

Oviposition Behavior of the Stingless Bees
XVIII. *Plebeia (Plebeia) emerina* and *P. (P.) remota*,
with a Preliminary Ethological Comparison of Some
***Plebeia* Taxa (Apidae, Meliponinae)**

Patricia M. DRUMOND*, Ronaldo ZUCCHI*, Sôichi YAMANE**
and Shôichi F. SAKAGAMI***

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Abstract

Plebeia (Plebeia) emerina and *P. (P.) remota* share with a consubgener *P. (P.) droryana*: 1- Brood cells typically combed. 2- Ovipositions always batched. 3- Brood cells synchronously constructed and provisioned. 4- Caste interactions distinctly agonistic and partly ritualized. 5- Worker eggs mostly laid on the comb before the climax of POP sequence. However, *P. remota* does not cover the combs with complete involucrum as *P. emerina* and *P. droryana* do, and their “hypnotic turning” practiced by workers of *P. emerina* and *P. remota* against the queen is slightly atypical than in *P. droryana*. In *P. remota* the excited crowding of workers on the comb during POP soon recovers when interrupted artificially.

Introduction

Following the description of the food provisioning and oviposition process (POP) in *Plebeia (Plebeia) droryana* (Drumond *et al.* in press), this 18th POP report deals with two consubgener, *P. (P.) emerina* (Friese) and *P. (P.) remota* (Holmberg). These three species are ethologically similar, sharing some important features. Therefore, the descriptions are given mainly comparatively, without repeating details on similar aspects. This is particularly so on *P. remota*. Because recently van Benthem *et al.* (1995) published a nice paper on the ethology of this species, so that only some notes supplementary to their article are described. An ethological synopsis of three species is given in Discussion, but “ethological features common to other taxa” and “ethological diagnosis”, which form the core of our serial reports, will be given after the POP of other observed consubgener would be described.

Quantitative data are summed up at the end of Results, but some data are briefly cited in earlier parts when it would facilitate the synoptic comparison.

*Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, 14040-901, Ribeirão Preto, SP, Brasil.

**Biological Laboratory, Faculty of Education, Ibaraki University, Mito 310, Japan.

***2-2-610, 1-6, Ainosato, Kita-ku, Sapporo 002, Japan.

Material and Methods

Plebeia emerina (Friese) is distributed through the south Brazilian coast from Rio Grande do Sul to Rio de Janeiro. The observed colony (= EM), whose origin was unknown, had been reared at the apiary of Ribeirão Preto, and was, then, transferred in an observation hive (Sakagami 1966) and observed in November by S.F.S. and R.Z., less intensively than *P. remota* (Holmberg), of which distribution is similar to that of *P. emerina*. One colony (R1) collected in Colombo, State of Paraná, was transferred in an observation hive at the same apiary, and observed from December to January 1971-'72 both by S.F.S. and R.Z. The behaviors in two colonies were compared with those of two colonies (=DD) of *P. droryana* (Friese) simultaneously reared at the same apiary. Later P.M.D. reanalyzed their unpublished protocols on these colonies and also observed another *P. remota* colony (R2) during March to May 1994.

As in our previous studies, the results were gradually improved by repeated observations of not individually marked bees, but with some video recording sessions by P.M.D. A part of discussion was jointly made by S.Y. The other taxa cited for comparison and other aims are informally mentioned by generic or subgeneric names. Those not found in references are citations from our unpublished data. Behavioral terminology mainly follows Sakagami and Zucchi (1974) and Sakagami (1982). Temporal organization of POP was explained in some previous papers, e.g., Wittmann *et al.* (1991) and Sakagami *et al.* (1993). As for *P. remota* the concordance or differences of our results with those of van Benthem *et al.* (1995) are shown with the abbreviations '≅ VB' or '≠ VB'.

Results

Nest Architecture and Related Aspects

1 - *Nest entrance diameter*: The entrance is wide, allowing the passage of more than one worker at one time in *P. emerina* as in *P. droryana*, while narrow, only one worker can pass through simultaneously in *P. remota* (≅ VB) as in *P. poecilochroa* and *P. nigriceps*.

2 - *Cell arrangement*: Both *P. emerina* and *P. remota* prepare "complete combs", which are adopted by many stingless bees, i.e., cells forming a comb neatly lie on the same plane, not as in "incomplete combs", e.g., *Friesella* (Sakagami *et al.* 1973) or in "clustered cell masses", e.g., *Leurotrigona* (Sakagami and Zucchi 1974) or *Tetragonula* (Sakagami and Inoue 1990, except *T. carbonaria*, Michener 1961, Yamane *et al.* 1995). The combs of *P. emerina* nests, including EM, was typically concentric (Fig. 1), as in two nests of *P. droryana* observed simultaneously (Fig. 2). As for the combs of *P. remota* van Benthem *et al.* (1995) wrote as concentric but occasionally found also a spiral tendency. Such plasticity is also suggested in R1 and R2, which showed both atypical spiralling (Fig. 3) and more concentric tendency (Fig. 4) in due course. Similar plasticity is found in some other genera.

3 - *Involucrum*: The two species clearly differ in the preparation of involucrum which separates brood combs and outer areas including storage pots. *P. emerina* prepares thick multi-layered involucrum as does *P. droryana* (Figs. 1, 2). In *P. remota* the combs are connected

with several pillars to the glass lid of the hive, whereas only partial and uni-layered involucrum

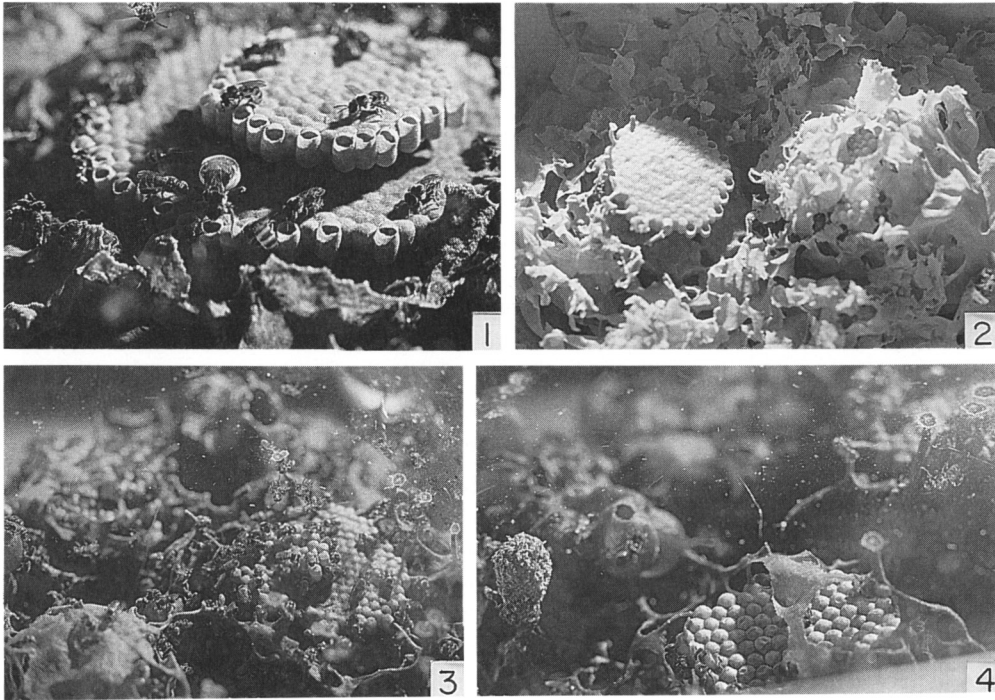


Fig. 1. Concentric combs of *Plebeia emerina*. At the center the queen is “waiting” in front of a complete (= collared) cell. All cells are already collared (= synchronously built). Involucrum was removed some days ago.

Fig. 2. Concentric combs of *P. droryana*. Involucrum covering left-hand younger combs is completely removed, but that covering the right-hand older combs only partly. The left-hand top comb has growing cells of the same stage, reflecting synchronous cell construction.

Fig. 3. Atypically spiral combs of *P. remota* with some pillars attaining to the glass lid of the hive. The older combs (left-bottom) are loosely covered with only one layer of involucrum.

Fig. 4. An old comb of *P. remota* with relatively regular arrangement and only partly covered with involucrum. Numerous pillars attached to the glass lid (right-above), and an oval heap of debris prepared on a pillar (left-below).

was occasionally prepared (Figs. 3,4, \approx VB) on the older combs like as in *Friesella* (Sakagami *et al.* 1973) and *Scaura latitarsis*. This difference between *P. emerina* (and also *droryana*) and *remota* evoked different troubles at POP observations. In *P. emerina* (and *droryana*) we had to remove involucrum occasionally (Figs. 1,2) as these layers disturbed closer observations, but this removal resulted in temporal interruption of POP. In *P. remota* many pillars attached to the glass lid made closer observations, and particularly photographing, difficult (see Figs. 3, 4), because the glass became dirty, smeared by bees at pillar preparation.

4 - Further, two peculiarities of *P. remota* R1 were observed, although whether these frequently occur or not is unknown. 4-1: Resin was deposited scatteredly in several places, each forming a small spot, not gathered at one or two definite heaps as in *P. droryana* colonies. 4-2:

There were four heaps of debris. One of these was oval and made on a pillar, high above the hive floor (Fig. 4, left-below).

Worker behaviors out of POP did not much differ from those in *P. droryana* except some aspects of queen-worker interactions given below.

Queen Behaviors and Queen-worker Interactions

Both queen behaviors and queen-worker interactions in *P. droryana*, *emerina* and *remota* are similar. Some of interspecific differences observed and described below might not be stably species-specific, possibly varying due to colony conditions. The observations by S.F.S. were made mainly in the later phase of the cell construction period and the POP period, while those by P.M.D. involved the whole POP period, that is, from the start of cell construction until the end of last cell operculation.

The following queen behavior traits, which were confirmed in all so far studied stingless bees, were observed also in the two species: 1- Avoidance by workers of the approaching queen more exaggeratedly than in honeybees. 2- Retinue gradually formed around the resting queen. 3- Rapid dashing followed by retreat repeated by each attendant facing the queen also much more exaggerated than in honeybees, involving occasional darting. 4- Absence of licking the queen by workers as honeybee workers do. 5- Virtual absence of feeding the mature queen by workers, being very frequent in honeybees, despite frequent trophallaxis among workers. (This aspect will be commented in Concluding remarks). Moreover, both *P. emerina* and *remota* share distinct queen-worker agonism with *droryana*, but with some differences:

1 - *The queen's stay on the comb*: The queens of both species (in *remota*, closely observed by S.F.S. in R1 (but overlooked in R2), visited the advancing front of the comb (Michener 1961) more frequently than those of *P. droryana* and often stayed there for a while, projecting the head slightly from the comb margin, occasionally gently lowering. This sitting-on-comb-margin lasted from 5 s to several min followed by walking.

2 - *Walking rhythm of the queen*: At walking the queens frequently changed the direction and speed, but this abruptness seemed less than in the queens of simultaneously observed *P. droryana*, possibly in part due to their larger metasoma. The time spent for 10 wing strokes, only measured in R1, was at walking 7-30 s (19.1 ± 6.3 , $n = 19$), longer than in *droryana* 4-20 s (9.4 ± 3.9 , $n = 197$) (Drumond *et al.* 1996), longer at resting 18-34 s (27.6 ± 5.1 , $n = 17$) and more at sitting-on-comb-margin (30, 32, 38, 40 s, $n = 4$). At any areas, either walking or staying, the queen moved her wings, more beating than vibrating.

In R2 P.M.D. traced the queen behavior in 19 occasions, each consisting of 1 h continuous observation. The percent ratios of various behaviors were as follows: Resting on combs, 48 % (In this case, the combs mean the whole brood area including old and new combs); on other areas (pots, etc.), ± 1 %; walking on combs, 49 %; on other areas, 1 %; out of sight, 1 %, i.e., she spent most of her time on brood area. However, in another set of 19 observations with similar durations the queen stayed 64 % of her time out of new comb though frequently seen on the brood area.

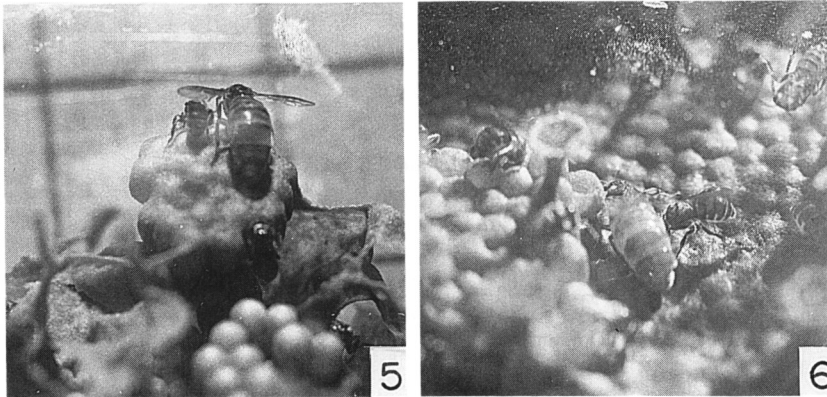


Fig. 5. Final phase of hypnotic turning in *Plebeia droryana* (left: worker, right: queen).

Fig. 6. Hypnotic barricading in *P. remota*.

3 - *Ritualized worker responses to the queen*: The aggressive treatment of workers by the queen was present in *P. emerina* and *P. remota* but seemed to be less intense and rarer than in *P. droryana*, of which incipient form is also seen in *Nannotrigona* (Sakagami *et al.* 1993, Fig. 2 C). In *P. droryana*, a worker cornered by the queen lowers her head and raises her metasoma by stretching mid and hind legs. Sometimes the behavior stops at this incipient crouching as frequently seen in *Nannotrigona* (Sakagami *et al.* 1993), but often goes further. Then, as if hypnotized, she turns around very gently until she is perpendicular or often parallel to the queen's head. Nextly she draws the legs which are turned to the queen and stretches those on the opposite side. As a result, the worker's body is inclined towards the queen (= hypnotic turning, Drumond *et al.* in press). In *P. emerina* and *P. remota*, this behavior (Figs. 5, 7 B) often appears typically, but the other two responses are also found: 1- Hypnotic head insertion. At face to face situation (Fig. 7 A), the worker straightforward approaches the queen, inserts her head below the queen's head and briefly keeps this posture there (Fig. 7 C, a superficially similar situation appears in *Melipona seminigra*, but here the queen, instead of the workers, is the actor riding on the latter. Beig and Sakagami 1964, Fig. 1 A). 2- Hypnotic barricading (Drumond *et al.* in press). Often a worker near a side of the queen darts her and stays there (Fig. 6) or at her front (Fig. 7 D) slightly lowering the head, as if preventing the queen's advance. These two acts also appear in *P. droryana* but seem rarer. All three acts (= hypnotic akineses) share the short but distinct, 'as if hypnotized' akinesis at the finale, although hypnotic turning is most conspicuous due to the precedence by a slow ritualized motion. Hypnotic akineses sometimes appeared frequently, e.g., 8 times on a comb in a POP during 18 min, soon before the first food discharge in a cell in *P. remota* (R1).

4 - *Relative frequency of various queen-worker interactions*: Various queen-worker interactions were casually counted and the results are summarized in Table 1. Observed cohorts are mentioned at the top of the table, and various interactions at the bottom. Interactions A show that: (1) Simple cases without h, d, b were most frequent (\cong VB). (2) Encounters ended by worker

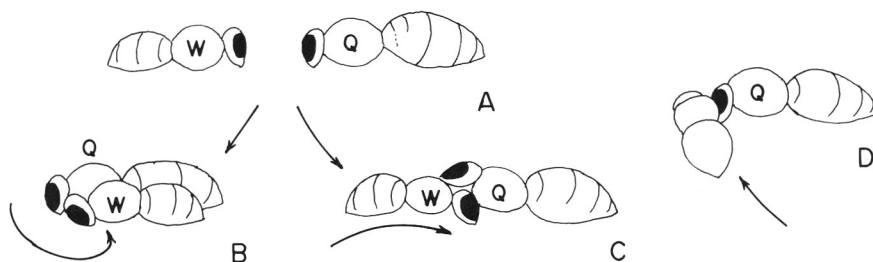


Fig. 7. Three types of hypnotic akineses : A. after the encounter between worker (W) and queen (Q), B. hypnotic turning, C. hypnotic head insertion, and D. hypnotic barricading.

departures were much more than queen departures, suggesting a hidden queen dominance. Interactions B show high frequencies of the cases including hypnotic akineses. The ratio is particularly high in rD. It is likely but can not be proved that this was caused by the high frequency of hypnotic turning, because three hypnotic akineses were not separately counted. It is possible but uncertain that the compared *P. droryana* colony was in a particular colony condition, which caused burst of hypnotic turning. Further comparative observations, which are not much difficult once behavioral differences would be recognized and defined, are requested to clarify this subtle but very interesting problem. Later P.M.D. observed in 25 occasions the first worker responses at encounter with the queen as follows: Simple escape 85 %, antennation 10 %, incomplete hypnotic turning (including hypnotic head insertion and hypnotic barricading) 2 %, darting or buccal contact each 1 %, oviposition 0.5 %, full hypnotic turning 0.3 %, crouching 0.2 %. In R2, the duration of buccal contacts was 1-7 s (3.4 ± 1.4 , $n = 44$). The result shows, besides the prevalence of simple escapes, a high behavioral variability in workers at the encounter with the queen, which may increase much in the subsequent course until the separation of behaviors as suggested in Table 1.

5 - *Worker oviposition and queen oophagy: A tentative comparison* (Figs. 8-12): In a classification of the worker ovipositions in the stingless bees, Sakagami (1982) mentioned that worker eggs of *Plebeia* are laid out of the POP. Later van Benthem *et al.* (1995, *P. remota*) and Drumond *et al.* (in press, *P. droryana*) published detailed accounts. In both species, worker ovipositions appear mostly on the comb earlier than the proper parts of POP. Our results essentially agree with those in these two articles but differ in some minor aspects, possibly due to the differences either in specific traits or in colony conditions. Hoping further clarifications, the results are tentatively compared below.

First, both *P. droryana* and *P. remota* and probably also *P. emerina* produce two types of worker eggs, oval-yellowish and thin-pale ones (Terada 1980). From analogy with the more distinct and functionally clarified worker-egg dimorphism in *Scaptotrigona depilis* (= *postica* auct.)(Akahira *et al.* 1970, Beig 1972), these two types were assumed to be trophic and male-producing eggs, respectively. However, due to their differences more subtle than in *S. postica*, these two types were not always distinguished in *Plebeia* at each observation, though it was observed that the queen of *P. droryana* can eat both kinds of trophic eggs (Drumond,

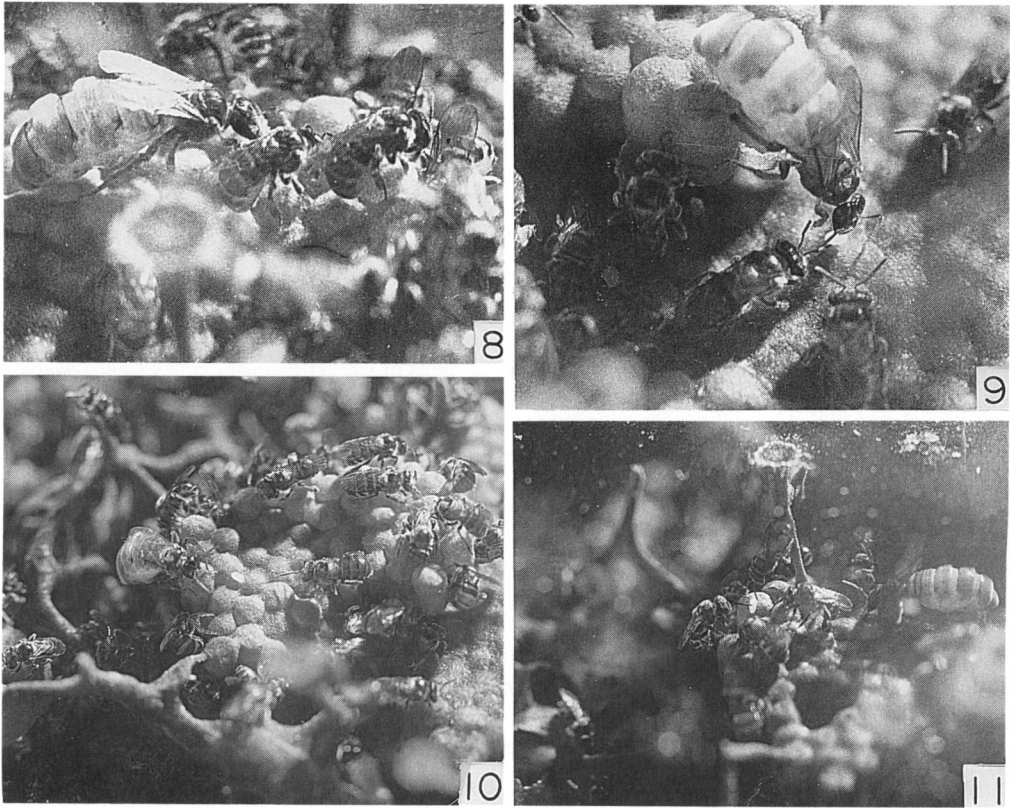


Fig. 8. A *Plebeia remota* queen tapping a worker who oviposited soon later (cited from Sakagami 1975, cf. also Sakagami 1982, erroneously shown as *P. droryana*).

Fig. 9. The same *remota* queen just eating an egg left by the escaping worker.

Fig. 10. A *Plebeia remota* queen (= that in Figs. 8, 9) rejecting a worker-born egg.

Fig. 11. The same queen approaching a worker egg, left on the comb and encircled by other workers.

unpubl.). This might be one of the most expected aspects to be solved in future, also in correlation with the acceptance or rejection of worker-eggs by the queen. Further, Drumond *et al.* (in press) observed two interesting facts at worker oviposition: 1- The appearance of a yellow droplet between the mandibles of the laying worker, which was soon reabsorbed during the oviposition. The queen never attempted to contact with this fluid. 2- The deposition of a metasomal fluid which was soon absorbed by a nearby worker. Such behaviors (1 and 2) were also observed in R2. A similar behavior is frequently seen in the queen of some stingless bees (*Scaptotrigona depilis*, *Trigona recursa*, etc.). It is still unknown whether these behaviors are homologous between two castes.

Except these cases, the comparison is made below on several features in the following results: DR (*P. droryana*: Drumond *et al.* in press), RVB (*P. remota*: van Benthem *et al.* 1995), R1, R2, EM (*P. remota*, R1, R2 and *P. emerina*, this study).

A. Oviposition timing:

Divided in 5 periods: 0 and I- before and during cell construction, II- all cells collared to start of provisioning, III- during provisioning, IV- after provisioning but before queen ovipositions (modified from Drumond *et al.* in press).

DR: $n = 36$ ovipositions (in %, 0=3, I=94, II=3); R1: $n = 26$ (I 20, II 6, observations made mostly in I and II); R2: $n = 181$ (0 not seen, I 65, II 31, III 2, IV 2); RVB: $n = 14$ (I+II 14); EM: $n = 11$ (I 11, obs. made only in I and II).

In all colonies, the concentration of ovipositions in the construction period (more in its later part) is obvious. Ovipositions during the food discharge (= provisioning) or postdischarge phases were rare, only 4 % in R2, nevertheless this trait mostly appears during these phases in other stingless bees which have this trait under the queenright state.

The number of worker ovipositions per POP was only one in RVB while often more in other cases, e.g., 1-4, exceptionally to 6 in R1. In RVB, however, the number increased in autumn and spring, i.e., at the seasonal end and start of the queen oviposition. The total observed worker ovipositions reached 138 against in the active season ($n = 14$ as listed above. Such seasonal difference should be studied also in other more tropical localities). Quite similar results were obtained in R2 also. Up to 12 eggs per cycle were counted during the months of March and April.

B. Oviposition site: I - on the upper surface or the outer margin of the comb, II - on the comb margin, III - on the margin of open and not yet provisioned cell (R1, EM), IV - on the margin of open and provisioned cell (R2).

DR: $n = 36$ (all I or II); R1: $n = 27$ (I=22, II=1, III=4); R2: $n = 180$ (I+II=98 %, IV=2 %), RVB: $n = 14$ (all I?), EM: $n = 11$ (I=7, III=4).

Evidently most worker eggs were deposited on the comb surface, but some ones on the upper margin of the open cell. In R2, types I and II occurred during periods I - III, while type IV during period IV. This aspect was not precisely studied in other cases.

C. Kinds of oophagy: Q: completely eaten by the queen, W: completely eaten by a worker or workers (Q was absent at the time), QW: eaten first by Q, then by a W or by WW, Q'W: rejected by Q, then eaten by W or WW, W'Q: rejected by W, then eaten by Q, Q'QW: first Q', then QW.

DR: $n = 98$ (Q 31%, QW 17%, Q'W 52%), R1: $n = 26$ (Q 12, W11, Q'W 1, W'Q 1, QQ'W 1), R2: $n = 179$ (Q 55%, W 43%, Q'W 2%), RVB: $n = 14$ (Q 6, Q'W 8), EM: $n = 11$ (Q 8, W3).

Descriptions are often incomplete but W or WW usually occurred after the egg was left, not eaten by the queen, and left for a few minutes (either rejected by Q or she was circumstantially absent). The rate of rejections by the queen is rather high in DR, R1, RVB than in other cases. In the latter, it is possible that earlier Q' was overlooked. A single case of Q'QW in R1 shows that

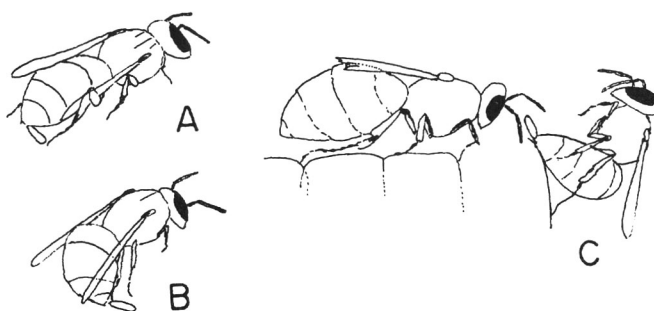


Fig.12. Three postures of worker oviposition in *Plebeia remota*. A: Relatively flat posture, B: Relatively raised posture (The posture in Fig.8 is intermediate between A and B), C: Worker oviposition at the upper margin of a collared but still not provisioned cell (The queen avoided to eat the egg).

the queen's attitude sometimes fluctuates during a short period.

D. Duration of worker ovipositions: DR: 1-7 s ($3.2 \pm 1.6, n = 50$), R1: 1-2 s ($1.6 \pm 0.5, n = 9$), R2: on comb surface, 3-14 s ($6.3 \pm 2.3, n = 92$), on cell margin, 31 s ($n = 1$), RVB: 2.5-4 s, EM: 1 s ($n = 2$).

Except a single case in R2, most other cases were seemingly also observed on the comb. For comparison, the durations of queen ovipositions are cited: DR: 1.2-5.8 s ($2.7 \pm 0.9, n = 100$, from Drumond *et al.* in press), R1: 2-6 s ($3.7 \pm 0.7, n = 75$), R2: 3-7 s ($4.7 \pm 0.8, n = 40$), RVB: 2.9-15.6 s, EM: 2.7 s ($2.6 \pm 0.7, n = 160$). The durations varied in both castes rather erratically.

E. Duration of queen oophagies: DR: 1.7 s ($3.0 \pm 1.8, n = 8$), R1: 1-2 s ($1.6 \pm 0.5, n = 9$), R2: 2-10 s ($4.0 \pm 1.8, n = 62$)(no data in RVB and EM). The duration of worker oophagies was measured only in R2 (3-27 s, $9.7 \pm 6.2, n = 41$). The duration is fairly variable.

F. Postures of worker oviposition: As for worker oviposition, Van Benthem *et al.* (1995) wrote as follows. "The large number of workers on the comb can create situations in which the normal flee-reaction of a worker for an approaching queen cannot take place. Then...the worker being cornered..., retreats a few steps, then lifts her head and thorax, curves her abdomen forward under the thorax and spread her wings while ovipositing on the comb surface. This was observed 14 times. The queen subsequently inspects the egg while the worker retreats another few steps and walk away". Our observations on 3 spp. coincide with the above description on the oviposition postures, particularly on wing spreading and metasomal curving, but otherwise are some differences, possibly suggesting some behavioral diversity than distinct specific differences. In DR, the queen often violently tapped the workers with antennae and fore legs, and solicited buccal contacts before the worker lays an egg. Such molestation was also seen in R1, though usually less violently. According to the behavior of the queen and the response of the worker, various postures were observed (Figs. 8, 12). In R2 the oviposition behavior was as in R1. Van Benthem *et al.* wrote, "The way trophic worker ovipositions take place in *P. remota*, offering as it were the egg to the queen while retreating on the comb, suggest an even more derived position than found in *P. droryana*, *P. emerina* and *P. saiqui*". This interpretation itself

Table 1. Relative frequencies of various queen-worker interactions in 3 cohorts

Cohorts	Rt	Rd	rD
Interaction	Interactions A		
DpW	53	7	6
xDpW	112	21	24
DpQ	3	-	2
xDpQ	23	13	8
	Interactions B		
h(hypnotic akineses)	59	12	31
d(darting)	22	2	-
b(buccal contact)	3	-	1
hd	7	2	-
hb	-	-	-
bd	20	4	3
hdb	3	2	-
with h	69	16	31
without h	45	6	4

Rt: Colony R1 of *Plebeia remota*, all observations combined.
Rd: = Rt, observations synchronously made with rD were extracted.

rD: Observations on a *P. droryana* colony made synchronously with Rd.

Interactions A: DpW-Departures of workers (usually hurriedly), not preceded by interacts other than short retreats. DpQ-Departures (usually slowly) of the queens not preceded by interactions other than workers' short retreats, xDpW, xDpQ-Ditto, but preceded by some other acts (see interactions B) before departures.

Interactions B: hd, bd- The occurrence of 2 or 3 acts before the departures (chronological orders were ignored, e.g., hd and dh were combined).

might be reasonable, but the fact that *P. remota* workers lay eggs at different situations and postures suggests that here the difference is not firmly species-specific but situation-conditioned.

In R1, some workers oviposited on the upper margins of the not yet provisioned cells, unlike done by many other stingless bee taxa with worker oviposition (Fig. 12 C). Concerning R2, however, it surely involved a provisioned cell, just done by many other stingless bee taxa with worker oviposition. One of us (S.F.S.) assumes this as representing a plesiomorphy before the appearance of the oviposition on the comb, although the laid eggs are not always eaten by the queen.

6 - Two observations on queen cells of *P. remota*: Except *Melipona* (cf. Kerr 1950, etc., Sakagami 1982) and *Trigona (Sundatrigona) moorei* (Sakagami *et al.* 1989), stingless bees prepare the queen cells at the periphery of well developed combs (or cell clusters). Such combs usually cease further expansion after receiving queen cells. Both provisioning and oviposition in the queen cells usually much delay than in normal cells. Here two somewhat anomalous observations in *P. remota* are cited.

R1 (8 March 1972): At 1000, together with dense involucre, most of 22 growing cells were experimentally removed. Possibly due to this interference, two queen cells, QC1 and 2, were constructed together with 6 normal cells. QC1 was first provisioned much earlier than the other cells and attended by a worker. The queen pushed away her, and took the laying posture but could not oviposit, possibly due to the insufficient food amount. Meanwhile the other cells including QC2 were provisioned. QC1 was additionally provisioned and both were oviposited not much later than the other cells, *i.e.*, without delay as in usual cases.

R2 (14 March 1994): A queen cell was constructed. When the queen approached, the body-inserting worker left the cell and defecated on the comb margin nearby. The queen soon left the cell. Some workers touched this yellowish feces and retreated. About 20 min later, the feces dried up and were scratched by some workers. Two other signs of such dried feces were found around the cell and also the 4th defecation occurred. Besides such event, however, sometimes the body-inserting worker left the cell without defecation. It should be kept in mind whether such facultative defecation can explain in part the well known delayed provisioning and oviposition in the queen cells or not. The duration of queen oviposition in the cell lasted 4 s, and of the phase \bar{d} ($=\bar{d}_d + \bar{d}_p$), subphases \bar{d}_d , \bar{a} , \bar{r}' , and \bar{d}_p ($=\bar{a} + \bar{r}'$) were 422, 118, 292, 12 and 304 s, respectively. Fifteen food discharges were counted.

Provisioning and Oviposition Process: General Accounts

P. emerina and *remota* share the following POP items with *P. droryana*:

1, 2 - Cells are always batched and cells of the same batch grow more or less synchronously (Type SyBe vs. ScBf, cf. Drumond *et al.* in press, Ethol. Diagnosis [=E. D] items D16, 17). Cells of the next batch are always started after cells of the prior batch were oviposited (cf. also Drumond *et al.* in press, Fig. 2)

3 - Food provisioning (P) and oviposition (O) proceed not successively for each cell, such as $P_1O_1, P_2O_2, \dots, P_nO_n$, but all cells are very rapidly provisioned, followed by ovipositions (synchro-

nous type D_y against successive type D_e in Drumond *et al.* in press, D18). Consequently, temporal orders of provisioning and ovipositions can be quite different among cells, e.g., such as P₁O₃, P₂O₁, ..., P_nO_n' (Fig. 15).

4 - Both provisionings and ovipositions are performed under a violent agitation, which governs the both castes, and are not confined to each cell but spreads over the whole comb, or even to pillars and the underside of the glass cover of the hive (Type generalized, A_g, against type localized, A_l, Drumond *et al.* in press, D28).

5 - Time spent for all ovipositions is longer than that for all food discharges: In R1 118-219 s (mean 158.0 ± 34.5, n = 10) vs. 25-184 s (114.1 ± 63.7, n = 11). In R2 113-711s (310.3 ± 207.4, n=6) vs 119-365 (200.2 ± 99.2, n=5). This difference is easily explained by two reasons. (1) Ovipositions start necessarily later than provisioning. (2) Total time becomes necessarily longer in ovipositions because these are performed successively by the single queen, whereas provisioning is achieved by many workers. Due to this temporal gap between two activities, the postdischarge subphase \bar{d}_p appears obligatorily in each provisioned cell until the arrival of the queen there (Fig. 15). During most part of \bar{d}_p , the provisioned cell is attended by a worker, though sometimes substituted by another.

6 - Most queen ovipositions are not preceded by her cell inspections as detailed later. This peculiarity was carelessly omitted in the ethological diagnosis of *P. droryana* (Drumond *et al.* in press).

7 - After the queen oviposition the cell is closed, either soon or rarely with a slight delay (x in Fig. 15) by a worker who inserts her metasomal tip into the cell and closes it with metasomal rotation as practiced by all so far observed taxa except *Friesella* (Sakagami *et al.* 1973), *Camargoia* (Zucchi, unpubl.) and *Plebeia* aff. *minima* (Drumond, unpubl.). Then the metasoma is withdrawn and operculation is continued with mandibles as usual.

All mentioned items except 6 are also shared by *Nannotrigona* (Sakagami *et al.* 1993), but this taxon differs from *Plebeia* in: (1) The queen spends most of her daily life on the combs, without leaving there even in the extra POP period. (2) The queen behavior during the generalized agitation on the combs is slower, and less abrupt and less agonistic than in *Plebeia*. (3) Worker eggs are always laid on the cell margin during POP agitation, never on the combs before POP. (4) Pre-oviposition cell inspection by the queen is always present.

Some Additional Qualitative Accounts

1 - In all 3 spp., insertion of fore-bodies in cells by workers are shallow, the anterior half of metasoma usually being visible. The appearance of deeper insertions suggests the approach of food provisioning. The time from the appearance of worker agitation to the start of provisioning was precisely measured only once in R1 (2.5 min). Worker agitation remarkably increases by the first food discharge in a cell (≅ *P. droryana*).

2 - The postdischarge workers quickly run away from the cell as in all other observed taxa. Unlike in the taxa with successive food provisioning in cells, the queen does not stay by cells at

provisioning. Therefore, the worker's postdischarge withdrawal does not direct against the queen but takes any directions from the cell in all 3 species.

3 - At temporal ebbs of worker agitation on the comb, an instant royal court appears around the queen, but she soon leaves the place and attendants do not follow her. Provisioned food is sometimes ingested by the queen in both species. The duration of intake was measured twice in R1 (5 and 7 s, respectively). In R2 it was 1-5 s (2.7 ± 1.3 , $n = 15$).

4 - Worker behaviors during the food discharge phase ($\bar{d} = \bar{d}_d + \bar{d}_p$), are formulated as $nIc + mA + n'Ic' + Att$, where Ic = worker body insertions, A = food discharges, Ic' = shallow worker body insertions, Att = worker cell attendance ($\hat{=}$ cell guard in VB), n , m , n' = number of performances ($n = 0-5$, 1.7 ± 1.7 , $n = 7$ in R1; $m = 6-10$, 7.4 ± 1.6 , $n = 9$ in R1, 8-17, 12.3 ± 1.8 , $n = 40$ in R2). During mA , Ic is often inserted 1-2 times but once seen more in R1: A_1-A_2 , $6Ic$, A_3-A_6 , $4Ic'$. Ic' is shallower than Ic because the cell had already been provisioned, and often accompanied with body rotation, which is rare before mA .

5 - Operculation usually starts soon after queen oviposition, that is, preoperculation subphase (\bar{s}_p) is very short even if occurs (1-63 s, 4.9 ± 8.7 , $n = 55$ in R2, while 0-3 s, 0.9 ± 1.7 , $n = 7$ in R1). In Fig. 15 the presence of relatively long (\pm over 2 s) \bar{s}_p is shown with x. The rotation subphase (\bar{s}_r) is the most stable part in operculation, lasting 33-56 s (45.3 ± 8.2 , $n = 8$) in R1 and 36-370 s (77.7 ± 51.2 , $n = 50$) in R2. In both R1 and R2, after withdrawal of metasoma, the operculator continues the work for a while with mandibles, but in both R1 and R2 her work was often very unsteady, either returning to a momentary rotation, or easily abandoning the work, either spontaneously or interfered by the queen. The worker, either the previous rotator or another one, recommences the work but again soon abandons it. Consequently there were found many unoccupied cells with small orifices, which was excentric or sometimes represented by two small holes. The work is more steady in *P. droryana*. Cells are rarely abandoned spontaneously. Even disturbed by the queen, soon one worker (either the previous one or another) recommenced the work so that cells are rarely unoccupied with small holes as in *P. remota* (R1). The period during which cells remained semiopen after the rotation subphase was measured in some cases (in sec): 75, 79, 100, 150, 240, 296, 337 ($\bar{x} + SD = 182.4 \pm 100.1$, $n = 7$ in R1). Although n is small, it is certain that such prolonged open state was never observed in *P. droryana* or in *P. remota* (R2). Unlike in many other meliponine species, cowork of 2 or 3 workers with mandibles on the same cell was rare in both species. No precise observation was made in *P. emerina* but the rotation subphase was clearly short as in *P. droryana*, and the presence of many semiopen cells as in R1 and R2 was not observed.

Batch Size and Spatiotemporal Sequence of Queen Ovipositions

Batch size was 6-26 cells (17.3 ± 5.8 , $n = 33$) in R1, 7-30 (17.8 ± 6.1 , $n = 56$) in R2, 5-40 (21.3 ± 6.5 , $n = 70$) in *P. droryana* (Drumond *et al.* in press), whereas 17-74 (47.1 ± 17.0 , $n = 10$) in *P. emerina*. Probably *P. emerina* can often realize fairly large batches.

Fig. 13 shows the spatiotemporal sequence of queen ovipositions in a batch with 21 oviposited cells in *P. remota*. The number of ovipositions in each minute was 6(A), 11(B), 2(C),

2(D). About one half of ovipositions were concentrated during the second minute. The running course of the queen is shown approximately, actually being more complicated by frequent changes of directions.

Fig. 14 presents the spatiotemporal sequence of queen ovipositions in a large batch (with 64 ovipositions) of *P. emerina*. Cells were built at the margins of 4 concentrically built combs (C) and the queen visited 4 combs alternately (A). The interval between two successive ovipositions sometimes prolonged (B: shown with arrows) but this did not necessarily caused by the queen's visit to a different comb, as shown by the absence of intimate correlation between the delay of oviposition and queen's visit to another comb (compare arrows with some horizontal bars connecting A and B). This shows that the queen perceived 4 combs as a unity without spatial differentiation. However, such capacity seems to have a limit. A cleptobiotic stingless bee genus, *Lestrimelitta*, is regarded as phylogenetically near to *Plebeia* (Michener 1990, Camargo and Pedro 1992). Its commonest species *L. limao* prepares a huge colony, with batch size of ± 200 , once attaining 250 (Sakagami, unpubl.). This species prepares a large brood area consisting of several both vertically and horizontally differentiated combs. The POP proceeds synchronously within each comb but successively between combs, i.e., the queen "perceives" the brood area as a

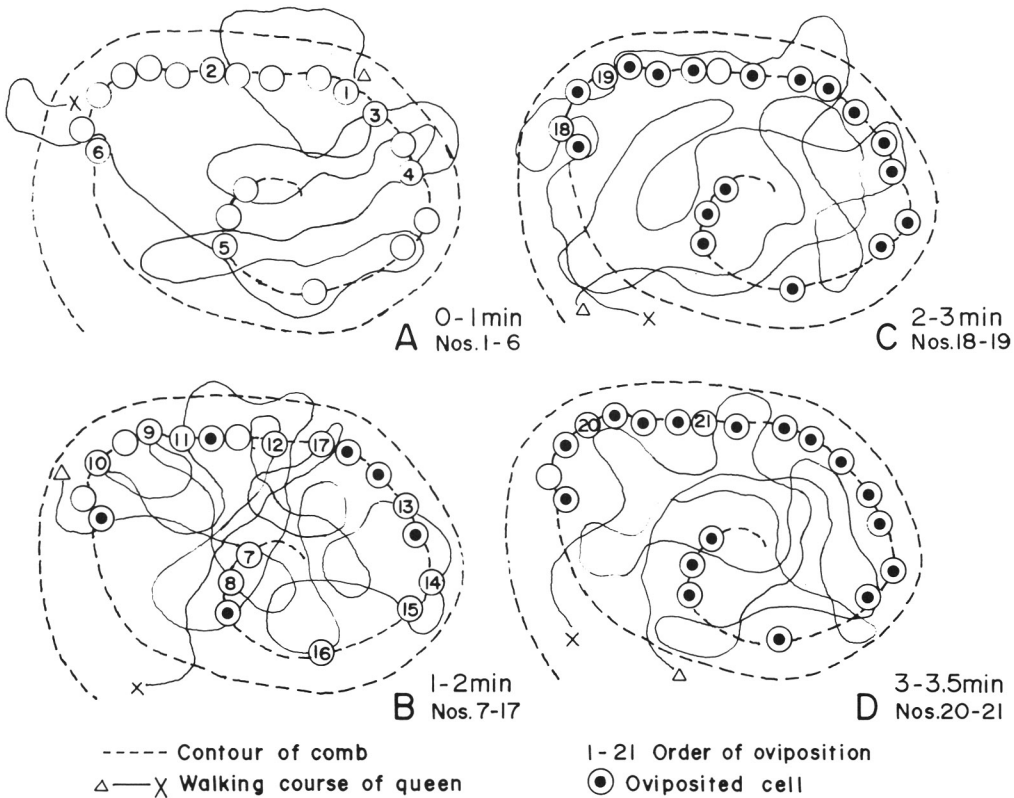


Fig.13. Spatiotemporal sequence of queen oviposition in a batch with 21 cells of *Plebeia remota*. The queen's continuous activity was shown in 4 successive maps separately (R1, February 24, 1972).

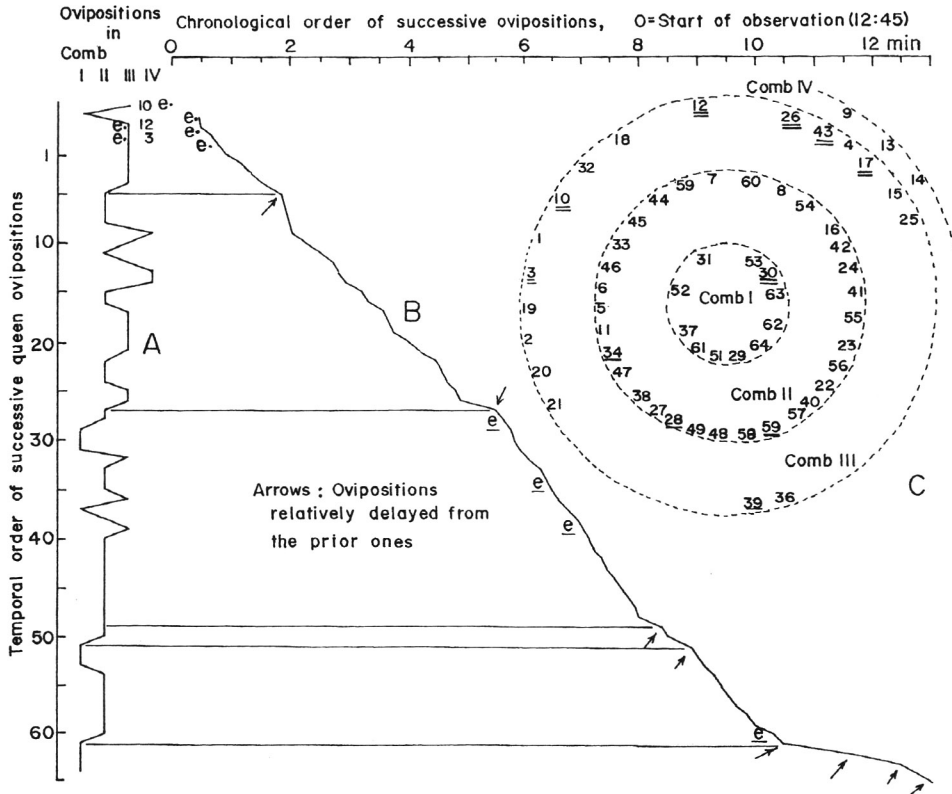


Fig.14. Spatiotemporal sequence of queen ovipositions in a large batch with 64 cells of *Plebeia emerina* (EM, 12:45, 8 January, 1972). A: Temporal order of ovipositions in 64 cells built on combs I-IV, showing the queen's wandering among 4 combs. e*=cell inspections not followed by oviposition, B: Chronological order of ovipositions. Arrows and horizontal bars=ovipositions relatively delayed from the prior ones, e= ovipositions preceded by cell inspections. Horizontal bars show the relation between queen's comb-to-comb wandering and the interval between the arrowed and prior ovipositions, C: Location of cells (1-64) on 4 combs (I-IV) and temporal order of ovipositions to them. $\underline{n} = \underline{e}$ in B and $\underline{n} = e^*$ in A and B.

spatially differentiated conjunct. However, the primordium of such differentiation already appears in *Plebeia*. When the brood area of *P. remota* showed spatial segregation more than that in Fig. 13, the worker agitation appeared at one area arrived at another with a delay.

Cell Attendants and Queen's Cell Inspections

Due to the afore-mentioned temporal gap (p.41) between provisioning and ovipositions in most cells, the queen does not arrive soon at the provisioned cells, i.e., the post-discharge subphase (\bar{d}_r) is obligatory in 3 *Plebeia* species as in *Scaptotrigona*, *Nannotrigona* and *Mourella*. During this subphase the cell is attended by a worker. Under normal reproductive conditions the attendance rarely continues over 2 min (but prolongs more before and after the inactive overwintering period, van Benthem *et al.* 1995). The cell attendant behaves approximately as in *Nannotrigona*. (In *Nannotrigona*, however, the postdischarge attendance is replaced by egg

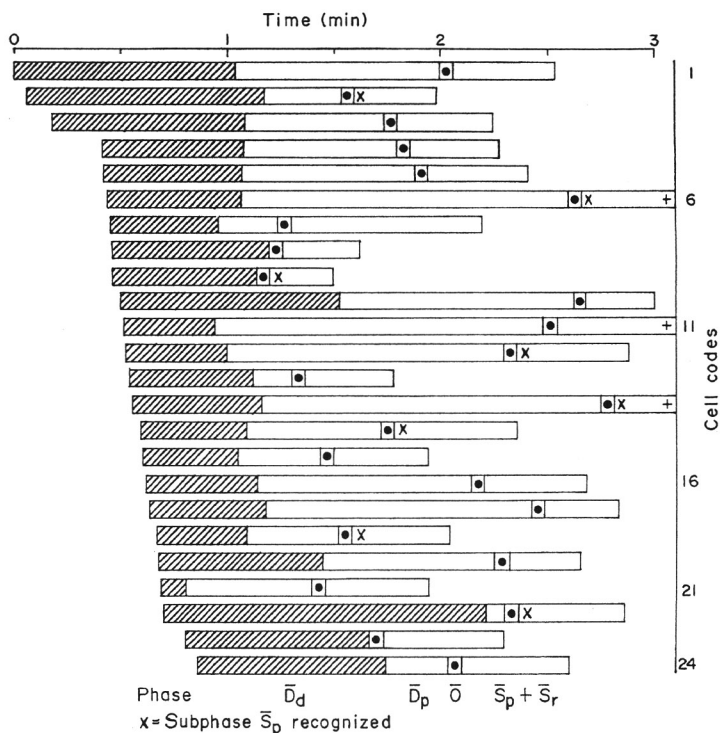


Fig.15. Temporal sequence of POP in a large batch with 24 oviposited cells of *Plebeia remota* (R2), showing the duration of \bar{D}_d (food discharge subphase), \bar{D}_p (postdischarge subphase), \bar{O} (oviposition) and s,s, (pre rotation and rotation subphases of cell operculum phase).

The duration of each oviposition was shown as 3 s in all cases (=approximate mean in 2 colonies, R1 and R2. cf. Comments on the duration of worker ovipositions). The other 3 subphases were shown with actually measured durations.

avoidance-attendance as soon as the provisioned cell receives one trophic egg or more.) She stands by the outside of the outer cell margin, occasionally inserts the antennae in the cell but does not touch the surface of the larval food. She keeps her immobility except for active shaking of antennae, and is indifferent to the other workers nearby. Sometimes another worker mechanically collides with her or more actively pushes her, but the attendant does not give way to the latter, though never counteracted aggressively.

On arrival of the queen, the attendant changes the attitude. She directs her head to the queen. Then she either soon gives way to the queen and flees away, somewhat behaving as in the postdischarge withdrawal from the cell seen in most taxa, or lowers the forebody as if covering the cell and resists to the queen's pushing, though usually soon pushed away. Only rarely she resisted strongly and the queen left away. Even in such a case, the attendant expressed no violent aggression to the queen as seen in *Scaptotrigona* (Sakagami and Zucchi 1963). There appeared to occur some subtle behavioral differences between *P. remota* (R1, R2) and two *P. droryana* colonies (no data were taken on *P. emerina*). In *P. droryana* the attendants appeared more resistant to the queen's pushing away. The queen of R1 simply used to push away the attendant, but the *P. droryana* queens often rode over the attendant to drive her away.

Table 2 shows the relationships among cell inspection by the queen (present = O or absent = O'), presence or absence of queen oviposition, and worker's resistance to the queen's pushing in 13 accurately traced POP in R1. As given in the left-hand column, total numbers of very weak, weak and strong resistance to the queen were 205, 48 and 3, respectively, i.e., most attendants gave way to the queen with little resistance. This result is somewhat different from that mentioned by van Benthem *et al.* (1995), who wrote that "when they guard the cells ready to be oviposited they actually resist the queen, defending their cells against her as if having proprietary rights". This assertion itself is correct, but the actual resistance seemed much weaker in our case. However, their results involve observations in the pre- and post-overwintering periods and in orphan conditions. Under such circumstances the resistance by the attendant might become stronger. Further precise comparison is expected.

Table 2 also shows that (1) most queen ovipositions were not preceded by her cell inspections (220 out of 256)(cf. also Fig. 14), (2) in 19 out of 34 cases with queen inspections she did not oviposit, and (3) in one half of the cases with queen inspections (either followed or not by ovipositions) some anomalies in cell conditions were observed, such as insufficient amount of larval food (*) or irregular cell margins (**).

Table 3 presents the temporal positions of queen cell inspections, either followed by ovipositions or not (= eO and eO') in 13 POP accurately traced on this aspect. It is soon recognized that eO' are concentrated in the earliest part of the POP sequence, except in batches 31 and 37, whereas eO appeared later in most batches, although still earlier than the mid-point of each POP, or exactly, 0.5 (batch size + no. of eO'). Only exception was no.17 (eO) in batch 37' where eO appeared in the last cell, but here the cell margin was somewhat irregular.

Consequently the drive for cell inspection by the queen, which is seen in all other studied taxa before each oviposition is still kept in the earlier part of worker agitation (cf. also Fig. 14, e*), but gradually drops in the course of food provisioning, though occasionally is still realized before some earlier ovipositions, or even later, when certain anomalous cell conditions were perceived by the queen (Table 3, batch 37', no.17). The opinion of van Benthem *et al.*, "the (cell-guard helping the queen to locate the cells still available for oviposition as a derived communicative function" is persuasive. Then the (gradual) omission of cell inspection by the queen may be an apomorphic trait, because both *Nannotrigona* and *Mourella* still keep this behavior under the worker agitation during the food discharge phase.

Table 2. Relationships among cell inspection by the queen, attendant's resistance and the presence (O) or absence (O') of queen oviposition in 13 accurately traced POP in R1

Attendant's resistance	Total	Queen inspection	
		+	-
Very weak	O 184	9+2*+4**	169
	O' 21	19+2*	
Resisting but pushed away	O 48		48
	O' 0	0	
Resisting and pushing away queen	O 3	0	3
Total	O 235	15	220
	O' 21	19	2
Grand total	256	34	222

*Cells seen insufficiently provisioned.

**Cells with margins slightly irregular.

Two Anomalous Sequences

In the course of POP studies in R1, two anomalous cases were observed, which seemed to be interesting to interpret the mechanism of POP sequence.

1 - *Ovipositions in a cell difficult to take the proper laying posture*: In batch 24 on 2 March 1972, 22 cells were normally oviposited and operculated, but one cell remained untreated. This cell was isolately built soon below the glass lid of the hive. Workers could work there freely. The cell was normally provisioned and attended by a worker, whereas the queen could not pass over the cell due to her swollen metasoma. During a 20 min qualitative observation, the queen repeatedly attempted to drive away the cell attendant, but her effort was vain as she could not pass through there. But the attendant eventually left the place and a few workers alternately inserted the head in the cell and absorbed the larval food a little when the queen was absent there. She repeated the following behavior chain: (1) Trying to take the laying posture, (2) Rotating around the cell wall, (3) Leaving the cell and running around the comb surface, nevertheless worker agitation had already dropped and most oviposited cells had been operculated. Once she laid an egg when rotating around the cell. The egg attached to the cell wall and eaten by a worker after repeated advances and retreats by other workers. Once a worker laid a large, probably trophic egg on the cell wall. It was eaten by another worker.

After then, observation continued for 35 min. During this period, the queen repeated cell inspections 10 times and cell inspections followed by futile oviposition posture 87 times(!), and ovipositions on cell wall 10 times. Actually eggs remained at her metasomal tip, then attached to the cell wall. These eggs were eaten by some workers after repeated avoidances. Once a worker started to close the cell with her mouth as often seen under orphan condition, not by rotating the metasomal tip as practiced in queenright colonies of all observed taxa except *Friesella* (Sakagami *et al.* 1973), *Camargoia* (Zucchi, unpubl.) and *P. aff. minima* (Drumond, unpubl.).

The appropriate interpretation of this sequence is still difficult, but the following facts should be pointed out: The queen's oviposition urge still continued after normal 22 ovipositions and during nearly 50 min after the cessation of worker agitation as shown by over 100 futile laying attempts. The observation also revealed her precise locality memory.

2 - *Delay of POP caused by destroyed cells*: Batch 31 on 10 March 1972: At 1120 there were 29 collared cells and one 4/4 high cell. The worker agitation was just starting. In opening

Table 3. Temporal order of incidences of cells inspected and oviposited (=eO) or not (eO') in the sequences of 13 accurately traced POP. Numbers mean the order of incidences of 3 cases, eO, eO' and e'O, though the last cases, which were most frequent, are not cited. By the presence of O', the total number of cases in each POP is slightly larger than batch size (= eO + e'O).

Batch code	Batch size	n (Temporal positions of the cases)
1	18	eO'(nos.1-6), eO (no.7)
2	26	eO'(nos.1-4), eO (nos.5-6)
8	25	eO'(nos.1-7, 9-10), eO absent
9	20	eO'(nos.1-4), eO (no.7)
13	32	eO'(nos.1-11), eO absent
15	23	eO'(nos.1-7), eO absent
22	20	eO'(nos.1-4), eO absent
29	27	eO'(nos.1-2, 8*, 9*), eO absent
31	16	eO (no.1), eO'(no.2)
37	16	eO (no.1), eO'(no.2)
37'	17	eO'(nos.1-3), eO (nos.14-17**)
38	16	eO', eO absent
39	21	eO'(nos.1-5), eO (no.7)

*Cells seen insufficiently provisioned.

**Cell margins seen slightly irregular.

the glass lid for another purpose, about 1/8 of brood comb was accidentally destroyed. Workers started to absorb the larval food flown out of the cells and their pre-POP agitation completely ceased. At 1130 most flown out fluid was absorbed but workers still continued to absorb the remainder. At 1200 most cells were repaired leaving heavily broken ones. At 1210 the queen became very excited, and started the cruising over the comb. At 1213 first food provisioning started and all cells were provisioned after 200 s (in normal cases ± 100 s, cf. p.41). At ± 1210 first oviposition took place but all cells were oviposited only after 6 min, much longer than in the 4 normal cases with comparable batch sizes (118, 121, 149, 211 s).

In this case the queen recovered her urge earlier, but worker agitation recovered later because many comb-workers participated in absorbing the flown larval food and repairing broken cells probably resulted in the delayed agitation, nevertheless they had super-enough larval food in their crops.

Blowing Away Experiments

Following the two casually observed anomalous cases of POP, the worker agitation at early POP was experimentally interfered. It was repeatedly mentioned that POP proceeds under a strong agitation of both queen and workers. The core of this agitation is the congestion of agitated workers on combs. To disturb this state, the glass top of the hive of colony R1 was opened and workers and the queen were blown away at various stages of POP. Then the glass top was quickly closed and how and when the agitation would recover was observed.

Fig. 16 shows the sequences in five experiments 1-5. Bees were blown away at the following timing: case 1: at the start of agitation and also at the earlier half of provisioning, case 2: at the start of food discharge, case 3: at the completion of provisioning in the first cell and all other cells more or less provisioned, case 4: after second and fifth ovipositions, case 5: after sixth oviposition. In the last case, bees were more strongly blown away and damage to the cells was not particularly avoided.

The result was very simple. In all cases except case 5, the suppressed agitation recovered within 10-30 s, and interrupted provisioning and ovipositions restarted soon. The queen was sometimes blown to the hive wall but soon returned to the comb, and the subsequent ovipositions proceeded normally. Even in case 5, the recovery delayed more (about 1 min) but the subsequent worker agitation and queen ovipositions continued normally.

Thus, it was confirmed that POP agitation of *Pl. remota* is very stable, resistant to the temporary change of outer conditions, and recovering soon when disturbed. Probably the other taxa with generalized agitation, *Nannotrigona*, *Scaptotrigona*, *Mourella* and other still not accurately documented groups may have more or less similar trait.

Quantitative Data

Each item is arranged in the order of variation range and, in the parentheses, arithmetic mean \pm sample standard deviation and sample size.

The following data were already given at appropriate places: Batch size (R1, R2, EM); time spent for 10 wing strokes in the queen (R1); duration of worker and queen ovipositions (R1, R2, EM); time spent for all ovipositions and food provisioning to all cells of a batch (R1, R2); no. of

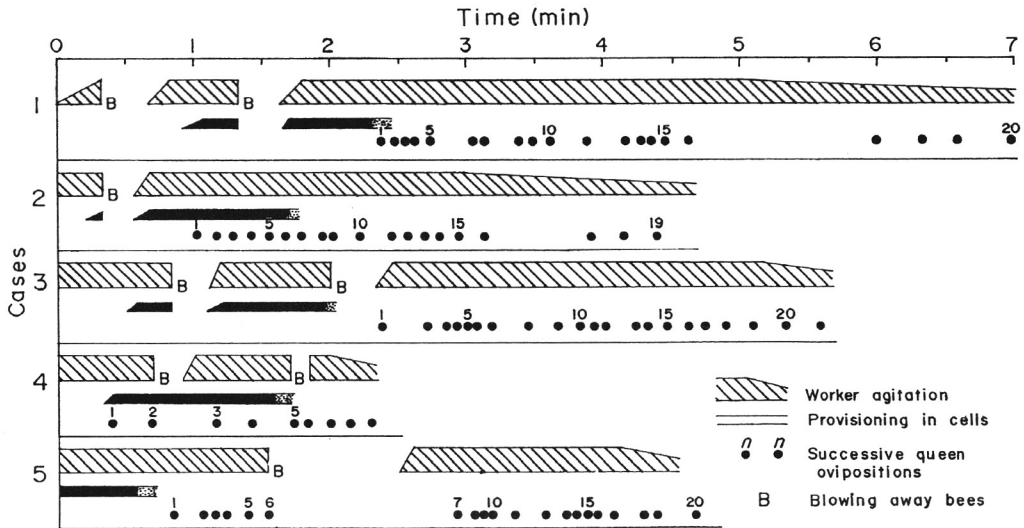


Fig.16. Results of blowing experiments in colony R1. Further explanations in the text.

food discharges per cell (R1, R2); duration of the subphase S_p and S_r in the operculation phase. Below the items are arranged in the order adopted in *P. droryana* (Drumond *et al.* in press). If the colony code was unmentioned, it means the lack of reliable data, for example, there is no more reliable data of EM.

Time for the construction of all cells of a batch: 3.5-5 h (4.0 ± 0.6 , $n = 8$) (R1); Interval between two successive POP (measured from the end of the last operculation of a previous POP to the start of the first queen oviposition in the next one (R1): 180-600 min (300.0 ± 153.0 , $n = 5$)(R1)[In R2, interval between two successive POP (measured from the start of the last queen oviposition of a previous POP to the first queen oviposition in the next one: 187-344 min (269.8 ± 35.8 , $n = 20$)]; Approximate daily oviposition rate (in R1, mean cell number oviposited between 2 POPs) : 48-240 cells (120.7 ± 55.6 , $n=9$), (in R2, mean batch size multiplied by 24 hours, and then divided by mean interval between 2 POPs) : 95 cells ; Duration of provisioning in each cell: 32-62 s (46.8 ± 12.4 , $n = 12$)(R1), 61-222 s (100.9 ± 37.2 , $n = 41$)(R2); Duration of provisioning (= food discharge) phase \bar{d} (= $\bar{d}_d + \bar{d}_p$, discharge and postdischarge subphases combined): 38-285 s (141.2 ± 8.3 , $n = 5$)(R1), 72-320 s (166.6 ± 67.7 , $n = 38$)(R2); Duration of postdischarge subphase ($\bar{d}_p = \bar{a} + \bar{r}'$): 6-240 s (94.4 ± 8.1 s, $n = 5$)(R1) 0-247s(69.1 ± 63.1 , $n=38$)(R2); Total POP duration from the appearance of first cell primordium to the end of the last operculation: 206, 232 min (219 ± 13 , $n = 2$)(R1), or from the start of \bar{d} to the end of \bar{S}_r : 8-30.1 min (13.1 ± 4.2 , $n = 45$)(R2).

Concluding Remarks

Ethological Features Common to 3 Species Cited

Besides many features common to other stingless bee taxa (e.g., given in Drumond *et al.* in press), *P. droryana*, *P. emerina* and *P. remota* share the following ethological features: 1- Cells

completely combed, 2- Involucrum more or less present, 3- Queen's walking very abrupt, with some aggressive treatment to workers encountered, 4- Appearance of hypnotic akineses in workers cornered by the queen, 5- Worker oviposition mainly on the comb surface earlier than proper part of POP, though occasionally ovipositing on cell margins as in many other taxa, 6- Cell construction always batched, 7- Cells of the same batch growing synchronously, 8- Food provisioning in cells synchronous, followed by subsequent and quite immediate queen ovipositions both made under high agitation, 9- Postdischarge (= post provisioning) subphase in each cell obligatory, because generally being attended by a worker until queen's arrival, 10- Queen's cell inspection before oviposition, being obligatory in all so far ethologically described taxa, mostly absent, 11- Cells operculated first by rotation, then with mandibles usually by single worker, not by 2-3 workers as in many other taxa.

Ethological Differences among 3 Species

Table 4 presents some ethological differences among *P. droryana*, *P. emerina* and *P. remota*, although behavioral observations are less complete in *P. emerina*. Clear and stable interspecific differences are scarce, requiring further critical observations, but this suggests that three species are ethologically very similar, not as in some other consubgenera described in subsequent papers.

Ethological comparison among 3 subgenera of the genus *Plebeia*

Leaving the preparation of ethological diagnosis of the three mentioned species and some others later, here the subgenus *Plebeia* (sensu Moure 1951, 1961) is compared with two other aberrant subgenera, *Friesella* and *Mourella*, which were ethologically well documented (Sakagami *et al.* 1973, Wittmann *et al.* 1991). An ethological comparison between *Friesella* and *Plebeia* s.str. was tentatively made in Sakagami *et al.* (1973). A revised comparison was given in Table 5, with indication of character polarities if possible. Both *Friesella* and *Mourella* are monobasic but the first subgenus may involve two 'ethospecies' (Camillo-Atique 1977, here only Type I was adopted for our comparison.). Further the following comments may help to understand the table.

1 - Character features of *Plebeia* s.str. were given only based upon the observations on three species, *P. droryana*, *P. remota* and *P. emerina*, although there are some other species which are ethologically slightly different as documented in subsequent papers.

2 - In ethological features cladistic polarities were tentatively mentioned relative to *Melipona*, which we regard ethologically most plesiomorphic (Zucchi *et al.* 1992, Sakagami *et al.* 1993, Yamane *et al.* 1995), despite possessing some indubitable apomorphies. Features considered more apomorphic than in *Melipona* were assigned as A₀, and those seemingly more apomorphic (mostly simplification by probable secondary disintegration) as A₁. Van Benthem *et al.* (1995) wrote "that the level of integration of the POP is a highly evolved trait in this species" (= *P. remota*). This is true but its POP is apomorphic only relative to *Melipona*, whereas a highly integrated POPs are plesiomorphic relative to many other taxa (e.g., *Friesella* and *Mourella*) with

secondary disintegrated POP (cf. Wittmann *et al.* 1991).

Table 5 clearly demonstrates astonishingly simplified (= degenerated = evolved) ethological syndromes of *Friesella* and *Mourella*, nevertheless they are morphologically not much different from *Plebeia* s.str. Michener (1990) synonymized these two subgenera sensu Moure (1951) with *Plebeia* s.str. This treatment might be valid on the morphological basis, but there is no reason to generalize that all ethological characters are necessarily inferior to morphological ones as distinctive criteria (cf. Sakagami and Yoshikawa 1968, on the recent papers see Zucchi 1994). Thus, *Friesella* and *Mourella* should be regarded as having the same status as *Plebeia* s.str., irrespective of they are considered genera, subgenera or the species groups.

Table 4. Ethological differences among *Plebeia droryana*, *P. emerina* and *P. remota*

Features	<i>droryana</i>	<i>emerina</i>	<i>remota</i>
Nest entrance	wide, more than 1 worker passable at one time		narrow, only 1 worker passable
Involucrum	dense		incomplete, occasionally absent
Pillars	scarce		many
Queen's stay on comb ^p	less frequent		more frequent
Hypnotic akinesthes ^p	hyp. turning more frequent	hypnotic head insertion and hyp. barricading relatively frequent	
Batch size ^p	small (21.3 ± 6.5)	large (47.1 ± 17.0)	small (17.3 ± 5.8, R1) (17.8 ± 6.1, R2)
Postrotation operculation	consistent	probably as in <i>droryana</i> but not precisely observed	not consistent

p: requiring further critical comparison.

Two Additional Comments

1 - *The rarity of queen feeding by workers in stingless bees*: Van Benthem *et al.* (1995) wrote as for *P. remota*: "The worker offers food to the queen. Queen and worker make contact with their mouthparts, and both show quick movements with the antennae and front legs". Such interactions were frequently observed in our colonies of *Plebeia*, but in no case resulted in the actual feeding. Throughout our continuous observations during many years with various stingless bee taxa, the real feeding queen by workers was extremely rare, nevertheless feeding by a worker to another was very common just as in worker-to-worker or worker-to-queen feeding in honeybees.

In the last three cases, the solicitant extends her glossa and inserts it into the mouth of the prospective donor. The latter opens mandibles and accepts solicitation. During the actual feeding both interactors become calm except mutual antennation. In honeybees, the duration of this actual worker-to-queen feeding ranged from a few seconds to more than 2.5 min ($\bar{x} = 44$ s) according to Allen (1960). Keeping this result in mind, one of us (S.F.S.) used to measure queen-worker interactions at the time from the cessation of active movements except antennation until the separation of both bees. It was very rare that this calm period continued more than 5 s in all species observed. This assures a remarkable difference between honeybees and stingless bees as for feeding the queen. Probably the queens of most stingless bee taxa should depend nutritionally on the intake of larval food and worker eggs (Sakagami *et al.* 1977). However, it is still enigmatic how the queens can obtain sufficient food to maintain the own survival and the high egg production, particularly in such taxa as *Frieseomelitta* (Da Cunha *et al.* 1986) and *Tetragonula* (Sakagami, unpubl.), where workers seldom develop their ovaries even in orphan colonies and food intake from the provisioned cells is also not very common.

2 - *Spectrum of ritualized queen-worker behaviors*: The highly complicated POP of the stingless bees are characterized by three peculiarities: Well temporally structured sequence, appearance of various types of worker ovipositions as a part of this sequence, and the presence of highly ritualized queen-worker interactions. Among these, the worker ovipositions were mostly well known by its simplicity, followed by the temporal structure of POP as a “general conversation” between the queen and workers. It was unfortunate that the third peculiarity, ritualized queen-worker interactions as a “particular or group-specific conversation” between the two castes, was most ignored until Zucchi (1993). In part this disregard must depend on an inevitable cause: Their ritualized queen-worker behaviors are very fascinating to any persons who once directly saw these with their own eyes, but are very difficult to describe appropriately with simple and short expressions. This complexity becomes highest in some taxa such as three *Plebeia* species treated in this paper. It is our opinion that these highly elaborated rituals are plesiomorphic, becoming gradually simplified until reaching a simpler level as seen in many *Tetragonula*, just comparable to the behavioral simplicity exhibited by the queen-worker interactions in the honeybees. By their diversity in such ritualized behavior, the stingless bees must be one of the most fascinating groups in the comparative ethology. But one final comment must be added to: Their highly differentiated ‘gestures’ should not function as visual stimuli as such in various elaborated courtship behaviors in birds, because the behaviors are here played within the darkness of the nests.

In only a few taxa, their behaviors were observed under the dark red lamp (*Scaptotrigona*, *Melipona*, etc. This type of observations should be made more with the behaviorally well documented taxa). The observed behaviors did not much differ from those seen during daytime through the glass window of the observation hives, though much more comparison should be undertaken. Possibly their peculiar behaviors viewed by us are perceived by their nest mates as the combination of chemical and tactual stimuli mainly through antennae and legs.

Table 5. Synoptic comparison of behaviors among 3 subgenera of *Plebeia*

Features compared	<i>Plebeia</i>	<i>Friesella</i>	<i>Mourella</i>
Morphological features (cf. Moure 1951)			
1. Body coloration	black to reddish	black	slightly metallic blue (unique in stingless bees) A
2. Facial yellow marks	well developed	absent A?	well developed
3. Mesosomal pilosity	poor and short	poor and short	dense and long
4. Propodeal surface	smooth and glabrous	reticulated and glabrous	punctate and hairy A
5. Sculpture on head and mesosoma	sparsely punctate, shiny	densely reticulo-punctate and mat	more or less reticulate but shiny
6. Marginal cell	slightly open A	nearly closed	nearly closed
Ethological features			
1. Nesting site	aboveground	aboveground	obligatorily subterranean A
2. Involucrum	more or less present	absent A	present
3. Cell arrangement	typically combed	irregularly semicombed A	typically combed
4. Queen walking	often quite erratic, accompanied by abrupt darting A ₀	moderate and relatively constant	A ₁
5. Worker responses to queen	highly exaggerated with frequent darting and/or peculiar hypnotic akineses A ₀	very calm, no particular responses except those seen in many other taxa	A ₁
6. Queen dominance behavior	+ A ₀	- A ₁	- , except peculiar darting at provisioning A ₀
7. Cell construction	synchronous A ₀	semisynchronous A ₁	successive A ₂
8. Batch formation	obligatory A ₀		facultative A ₁
9. Food provisioning in cells	synchronous A ₀	successive A ₁	synchronous A ₀
10. Pre-provisioning worker agitation	generalized and high A ₀	localized and weaker though with "frozen rosette", a peculiar akinesis around the attended cell A ₁	generalized and high A ₀
11. Cell inspection before queen oviposition	only in some earlier oviposited cells A	always present P	
12. Worker oviposition in queenright colonies	mainly on comb before peak of POP agitation, occasionally on cell margin and in cell during operculation A*	only during operculation in populous colonies	on cell margin during POP A ₀
13. Worker rotation in cell operculation	present	absent A	present

P: Plesiomorphic; A: Apomorphic (apomorphy as A₂ > A₁ > A₀).

* Either plesiomorphic (Zucchi 1994) or apomorphic (Sakagami, unpubl.). This aspect will be detailed in a later paper.

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