

	Alone in POP		In pairs			3 queens present in POP			Totals	
Queen	Eats	Lays	Frequency	Eats	Lays	Frequency	Eats	Lays	Eaten	Laid
R	11	34	14 + 16	3	11	7	0	0	14	45
Gr	2	6	14 + 5	0	12	7	0	3	2	21
W	5	16	16 + 5	0	12	7	0	4	5	32

normal conditions the low frequency of actual food transmission and the short duration of it precludes a significant role of trophallaxis in the nourishment of the queen.

At the individual level it was noted that initially queen Gr requested trophallaxis much more often. This queen also spent more time in consuming food from the cell, and ate 4 of the 5 trophic eggs laid in the first period of six days. Nevertheless, over this period of time her egg production was a bit lower (n = 17) than that of queen R (n = 20). This demonstrates that there is no immediate response in terms of eggs produced compared to the advantage in the consumption of food. During the second period, queen R became the principal egg layer; this was combined with the highest ratio of trophic eggs consumed/eggs laid (Tab. Va).

A further analysis shows the impact of trophic eggs from another perspective, namely through the effect multiple queen POPs had on the probability that workers would lay trophic eggs. During the first 6 days (period 1999-I), two or three queens were present at 9 out of 40 POPs, while for the same colony in the second period this was the case in 46 out of 98 POPs. In this period (compare Tab. Vb) queen R was present in 71 of the POPs, laid an egg in 45 of these cells and consumed 14 of the trophic eggs. Queen Gr was present in 32 POPs, laid an egg in 21 of them and consumed 2 trophic eggs, while for queen W these values were 44 times present, 32 eggs laid and 5 trophic eggs consumed. The relative frequencies at which the three queens laid an egg, in relation to their presence was almost identical (R: 0.66; Gr: 0.63 and W: 0.68). However the ratios of trophic eggs consumed to eggs laid differed among the queens (R: 0.31; Gr: 0.09 and W: 0.19). Queen R was more frequently alone at the cell than the other queens (χ^2 = 7.8, d.f. = 2, $P < 0.05$). With just one queen at the cell the probability of receiving a trophic egg was higher (χ^2 = 8.9, d.f. = 1, $P < 0.01$). Queen R's higher rate of egg laying is the consequence of being more active. Because of this, she is relatively more often alone during the POP, and this led to a more favourable ratio of trophic eggs versus eggs laid.

The 1998-A colony provided us with information on the uptake of food from the brood cell during the postprovisioning stage of the POP. In multiple queen POPs, the queens differed behaviourally. In a total of 507 POPs, we observed 838 times that food was taken by a queen. In 490 POPs (96.6% of all POPs) this occurred immediately before the queen oviposited. In 348 cases, food consumption had also taken place at an earlier moment. In POPs with just a single queen attending, this occurred 73 times (in 30% of such POPs). In POPs with two queens, the queen that would oviposit later had already taken food before in 60% of the POPs, the other queen only 35% of times ($\chi^2 = 13$; d.f. = 1, $P < 0.01$). In 65 POPs three queens were present; 72 times an early food consumption was registered: 46 times by the queen that would oviposit later on against only 26 times for the other two queens taken together ($\chi^2 = 30$; d.f. = 1, $P < 0.01$). Similar figures and differences were found for some of the other colonies. This showed that queens that were together at a POP, may be there for different reasons: oviposition, obtaining larval food, or obtaining a trophic egg. As a consequence, they behave differently.

Differences in the behaviour of queens may have a more permanent character. In colony 1998-B, the durations of the preprovisionings were measured and, on average, were found to be different for the queens. For queen Bl the shortest durations were found, 3.3 min on average (mode 2 min), queen W had 5.0 (mode 3) min and for Gr the average was 7.2 (mode 6) min. In colony 2000-I, queen W was almost incapable of stimulating workers to deposit the first regurgitation and, consequently, in her POPs the preprovisioning stage was either long, or, when short, might have been the result of this queen being joined by another more stimulating queen. Perhaps such differences have their origins in the details of the antennal beatings by the queens once workers performed their deep insertion.

The effect a queen has on the frequencies of receiving a trophic egg by delaying her oviposition is illustrated with data from colony 1998-B. If the postprovisioning stage is brief, because the queen laid her egg soon after the last cell provisioning, very often there was no time for a worker to lay a trophic egg. If however the queen delayed oviposition, usually one or more trophic eggs were produced by the workers and consumed by the queen (Fig. 2). If there was just one queen, the average duration of the postprovisioning stage was found to be longer, and consequently, the incidence of finding a trophic egg in the cell was much higher than if there were multiple queens. Interestingly, queens often differed in their behaviour when alone at the cell. For instance in colony 1998-B, queen W did not wait long for a trophic egg in the postprovisioning stage of a POP. She always laid her egg within 6 min, whereas the other two waited longer, for up till 19 and 26 min, respectively. Queen Bl was always successful, whereas in 9 of the POPs of queen Gr with a postprovisioning stage of over 6 min, a total of only 4 trophic eggs were laid.

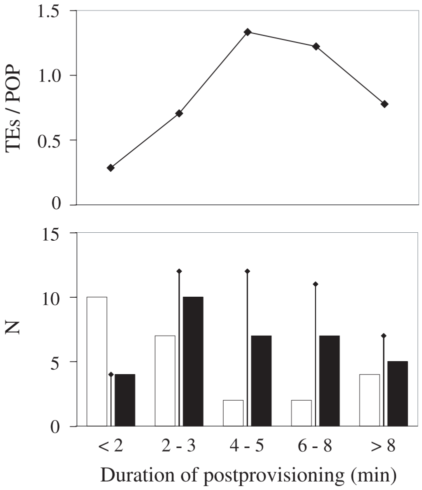

Figure 2. The relation between the duration of the postprovisioning stage and the probability of workers laying a trophic egg in the cell. Data are from colony 1998-B, and concern only POPs with a single queen. Below: the number (N) of POPs without (open bars), and with (black bars) trophic eggs (TEs) in relation to the duration. Between each pair of bars the total number of trophic eggs laid in that category of duration is indicated by a line. Above: the implicit functional relationship between the ratio of trophic eggs made available per POP and the duration of the postprovisioning stage of the POP, indicating an optimum in the success of a queen if she postpones her egg laying.

In Figure 3 the relations between the intake of trophic eggs and egg production of the queens is given. This figure shows that there are important differences in the daily egg production and the ratios of trophic eggs versus eggs laid among the queens, both within and among colonies. An analysis of these data by means of a general linear model in which egg production is the dependent variable and

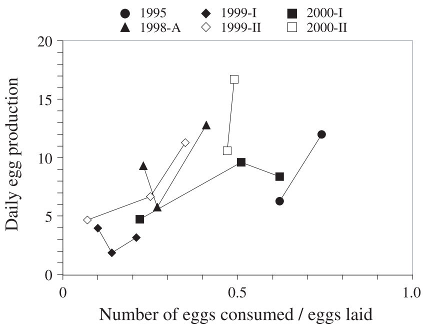

Figure 3. The relationship found amongst the queens of our colonies between a queen's daily egg production and the number of trophic eggs consumed per reproductive egg laid. Data points for queens of the same colony are connected by a line for ease of reference only.

ratio of trophic eggs consumed versus eggs laid and colony are the independent variables, shows that there is no significant interaction effect of colony and egg ratio on egg production ($F_{(5,4)} = 1.33$, $P = 0.40$). This means that the linear relationship between the ratio trophic egg/eggs laid and egg production is not influenced by the colony. We therefore, subsequently tested the model that did not include the interaction effect. Using this model to explain the observed data on daily production, it was found that egg production is significantly influenced by the ratio trophic eggs consumed versus eggs laid ($F_{(1,9)} = 7.73$, $P = 0.021$) and that the difference in average egg production between the colonies is not ($F_{(5,9)} = 3.25$, $P = 0.060$).

4. DISCUSSION

The data from colony 1998-A has been previously analysed (Velthuis et al., 2001) and showed that a queen's presence in a POP in this colony, either singly or in combination with other queens was random. The queens differed in their proportions of being single or in a combination with other queens; this only depended on the number of POPs attended. In the same analysis it was found that the probability of a worker laying a trophic egg was lower in a POP with multiple queens compared to those attended by just one queen. The most active queen, therefore, had a more favourable ratio of ingesting trophic eggs compared to the number of eggs she produced. It was also found that the probability of laying the egg, in cases where there were two queens, did not differ from $P = 0.5$, and in cases where there were three queens, it did not differ from $P = 0.33$.

The present seven sets of data allowed us to investigate whether the earlier conclusions can be generalised. First we dealt with the randomness of the encounters of queens during a POP. Randomness was confirmed for two more data sets involving three queens, but was not confirmed in a third (2000-I). In this colony the incapability of queen W to provoke the provisioning of the cell resulted in a raised frequency of multiple queen POPs and a significant χ^2-value for the distribution as a whole.

The second element in the investigation was the question concerning the frequencies with which workers lay their trophic eggs: is there a difference between POPs with a single queen and those with multiple queens? Such a difference was found in all colonies: in POPs with a single queen the probability of receiving one or more trophic eggs was 2–4 times higher than in POPs with multiple queens.

Random encounters and differences in the number of POPs attended were the cause of different ratios of trophic eggs consumed versus eggs laid, which in itself could be a stabilising factor for the different reproductive outputs of queens in a colony. Once differences emerge, they are maintained for some period of time, but they need not be stable. Random processes could invert the sequence of productivity of the queens. At this point the comparison of the two 1998 samples of the same colony is relevant. After half a year the order of the queens (in the number of eggs laid by each of them) was still the same, queen Bl > queen Gr > queen W. Therefore, these differences are perhaps not just the result of some random process, but are the consequence of lasting quality differences among these three queens.

This brings us to the relation between nutrition and egg laying of a queen. Three sources of food were distinguished: the food obtained during trophallaxis, the larval food taken during and after the provisioning stage, and the trophic eggs laid during the postprovisioning stage. Trophallaxis and food from the cells comprise the same mixture of carbohydrates and pollen stored in the worker's stomach. Of these two sources trophallaxis usually is infrequent and of short duration, while food from the cells is available without workers having control of the amount a queen can ingest. Trophic eggs appear to be a superior kind of nutrition for a queen, but in this case the queen again depends on the workers that can lay them.

The year 1999 provided us with insight into another function of trophallaxis. We witnessed extremely high frequencies of trophallaxis requests by the queens (Tab. III). During the first 6 days, queen Gr asked for it 591 times, queen R only 196 times; both had a success rate of 0.4. The success rate of requests was low during the extra oviposition periods, but became high when a POP was to begin (Tab. IV). These features demonstrate a different function for trophallactic requests: the queen stimulates workers to collect food from other workers, which in turn will also search food, in part taking it from storage pots (compare Sommeijer et al., 1985). As a consequence the workers may prepare for the provisioning stage by collectively storing more food in their stomachs. This makes it possible that during the POP, food transfer among workers can be easily initiated. At the same time, the success rate of begging informs the queen about the preparedness of the workers, and if it is low she could eventually return to a resting place. Trophallaxis, therefore, is not primarily a way of getting nourishment. This picture includes the idea that workers, without stimulation by the queen, prepare themselves for the event of provisioning a cell, be it perhaps at a lower pace.

The amount of food taken from the cell during the POP is probably much bigger compared to what is received through trophallaxis. Uptake of food is almost an obligatory act immediately before oviposition, but may occur also earlier, independent from egg laying. As Table IIIC shows, in a colony where the egg production rate was changing rapidly, the average duration of this food uptake per egg laid was rather constant. Food from the cells appears to be the basic energy resource for the activity of the queen, including oogenesis.

The availability of trophic eggs was quite different among the colonies studied. The ratio of the number of trophic eggs consumed over the number of eggs laid by the queens varied from 0.13–0.76 (Tab. II). Only the lowest of these values was associated with a low average of queen-laid eggs per day. An exchange ratio of 0.21–0.76 combined with egg production rates from 19.0–27.4, and there is no correlation between these two parameters at the colony level. Does this undermine the earlier conclusion that the consumption of trophic eggs is essential for higher egg production rates in queens?

Let us compare the ratios of these two types of egg at the individual level within each colony. In two of the colonies studied the difference among the queens in the number of eggs consumed compared to the number of eggs produced was statistically significant: 1998-A ($\chi^2 = 8.94$; d.f. = 2, $P < 0.05$) and 2000-I ($\chi^2 = 19.40$; d.f. = 2, $P < 0.01$). In these colonies, the majority of POPs (54 and 63%, respectively) had multiple queens. This factor boosts the differentiated consumption of trophic eggs among the queens: trophic eggs appear most frequently in single queen POPs, a category in which the most active queen is overrepresented. In the other colonies, the proportion of multiple queen POPs varied from 0.12–0.38. In combination with the sample size of POPs, this factor is considered responsible for obscuring the correlation between productivity and egg ratios in these colonies. However, as Table II shows, the queen that was most active in egg laying tended to have a relative share in trophic egg consumption that was higher than her share in egg laying. This is supported by the conclusion derived from Figure 3. The statistical analysis of these data demonstrated a significant correlation between the daily egg production and the ratio of trophic eggs consumed versus eggs laid. But there is an important difference in this ratio among queens, even when they have a high production. In conclusion, the link between the ingestion of trophic eggs and the increased production of eggs must be an indirect one. Perhaps the fat bodies of the queens harbour a large reserve of proteins, and it is this reserve that could enable the maintenance of a high daily rate when the intake through the consumption of eggs is temporarily reduced. However, a production rate of less than five eggs per day is always associated with a low exchange rate, indicating that the food taken from the brood cell, available ad libitum, is not sufficient for attaining high production rates. This might be due to a much longer time needed to digest the pollen in this food compared to that for the digestion of trophic eggs.

That queens have a special interest in receiving trophic eggs instead of larval food can also be concluded from the observation that those queens that do not lay the egg in multiple queen POPs ingest less larval food than they would do as an egg layer. They are there in the prospect that there may be a trophic egg, and they are less interested in the possibility to produce an egg themselves. This dual motivation for attendance at POPs is also reflected in the variable duration of the postprovisioning stage: if egg laying is the prevalent motivation, then the queen will lay her egg almost immediately. If, however, she is waiting for a trophic egg, she withdraws from the cell, thereby enhancing the probability that a worker will lay. If needed she may postpone oviposition for many minutes.

It remains to be clarified whether a queen waiting for a trophic egg is able to perceive the presence of a worker capable of laying such an egg. For an observer, such a presence was often visible, due to the behaviour of the worker. However, under natural conditions, inside the nest there is darkness. Mechanisms for detection can be expected to be based on odour differences among workers. We often witnessed the presence of such a prospective laying worker in cases where the queen laid the egg very early in the postprovisioning. The queen either neglected the information or may not have noticed it.

One theoretical possibility needs to be mentioned, namely that workers recognise one of the queens as their mother and attune their behaviour accordingly, in the interest of their fitness. Alonso et al. (1998) addressed that question and found no preference for the queens among individually marked workers, as expressed in any aspect of their POP behaviours. From our observations we can only confirm that conclusion: a prospective laying worker, unable to lay her egg because the queen has already laid, is usually present in the next POP and tries again, irrespective of the identity of the queen she encounters. We did not find indications that could stimulate us, or others, to combine direct behavioural observations with an analysis of genetic relatedness of the individuals involved. Such a refined tuning of worker behaviour and fitness aspects appears unlikely, because the polygyny of *M. bicolor* is only a facultative one, indicating that there is not a strong selection pressure leading to or maintaining that condition. In addition, in polygynous colonies the queens are likely closely related, being mother-daughter or sister combinations.

In conclusion we can affirm that the competition among the queens is of the scramble type: there is no behavioural dominance of one queen leading to exclusion of the others, no difference in the number of eggs laid by each of the queens when they are together at POPs and no mutual egg destruction. The divergence in the number of eggs laid leads to a disparity in the number of single-queen POPs among the queens. As a consequence, the most productive queen obtains a disproportionately large share of the trophic eggs produced by the workers. It is this superior protein source that enables the most productive queen to maintain her top position, but perhaps there are also fundamental differences at the basis of their divergent productivities. Perhaps queens differ in the efficiency of the conversion of food into eggs or in the amount of proteins stored in their fat bodies, that enable them to overcome periods during which protein intake is mainly in the form of pollen grains.

We are only beginning to comprehend the adaptiveness of polygyny. Apparently, there is no advantage of polygyny for the queens. We found no indications that polygynous colonies are larger and produce more brood than monogynous ones. In fact, the highest productivity, 29.9 cells per day, was encountered in a monogynous colony. The productivity of

M. bicolor is not different from other *Melipona* species. This is understandable insofar as productivity is mainly determined by the workers, as they govern the rate of cell production and provisioning. As long as the egg maturation rates of the queen(s) are potentially higher than the cell production rates of the workers, the workers are the limiting factor. Workers are also in a position to regulate egg maturation rates in the queens by adjusting the rates with which they provide the queen with trophic eggs. In polygynous colonies of *M. bicolor*, the proportion of multiple queen POPs becomes a colony level instrument in the adjustment of the individual queen productivities. But what could be the advantage? In all other monogynous species, colony level regulation appears to be equally efficient. Given that there is no advantage of polygyny at the level of the queens, and polygyny reduces the relatedness of workers with the brood they raise, we should look for an explanation at the colony level.

There is perhaps an advantage of polygyny in situations where a colony has to recommence its production of brood cells, such as occurs after an extended period of food shortage. Under such conditions, queens may be limited in their egg production, depending solely on the larval food from brood cells. In the absence of trophic eggs, polygyny allows for a higher daily production rate for the colony. Brief periods of massive flowering of favourite food plants, in alternation with periods of food scarcity, could be the ecological condition associated with polygyny. Unfortunately, there are no field observations on the natural ecological conditions of *M. bicolor* that could support this reasoning. Therefore, at the moment this suggestion remains speculative, and exciting new information can be expected from future studies.

ACKNOWLEDGEMENTS

This study was made possible by a thematic research grant of the scientific funding body of the State of São Paulo, FAPESP. Annemiek Roeling assisted in the collection of data in 1998, and in the analysis for that year. Olga Cepeda took part in data collection in 2000, while Dick Koedam was very much involved in the observations made in the years 1995 and 2000. Dr. Joop Faber advised us in the statistical analysis of the frequencies of egg laying by the queens in the polygynous colonies. Berend-Jan Velthuis furthered the preparation of the manuscript. We express our gratitude for this support.

Résumé – La polygynie de *Melipona bicolor* : compétition acharnée parmi les reines. L'abeille sans aiguillon *Melipona bicolor* a la caractéristique unique parmi les abeilles d'être polygyne facultative. Il n'a pas été montré que les colonies polygynes étaient plus productives que les monogynes. Dans une colonie polygyne les reines partagent donc les cellules de couvain produites par les ouvrières plutôt qu'elles ne déterminent le nombre de nouvelles cellules. Individuellement les reines alternent périodes d'activité et de non activité, peut-être en liaison avec les cycles de maturation des œufs et les cycles de ponte. Dans les périodes d'activité, elles apparaissent sur les rayons où les cellules sont en construction et elles participent au processus d'approvisionnement et de ponte (POP) des cellules. Une reine peut être seule ou avec une ou deux autres reines. Le tableau I donne les fréquences auxquelles chaque reine était présente dans les cinq colonies étudiées. Les colonies différaient entre elles par la proportion de POPs à plus d'une reine. Sauf pour une colonie, la présence des reines, qu'elles soient seules ou à plusieurs, pouvait s'expliquer en estimant un processus aléatoire d'apparition des cellules, étant données les différences de niveau d'activité des reines.

Quand elles étaient ensemble, chaque reine avait en moyenne la même chance de pondre un œuf, ce qui montre l'absence de dominance. Mais elles participaient pour diverses raisons. Une reine peut s'approcher d'une cellule parce qu'elle est prête à pondre un œuf. Elle peut prendre de la nourriture larvaire ad libitum lorsqu'elle est sur la cellule. Elle peut aussi être intéressée par l'obtention d'un œuf trophique. Pour l'obtenir la reine doit donner une chance aux ouvrières de le pondre en reportant à plus tard sa propre ponte (cf. Fig. 2) et en se retirant de la cellule. La fréquence des œufs trophiques dans les POPS à plusieurs reines est donc moindre que dans les POPs à une seule reine (Fig. 1).

Les colonies différaient par le taux auquel les œufs trophiques étaient disponibles pour les reines. Le tableau II résume le nombre de POPs observés, leur nombre par jour, le rapport œufs trophiques/œufs pondus par les reines et la proportion de POPs à reines multiples. Aucune paire de ces variables n'est apparue corrélée. Pourtant, pour la reine la plus active, nous pouvons conclure que sa part dans la consommation d'œufs trophiques est égale ou plus forte que sa part dans la ponte. La reine la plus active d'une colonie était proportionnellement plus impliquée dans des POPs à reine unique que les reines moins actives et donc elle recevait relativement plus souvent un œuf trophique. Le niveau d'activité d'une reine a donc des conséquences nutritionnelles.

Le tableau II présente les données d'une colonie qui a redémarré une production de couvain après un arrêt de quelques semaines. La demande de nourriture aux ouvrières ou trophallaxie a eu lieu très fréquemment, alors qu'il s'agit d'un comportement habituellement très peu fréquent. Le taux auquel les reines recevaient cette nourriture a augmenté avec les POPs (Tab. IV). La trophallaxie peut donc avoir un autre but. En quémandant de la nourriture les reines stimulent les ouvrières à devenir plus actives. Quand le taux de production de cellules est revenu à des valeurs normales, la fréquence de la trophallaxie est retombée. Le taux d'œufs trophiques consommés par rapport aux œufs produits semble être important pour le taux de production d'œufs d'une reine. La figure 3 montre qu'au sein des colonies et entre elles ces deux variables sont corrélées, mais la variance est élevée. Outre les conséquences nutritionnelles du niveau d'activité des reines, il y a probablement aussi une différence qualitative durable entre les reines. Ceci est indiqué par les différences de durée du stade initial de POP et par le classement des reines par rapport à leur taux de ponte : il est resté inchangé entre les deux échantillons de la colonie de 1998 prélevés à six mois d'intervalle.

La répartition de la ponte parmi les reines est donc basée sur le hasard plutôt que sur la dominance. Bien que nous ayons donné un aperçu des mécanismes qui sous-tendent cette compétition acharnée pour la ponte, l'importance adaptative de la polygynie pour cette espèce reste grandement mystérieuse. La fonction de la polygynie, tant est qu'il y en ait une, ne peut être comprise que lorsque les conditions écologiques spécifiques à cette espèce seront connues.

***Melipona bicolor* / abeille sans aiguillon / polygynie / compétition / trophallaxie / œuf trophique / Apidae / Meliponini**

Zusammenfassung – Polygynie bei *Melipona bicolor*: Dichtekonkurrenz (scramble competition) zwischen Königinnen. Die Stachellose Biene *Melipona bicolor* ist fakultativ polygyn und damit ein einzigartiger Fall unter den Bienen. Polygyne Kolonien sind jedoch nicht produktiver als monogyne, was darauf zurückzuführen ist, dass die Königinnen in polygynen Kolonien sich die von den Arbeiterinnen gebauten Brutzellen teilen und nicht selbst die Anzahl neugebauter Zellen bestimmen. Bei einzelnen Königinnen alternieren Phasen der Aktivität und Inaktivität, und dies ist eventuell auf eine zyklische Eireifung und Eiablage zurückzuführen. Während der Aktivitätsperioden befinden sich die Königinnen auf den Brutwaben, auf denen Zellen gebaut werden, und sie nehmen am Prozess der Verproviantierung der Zellen und der Eiablage (Provisioning and Oviposition Process, POP) teil. Eine Königin kann hierbei allein oder zusammen mit anderen Königinnen angetroffen werden. Tabelle I zeigt die Häufigkeiten, mit denen jede der Königinnen in den fünf untersuchten Kolonien in POPs präsent war. Die Kolonien unterschieden sich in den Anteilen der POPs mit einer oder mehreren Königinnen. Mit Ausnahme einer Kolonie konnte die Anwesenheit der Königinnen im POP, ob alleine oder zu mehreren, als ein Zufallsprozess interpretiert werden, der abhängig ist vom jeweiligen Aktivitätsniveau der Königin.

Wenn mehrere Königinnen an einem POP teilnahmen, hatte jede die gleiche Chance, ein Ei abzulegen, was darauf hinweist, dass Dominanzinteraktionen keine Rolle spielten. Die Königinnen scheinen jedoch aus verschiedenen Motiven an POPs teilzunehmen. Eine Königin kann zu einer neugebauten Brutzelle kommen, weil sie ein ablagereifes Ei hat, sie kann aber auch daran interessiert sein, ad libitum von dem frischen Larvenfutter zu fressen, oder aber ein Nährei zu erhalten. Um an ein Nährei zu kommen, muss die Königin jedoch einer Arbeiterin eine Chance geben, ein solches zu legen. Dazu zögert sie in der Regel ihre eigene Eiablage hinaus und entfernt sich etwas von der Brutzelle (siehe Abb. 2). Sind aber zwei Königinnen an der Brutzelle, dann müssen beide bereit sein zu warten, wenn sie an einem Nährei interessiert sind. Aus diesem Grund ist die Häufigkeit der Ablage von Nähreiern in POPs mit mehreren Königinnen geringer als bei POPs mit nur einer Königin (Abb. 1).

Die Kolonien unterschieden sich in der Rate, in der Nähreier für die Fütterung der Königinnen abgelegt wurden. Die Zusammenstellung in Tabelle 2 zeigt die Zahl der beobachteten POPs und ihre Zahl pro Tag, sowie das Verhältnis von Nähreiern zu Eiern, die von den Königinnen gelegt wurden, und den Anteil an POPs mit mehreren Königinnen. Kein Variablenpaar scheint hierbei eine Korrelation aufzuweisen. Für die jeweils aktivste Königin können wir jedoch schliessen, dass ihr Anteil an gefressenen Nähreiern gleich oder höher ist als ihr Anteil an abgelegten Eiern. Die jeweils aktivste der Königinnen war auch häufiger in Einzel-POPs präsent als die weniger aktiven Königinnen, und das ist der Grund, dass sie an relativ mehr Nähreier kam. Die Aktivität der jeweiligen Königin hat also Auswirkungen auf ihren Ernährungsstatus.

Tabelle III gibt Auskunft über eine Kolonie, in der die Brutaufzucht nach einer mehrwöchigen Pause wieder in Gang kam. In diesem Fall fanden wir häufig Trophallaxissituationen, in denen Königinnen von den Arbeiterinnen Futter erbettelten. Unter normalen Bedingungen war dies nur selten zu beobachten. Diese Trophallaxisrate stieg unmittelbar vor Beginn des POPs an (Tab. IV). Trophallaxis scheint demnach eine zusätzliche Funktion zum blossen Nahrungsaustausch zu haben. Indem sie um Futter betteln, könnten die Königinnen nämlich die

Arbeiterinnen zu erhöhter Aktivität anregen. Sobald die Brutzellproduktion wieder ein normales Mass erreichte, ging die Trophallaxishäufigkeit zwischen Königinnen und Arbeiterinnen dann auch wieder auf ein niedriges Mass zurück.

Das Verhältnis von Nähreiern, die eine Königin konsumiert, zu Eiern, die sie ablegt, scheint wichtig zu sein für die Rate der Eiproduktion einer Königin. Abbildung 3 zeigt, dass innerhalb und zwischen den Kolonien die Konversionsrate von Eiern und die Produktivität trotz hoher Variabilität korreliert sind. Neben den Auswirkungen des Ernährungsstatus auf den Aktivitätsgrad einer Königin scheint dieser auch zu langfristigen qualitativen Unterschieden zwischen den Königinnen zu führen. Dies drückt sich aus in den Unterschieden der Dauer der POP Anfangsstadien und in der Ähnlichkeit der Reihenfolge der Eilageraten der Königinnen in Kolonie 1998, für die der zweite Datensatz ein halbes Jahr nach dem ersten erhoben wurde.

Die Aufteilung der Eiablage zwischen Königinnen basiert demzufolge eher auf dem Zufall als auf Dominanz. Obwohl wir die Mechanismen beleuchten konnten, die dieser Dichtekonkurrenz (scramble competition) in der Eiablage zugrundeliegen, ist die adaptative Bedeutung der Polygynie in dieser Art noch eine völlig offene Frage. Die Funktion der Polygynie kann, insofern es eine gibt, nur verstanden werden, wenn die spezifischen ökologischen Bedingungen dieser Art untersucht sind.

Polygynie / Stachellose Biene / *Melipona bicolor* / scramble competition / Trophallaxis / Nähreier / Apidae / Meliponini

REFERENCES

Alonso W.J., Lucena T., Velthuis H.H.W., Imperatriz-Fonseca V.L. (1998) Do *Melipona bicolor* (Apidae, Meliponinae) workers distinguish relatedness among different physogastric queens? Apidologie 29, 503–512.

Bego L.R. (1983) On some aspects of bionomics in *Melipona bicolor bicolor* Lepeletier (Hymenoptera, Apidae, Meliponinae), Rev. Bras. Entomol. 27, 211–224.

Herbers J.M. (1993) Ecological determinants of queen number in ants, in: Keller L. (Ed.), Queen Number and Sociality in Insects, Oxford University Press, Oxford, pp. 262–293.

Hölldobler B., Wilson E.O. (1990) The Ants, Springer Verlag, Berlin, Heidelberg.

Keller L. (Ed.) (1993) Queen Number and Sociality in Insects, Oxford University Press, Oxford.

Kerr W.E. (1949) Algumas comparações entre a abelha européia (*Apis mellifica* L.) e as abelhas nativas brasileiras (Meliponini), O Solo (Oct. 1949), 39–47.

Michener C.D. (1974) The Social Behavior of the Bees, Belknap Press, Harvard University Press, Cambridge.

Michener C.D. (2000) The Bees of the World, John Hopkins University Press, Baltimore.

Nogueira-Neto P. (1997) Vida e Criação de Abelhas Indígenas sem Ferrão, Nogueirapis, São Paulo.

Rosengren R., Sundström L., Fortelius W. (1993) Monogyny and polygyny in Formica ants: the result of alternative dispersal tactics, in: Keller L. (Ed.), Queen Number and Sociality in Insects, Oxford University Press, Oxford, pp. 308–333.

Sakagami S.F. (1966) Techniques for the observation of behavior and social organization of stingless bees by using a special hive, Papeis Avulsos Zool., São Paulo 19, 151–162.

Sakagami S.F. (1982) Stingless bees, in: Hermann H.R. (Ed.), Social Insects, Academic Press, New York, Vol. 3, pp. 361–423.

Silva D.L.N. da (1972) Considerações em torno de um caso de substituição de rainha de *Plebeia* (*Plebeia*) *droryana* (Friese, 1900) (Hymenoptera, Apidae), Homenagem a W.E. Kerr, Rio Claro, SP, Brasil, pp. 267–273.

Silva D.L.N. da, Zucchi R., Kerr W.E. (1972) Biological and behavioural aspects of the reproduction in some species of *Melipona* (Hymenoptera, Apidae, Meliponinae), Anim. Behav. 20, 123–132.

Silveira F.A., Melo G.A.R., Almeida E.A.B. (2002) Abelhas Brasileiras, Sistemática e Identificação, Belo Horizonte.

Sommeijer M.J., de Bruijn L.L.M., van de Guchte C. (1985) The social food flow within the colony of the stingless bee, *Melipona favosa* (F), Behaviour 92, 39–58.

Velthuis H.H.W., Roeling A., Imperatriz-Fonseca V.L. (2001) Repartition of reproduction among queens in the polygynous stingless bee *Melipona bicolor*, Proc. Exp. Appl. Entomol., NEV Amsterdam 12, 45–49.

Witter S., Wittmann D. (1997) Poliginia temporária em *Plebeia wittmanni* Moure and Camargo, 1989 (Hymenoptera, Apidae, Meliponinae), Biociências Porto Alegre 5, 61–69.

Zucchi R. (1993) Ritualized dominance, evolution of queen-worker interactions and related aspects in stingless bees (Hymenoptera, Apidae), in: Inoue T., Yamane S. (Eds.), Evolution of Insect Societies, Hakuhinsha, Tokyo, pp. 209–249.

Zucchi R., da Silva-Matos E.V., Nogueira-Ferreira F.H., Azevedo G.G. (1999) On the cell provisioning and oviposition process (POP) of the stingless bees – Nomenclature reappraisal and evolutionary considerations (Hymenoptera, Apidae, Meliponinae), Sociobiology 34, 65–86.