Title	Oviposition Behavior of an Aberrant African Stingless Bee Meliponula bocandei, with Notes on the Mechanism and Evolution of Oviposition Behavior in Stingless Bees (With 16 Text-figures and 6 Tables)
Author(s)	SAKAGAMI, Shôichi F.; ZUCCHI, Ronaldo; ARAÚJO, Virgílio de Portugal
Citation	北海道大學理學部紀要, 20(4), 647-690
Issue Date	1977-05
Doc URL	https://hdl.handle.net/2115/27625
Type	departmental bulletin paper
File Information	20(4)_P647-690.pdf

Oviposition Behavior of an Aberrant African Stingless Bee *Meliponula bocandei*, with Notes on the Mechanism and Evolution of Oviposition Behavior in Stingless Bees[1]

By

Shôichi F. Sakagami, Ronaldo Zucchi and
Virgílio de Portugal Araújo

Zoological Institute, Hokkaido University, Sapporo, Departamento de Biologia,
Faculdade de Filosofia, Ciências e Letras de Ribierão Prêto, Universidade
de São Paulo, Ribeirão Prêto and Instituto Nacional de
Pesquisas da Amazonia, Manaus

(*With 16 Text-figures and 6 Tables*)

Meliponula bocandei (Spinola 1853) is a singular African stingless bee, showing morphological features intermediate between *Melipona* and the other stingless bees (Wille 1963). As a continuation of our serial reports on the oviposition behavior of stingless bees, the present paper deals with behavioral observations of this species, only a summary of which was previously reported (Sakagami and Zucchi 1966). In this occasion, our previous hypothesis on the mechanism underlying the oviposition process of stingless bees will be revised, accompanied with some discussions on related problems.

It was unfortunate that this species, highly aberrant in behavior from other stingless bees, was studied at an earlier stage of our serial observations, when our comparative knowledge was inevitably imperfect. But the basic behavioral characters given below will be useful for further detailed studies.

In subsequent pages the other species observed by us and cited for comparisons will be shown, unless necessary, with generic or subgeneric names alone, according to the system by Moure (1951, 1961) adopted in our serial reports. (Other systems were recently presented by Wille and Michener 1973, and partly by Sakagami 1975). The species names, authors and full citations of the behavioral accounts are found in the references at the end of the paper. The generic and subgeneric names not found there are those cited from unpublished data of ours or our students. For simplicity these taxa are henceforth regarded as genera. The terminology on behavioral details revised by Sakagami and Zucchi (1974) will be adopted in subsequent descriptions.

---

[1] Behavior studies of the stingless bees, with special reference to the oviposition process. X.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 20 (4), 1977.

The observed colony was introduced from Luanda, Angola, to Brazil, and had been kept in the apiary of Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Rio Claro, the State of São Paulo, in a hive following the pattern developed by Araújo (1956). For the behavior study, the colony was divided and the old queen accompanied with brood, food and adult workers was transferred in an observation hive (Sakagami 1966) and observed mainly from December 26, 1962 to February 28, 1963.

Meliponula is a very productive bee. Its nests often become fairly large, the extent of brood area occasionally attaining 15～18 cm in diameter and 20～25 cm high (Araújo (1955). It is possible that the behavior in the small colony kept in an observation hive deviates in some aspects from that in natural nests. But from observations of oviposition behavior in *Melipona rufiventris* Lepeletier under diverse colony conditions, Camilo-Atique (1974) clarified the persistence of qualitative aspects of behavior patterns and their articulation, though quantitative aspects varied according to colony conditions. This would be valid for *Meliponula*, too.

1. Behavior out of oviposition process

1.1. *General and miscellaneous accounts*

Meliponula builds incomplete combs (Fig. 1), the type being relatively rare in stingless bees, known only in some limited genera, *Friesella*, *Duckeola*, etc. Cells are juxtaposed one another but not neatly lie in the same plane. Each cell retains spherical contour at the top and the bottom, exhibiting an uneven appearance of comb surface. Combs expand irregularly, not concentrically as in typical comb builders and are occasionally but not always surrounded with involucrum. In general appearance as well as in size and coloration, combs are similar to those of *Duckeola* (Kerr et al. 1966). In March 1963, a piece of *Duckeola* comb, ca. 3×4 cm sq., was given to the nest. It was destroyed within a day. A piece of honeybee comb with stored honey was accepted and covered with cerumen, but destroyed after honey consumption.

Although no detailed observation on intranidal worker behavior was made, no particular difference from other genera was noted. General motor pattern

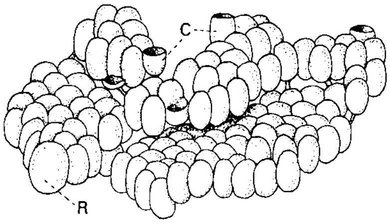

Fig. 1. Combs of *Meliponula bocandei*. C = Cells in construction, R = Royal cell.

resembles that of *Melipona,* not so sluggish as in *Duckeola* but slower than in most other genera. At walking wings are not rhythmically fluttered and food regurgitation between workers is often made with a twisted posture as in other genera. Feces and other debris are accumulated on the floor of the hive, forming several heaps, which are actively carried away on the wing during daytime. For this active removal the colony did not suffer the damage by the phorid fly, *Pseudohypocera kerteszi* (Enderlein), the worst enemy of stingless bees in southern Brazil, depsite many flies invaded the colony and workers did not pay much attention to them. Newborn workers were mostly helped by older sisters at emergence. As in other genera, at ventilation the head is directed against, not to the nest entrance.

Usually four to five workers stay at nest entrance as guards. They exhibit spontaneous repetition of fore- and backward movements, but never so overtly as in *Melipona quadrifasciata.* Guards seldom examined the homing foragers, responding with a mild retreat. When interfered they first retreat. By continuous stimulation with a stick, some guards advance, raising the fore body and fore legs and opening mandibles, dart and gnaw the stick. No further aggression nor ritualized defensive posture such as the warning fanning *(Scaptotrigona, Melipona seminigra),* etc. are exhibited. From pigmentation is concluded that most

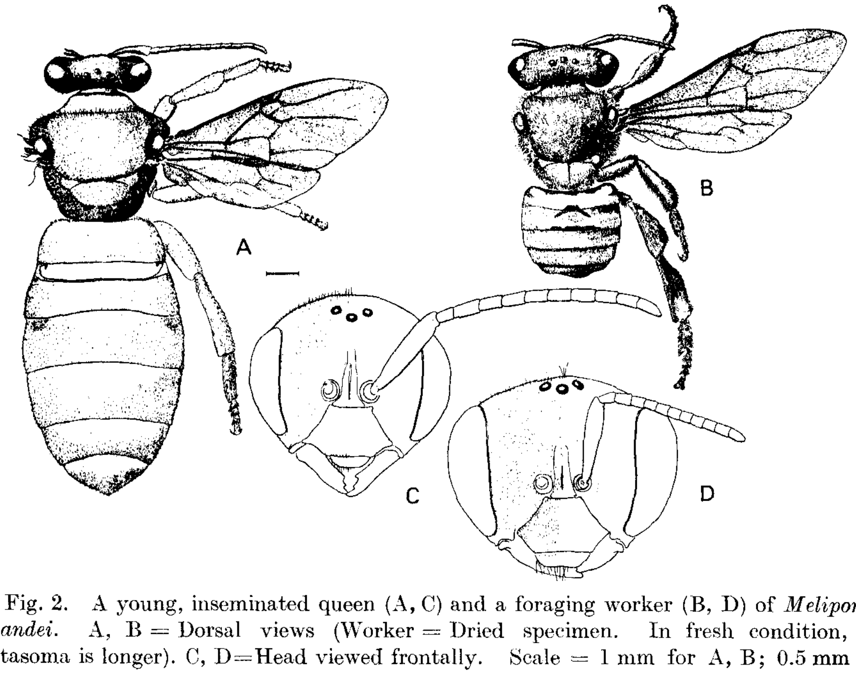

Fig. 2. A young, inseminated queen (A, C) and a foraging worker (B, D) of *Meliponula bocandei.* A, B = Dorsal views (Worker = Dried specimen. In fresh condition, the metasoma is longer). C, D = Head viewed frontally. Scale = 1 mm for A, B; 0.5 mm for C, D.

guards are at ages intermediate between house and foraging bees. Once a pollen laden worker participated in guarding for more than one hour.

1.2. *Queen behavior*

The queen and worker are shown in Fig. 2 and differences in some metric characters in Table 1. The head of the queen is a triffle narrower than in workers, but the mesosoma is slightly wider. The queen walks more rapidly than in *Melipona* and *Scaptotrigona.* Wings are beaten rather sporadically at walking but rhythmically at resting. Beats are single-stroked without vibration. The time spent for ten strokes are 4.0 sec (1 case), 4.5 (3), 5.0 (2), 6.0 (6), 6.5 (3), 7.0 (5), 9.0 (2) and 10.0 (1). The queen behavior is characterized by two features which are only rarely observed in other genera. One is the violent and constant shaking of antennae distinctly longer than in workers (Fig. 2, Table 1), irrespective of walking or resting, which is only similar to the behavior presented by the queen of *Axestotrigona,* another African group. The other is the incessant cruising throughout the hive, not only on the floor, combs and storage pots but even beneath the glass lid. Moreover, she frequently visited the entrance corridor connecting the hive to outdoors, even reaching the nest entrance. This was first

Table 1. Size difference between queen ($n=1$) and worker ($n=10$) in some metric characters (in mm) arranged in the ascending order of queen/worker ratio (L=length, W=width).

Character	Queen	Worker		Queen/worker ratio
		Mean	SD	
Eye L	1. 556	1. 926	0. 033	0. 808
Hind basitarsus W	0. 629	0. 744	0. 047	0. 846
Mesoscutellum L	0. 741	0. 874	0. 065	0. 847
Hind tibia W	0. 963	1. 133	0. 047	0. 850
Head L	2. 370	2. 659	0. 119	0. 891
Head W	3. 037	3. 356	0. 067	0. 905
Fore wing L	6. 444	6. 763	0. 094	0. 953
Mid tibia L	1. 852	1. 926	0. 047	0. 962
Hind wing L	4. 963	5. 089	0. 105	0. 975
Mesoscutum L	2. 000	2. 037	0. 076	0. 982
Mesosoma W	3. 926	3. 474	0. 102	1. 130
Mid femur L	2. 148	1. 896	0. 068	1. 133
Hind basitarsus L	1. 556	1. 363	0. 089	1. 141
Scape L	1. 444	1. 230	0. 049	1. 175
Hind tibia L	2. 815	2. 363	0. 070	1. 191
Hind femur L	2. 630	2. 104	0. 076	1. 250
Mid basitarsus L	1. 704	1. 318	0. 055	1. 292
Pedicel+Flagella L	3. 286	2. 428	0. 055	1. 354
Flagellomere I L	0. 370	0. 258	0. 021	1. 437
Malar L	0. 222	0. 151	0. 059	1. 470
Flagellomere II L	0. 333	0. 221	0. 004	1. 510

suspected as an instability caused by some abnormal colony conditions. But colony activities including ovipositions were normal. About 40 days after introduction in the hive, visits with the corridor disappeared (Table 2), but walking beneath glass, found only exceptionally in other genera, continued.

Fig. 7 presents the temporal sequence of queen activities during 150 min, involving three ovipositions, and Fig. 3 shows the spatiotemporal sequence of queen activities for 175 min, involving four ovipositions. Both visualize restless cruising, which covered most areas of the hive. The percentage ratios of various activities in six continuous observations are given in Table 2. In all cases cruising exceeded 65% of the observed duration. Although no systematic observation was made with other genera, such excess cruising was not seen except for *Nannotrigona* and *Geotrigona*, the queens of which tended to spend their most daily life by cruising on new combs. A brief continuous observation in *Duckeola* also showed the prevalence of resting against cruising. As seen in Table 2 and Figs. 3 and 7, the resting places were fairly definite. From December 1962 to early January 1963, first an area on the hive floor near a piece of cold comb, and later an area on storage pots (X and Y in Table 2 and Figs. 3, 7) were preferred, both about 3×3 cm. sq. in the maximum extent. Resting at other places was exceptional.

Another conspicuous feature seen in Table 2 is the low percentage spent by

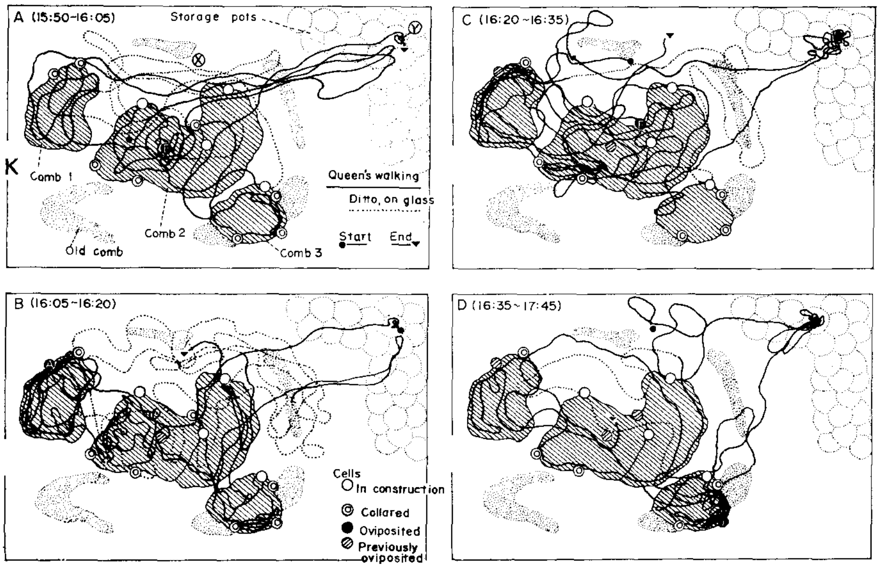

Fig. 3. Spatiotemporal tracing of queen activities for 115 min (Jan. 24 '63), involving four ovipositions (Cells E, A, F, I). X, Y=Resting places of the queen (cf. text), K= Entrance to flight corridor. Queen resting (mostly at Y) increased in the later period, D.

Table 2. Percentage ratios of various activities in four continuous observations of during a 15 sec interval, the interval was represented by the activity

Date (all in January)	Total duration of observation (in min)	Percentage time			
		Cruising on			
		corridor	storage pots	beneath glass lid	hive floor
15	130	+	8.1	7.4	23.2
16	30	3.6	—	12.5	18.0
18	30	1.6	12.6	—	30.3
19	35	6.7	2.3	15.7	20.0
21(Fig. 7)	220	—	3.8	9.3	38.8
24(Fig. 3)	175	—	6.9	18.4	19.3

waiting by the cell before or during the oviposition process, reflecting the behavioral peculiarities described in Section **2.**

1. 3. *Queen-worker coactions*

As in other genera responses of workers to the queen are characterized by their exaggerated manner compared with those in honeybees. In the areas other than new combs most workers encountered with the cruising queen hurried a few centimeters away or hid among combs, pots, etc. The response is similar on new combs but some workers, especially those "cornered" by the proceeding queen, face the queen and often dart her, whereas the queen does not show any aggressive acts (Fig. 4, A). Most workers encountered with the resting queen also avoid her (Fig. 4, C, I-II), but a royal court consisting of four to six attendants is gradually formed at her prolonged resting. Each attendant repeatedly moves her body fore- and backwards, accompanied or, more often, not by locomotion with legs (Fig. 4, C, III). Some workers touch the queen with antennae. Then they usually make an overt retreat. Darting (Fig. 4, A, C-IV) was frequent but licking the queen was never observed.

The queen occasionally begs workers for food. Short buccal contacts are observed at the royal court or on combs. But among 23 contacts precisely observed, real food delivery was confirmed only once. In four cases, contact lasted 6 sec (2 cases), 7 (1) and 8 (1), but the delivery was dubious. In another case, delivery certainly did not appear in spite of the contact lasting 8 sec. In two additional instances, the worker opened her mandibles and the queen inserted her distended glossa therein. The contacts lasted 4 and 7 sec respectively, but no food transfer was observed. Out of food solicitation, the queen does not show any particular responses to workers, including ritualized dominance characteristic of *Melipona*.

To confirm differential appearance of darting, responses of workers in encounters with the queen were counted under different situations.

queen behavior. Duration was rounded to 15 sec. (When two activities appeared lasting more than 8 sec). Resting places X, Y, cf. Fig. 3.

spent by							
		Waiting by	Resting on				
combs	*T*	cells	corridor	glass	X	Y	*T*
29.1	**67.8**	2.6	3.9	7.2	18.5	—	**29.6**
39.1	**73.2**	—	5.4	—	21.4	—	**26.8**
42.0	**86.5**	—	1.1	3.4	9.0	—	**13.5**
26.2	**70.9**	—	—	2.3	26.8	—	**29.1**
24.5	**76.4**	3.5	—	—	14.0	6.1	**20.1**
40.6	**85.2**	3.4	—	—	—	11.4	**11.4**

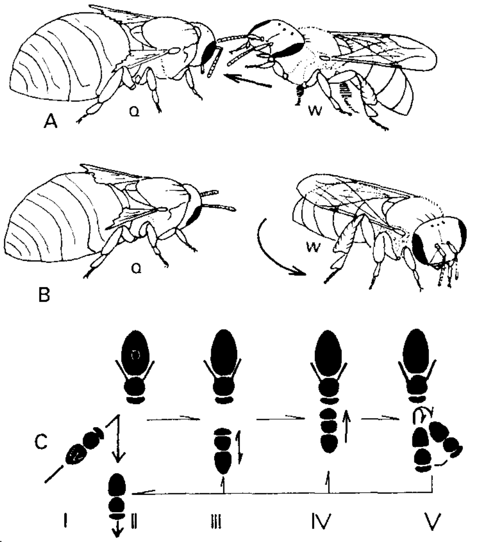

Fig. 4. Behavior in queen (Q) and worker (W) encounter. A. Darting by worker to queen. B. "Turning against" by worker. C. Sequence of various responses by worker. I=Arrival of worker. II=Avoidance. III=Repeated fore- and backward movements. IV=Darting. V="Turning against" (Thick arrows=Direction of behavior. Thin arrows=Sequence).

	Queen	Worker	
		Escape	Darting
At walking	corridor	6	–
	glass lid	62	–
	hive floor	59	1
	combs	40	51
At resting	hive floor	64	34

The prevalence of darting on combs or at the royal court is obvious. Darting is directed sometimes to the metasoma, but mostly to the head of the queen. The fore body and fore legs are raised and mandibles are widely opened (Fig. 4, **A**).

Actual clash is rare as the queen slightly retreats, but the violence of the act is equal or sometimes superior to that exhibited by *Scaptotrigona* and *Melipona*. Darting in front of the queen (*D*) is often repeated up to three times, followed by a neat "turning against" the queen (*T*, Fig. 4, B). At that time the head is directed against the queen, or at least kept at an angle more than 90° from her (Fig. 3, V, IV). The sequence is often repeated, e.g. in some actual cases, 2*D*-*T*-2*D*-*T*-2*D*-*T*-2*D*, *D*-*T*-2*D*-*T*-3*D*, etc., expressed as *m*(*nD*.*T*). Thereafter, the worker again performs fore- and backward movements or leaves away (Fig. 4, C). Although not accompanied with overt acts such as wing fluttering (*Scaptotrigona*), neatness of *T* and rhythmicity of the whole sequence gives a ritualized appearance.

The other miscellaneous observations on the reproductives are briefly cited:

1) Young males remained in the hive. Most of them rested on the underside of the glass lid. In encounters with the queen, they escaped hurriedly. No other responses as found between queen and workers were noted. 2) Once the queen defecated on the hive floor. The paste like excrement was licked by a worker.[1] 3) Some royal cells were built but food provisioning and oviposition were not precisely observed. The first one appeared on January 8, after 60 worker cells were built. It took 58 min to be provisioned, oviposited and operculated. Royal cells are distinctly larger than worker cells (Fig. 1, R). Removal of waxy walls so delayed that dark coloration remained after the same aged worker cells became yellowish by removal of the waxy coat. Often royal cells were destroyed a few days after being oviposited. 4) Emergence of a new queen was observed. It evoked a violent total excitement of workers, including those in flight corridor, mostly being foragers. The new queen was soon killed. It is unknown whether this is a rule in *Meliponula* or not. Such violent excitation was never observed in *Melipona*, which frequently practices virgin queen slaughter.

2. Oviposition behavior

2. 1. *Cell construction*

The type of cell construction is successive (S_c), i.e. cells are started not regularly so that various stages of cells are found at a given time (Fig. 5). As in other genera cells are built by summation of successive activities of young workers. The task abandoned half-way by a worker is succeeded by another. Possibly in part due to the small population size, however, half-built cells often remained unoccupied by workers, the inner walls of which were already smooth even in those 1/4 high (compared with oviposited cells). Time spent for the completion (=provided with constriction and knife-edging of the cell orifice) was not measured. When the performance was relatively active, time required for each of the following stages, start - 1/4 high - 1/3 - 1/2 - 2/3 - 3/4 - 4/4 - completion, was approximately 30 min, so that one cell would be completed 3～4 hrs after the beginning. But

---

[1] On this occasion we mention that we have so far never observed and, though no complete bibliographical survey was made, have failed to find any related record in previous studies on defecation of honeybee queens.

many cells remained at intermediate stages for a considerable time, whereas some ones started later were completed and received eggs earlier (Fig. 5, especially E and G).

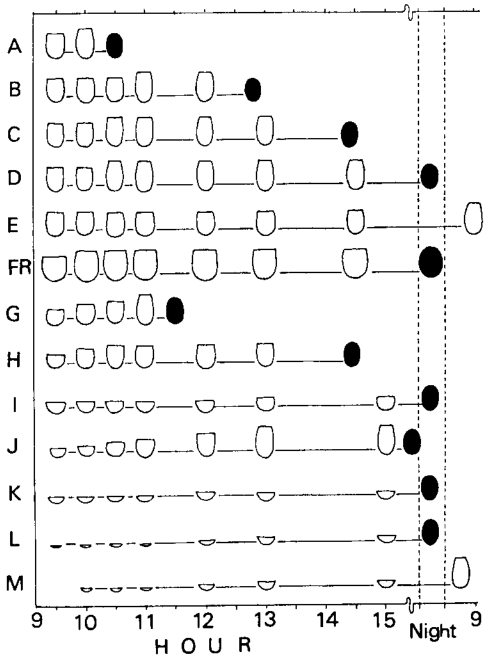

Fig. 5. Temporal sequence of cell construction (Jan. 15 '63). A~M=Cells arranged in the descending order of the start of construction and oviposition. FR=Royal cell. Black=Provisioned and oviposited.

2.2. *Oviposition rhythm*

At least in the observed colony, the oviposition rhythm showed the type predominantly singular (B_s), so far confirmed only in *Melipona*, i.e. each oviposition was usually separated by a sufficiently long interval, only rarely forming a batch. The interval between two successive ovipositions was mostly 30~100 min as follows:

Time (min)	less than 10	11~20	20~30	31~60	61~120	more than 120
Actual cases	2	11	22	32 50	64 94	128
(min)	3	14		35 53	64 116	
	6	20		43 55	77	
	10			45 56	82	
				48	91	
Total	4	3	1	9	7	1

Sometimes more than two ovipositions were continuously observed with the following intervals (min): January 15 (64–77–91–82–53), Jan. 16 (45–56–64), Jan. 17 (94–50–14), Jan. 21 (128–11, Fig. 7), Jan. 24 (14–22–10, Fig. 3). From these figures oviposition rate was calculated for some days:

Date (Jan.)	15	16	17	19	21	24
Oviposition rate/hr	1.04	1.10	0.74	0.40	0.62	0.62

In average 0.76/hr or 18.24/day was obtained, which was comparable to the rates in the colonies of *Melipona* reared in observation hives. But as colonies of *Meliponula* seemingly become larger than the average colony of *Melipona*, the rate will be much higher in populous colonies, with a corresponding shortening of intervals between ovipositions. It is not excluded that oviposition rhythm in larger colonies approaches type B_f (facultatively batched, e.g. *Tetragona*).

In a recent paper (Sakagami and Zucchi 1974), we regarded the daily life of stingless bee colonies as a sequence of several phases in relation to oviposition process. Some genera well follow the scheme presented but some others not always. *Meliponula* shows the most conspicuous deviation by its behavioral peculiarities. Leaving comparisons with other genera in **3. 2.**, the oviposition process is described below in the sequence of predischarge, discharge, postdischarge behaviors, followed by oviposition and operculation.

2.3. *Predischarge behavior*

As in other genera, structurally completed cells release an excited gathering of 3∼5 workers. They alternately insert the fore bodies into the cell (Fig. 13, A), which represents, in our interpretation, intention or subliminal food discharge act. In *Meliponula*, or possibly in the observed colony, the appearance of this excitement was quite irregular. For instance, on Jan. 17, cell G was already provided with the collar at 9:30, but left without formation of excited attendants until 15:10 (oviposited at 16:11), nevertheless three other cells were successively provisioned and oviposited during this time. Fig. 3 also shows that queen visits were more frequent for cells A and I than E and F. When there were more than one completed cell, worker excitement developed rarely at more than two cells, possibly in part affected by the small population size.

At body insertion the worker often rotates herself about 1/2–2/3 circumferences along the cell margin, which is frequently followed by a similar counter rotation. The duration of insertions and their intervals were measured in some cases:

Duration (sec)	Insertion	Interval between two insertions	Insertion in a cell before appearance of definite excitement (Fig. 6)
1 or less	12	206	36
2∼4	26	20	31
5∼8	67	11	29
9∼16	109	13	19
17∼32	71	3	10
33∼54	9		1

The average duration of each insertion becomes longer in parallel with increased excitement, as shown by the comparison of duration in such cells with a cell observed before the appearance of definite excitement for 45 min (Compare Fig. 6 with Figs. 10～11). Comparison of these figures also reveals that after the appearance of excitement the withdrawal of a body-inserting worker from the cell is soon replaced by another insertion. In general the average duration of body insertions, mostly ranging 5–14 sec, is longer than in other genera, roughly comparable to that in the arousal phase (previously *prefixation phase*) in *Melipona quadrifasciata*.

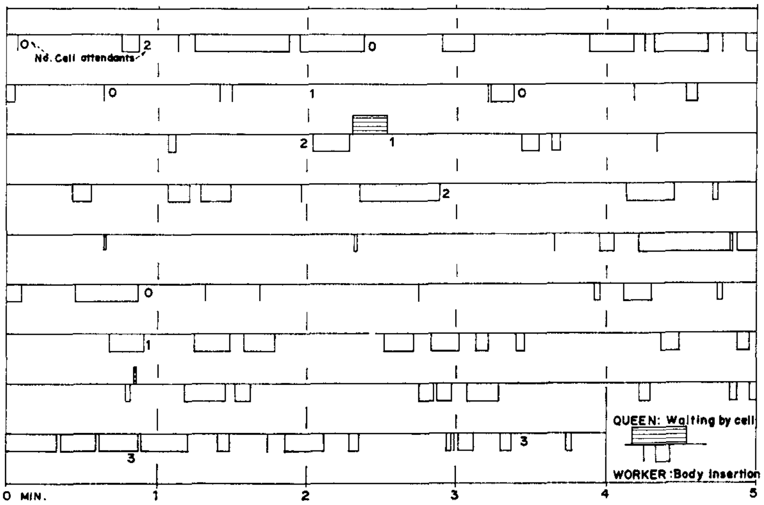

Fig. 6. Temporal sequence of queen and worker behavior at a cell before the appearance of worker excitement (Compare with Fig. 10, No. 12,″ Fig. 11, No. 13).

At body insertion the body trembles conspicuously except during rotation. Workers surrounding the cell also tremble and perform characteristic fore- and backward movements (Fig. 13, A), quite resembling the rocking movement practiced by honeybee workers (Alfonsus 1932, Sakagami 1953). This behavior appears also in some other genera, but not so exaggeratedly. In *Bombus atratus* Franklin, such trembling appears when the worker is opening the larval waxy coat, but it disappears during the feeding proper.

In other genera the queen sooner or later arrives at the cell attended by excited workers and *waits* thereby. She may leave there but again return. Larval food is discharged by workers in the cell always under queen waiting. Even in some genera which provision several cells synchronously (type D_y), at least the first cell is provisioned under queen waiting, or the cells are provisioned after persistent cruising of the queen over the comb. In *Meliponula*, however, the queen makes only brief visits with the cell. Arriving at the cell attended by excited workers, she usually leaves it after a brief stay there. After cruising she may again visit the cell but usually leaves it soon, exhibiting the type Cw as to the relative duration of cruising and waiting (C>W). The queen of *Trigonisca* sometimes abandons the cell in spite of a high excitement of cell attendants. But her waiting is long and the behavior sequence is not so erratic as in *Meliponula*.

Now it appears a trait with no similar one among all other genera so far studied: *Food discharge is released without queen waiting.* Moreover, after visiting the cell already provisioned with food, the queen does not always soon oviposit in it. Often she leaves it and re-starts cruising. In such case the oviposition takes place only after repeated visits with the cell. Thus, postdischarge delay appeared in all observed cases. This curious sequence is explained with Fig. 7, which presents a continuous observation of queen behavior for 150 min, involving three ovipositions in Cells D, I, G.

1) At the start of observation D was already provisioned. It was oviposited after repetition of three queen visits. 2) First visit with I was made 32 min after the start of observation, later followed by frequent additional ones. 3) At 92 min G received food inspite of only two prior visits (87 and 89 min), much less than in I. 4) Thereafter the queen continued to visit I more frequently. At 109 min I received food partly, and was filled

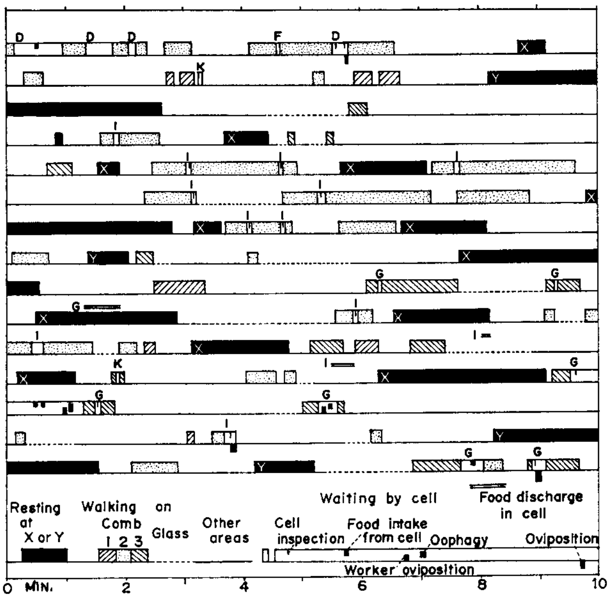

Fig. 7. Temporal sequence of queen activities for 150 min (Jan. 21 '63, 14:00–16:30) involving three ovipositions (Process Nos. 21~23, Cells, D, G, I). Cell D already filled with food at the beginning of observation. Cells D, F, I on Comb 2, G, K on Comb 3 (cf. Fig. 3, A).

up with food at 116 min. 5) The queen visited G at 120 min and waited there for a relatively long time, but again left it, followed by a short visit at 126 min. 6) At 134 min the queen visited I and soon oviposited in it. 7) At 148 min she visited G, but soon left it. At 149 min she revisited it and soon oviposited.

The whole sequence after oviposition in D is summarized as follows (*D*=food discharge, *O*=oviposition, *w*=short waiting, *W*=waiting lasting 15 sec or more):

Chronological sequence since start of observation (min)

Cell	30~50	~70	~90	~110	~130	~150
I	*w w w w*	*w w w w*	*w*	*w w* D_1	D_2	*O*
G			*w w*	*D*	*W w*	*W O*
K					*w*	

Another somewhat anomalous case is cited (Jan. 18):

1) At the start of observation (15:10) Cell A had already been provisioned, B was in pre-collared stage, C 3/4 high and D′ nearly so. 2) After several visits with A by the queen (including a prolonged food intake for 54 sec), B received food at 16:07 and C at 16:21, despite the collar was not well prepared, presenting irregular and rough contour. A also received additional food. 3) The queen continued visiting three cells with the relative frequency A>B>C. 4) At 16:28 the queen oviposited in B, at 17:18 workers started removing the food in A; C remained in the same condition and D′ received food at 17:18. 5) D′ received an egg at 17:27. At 17:30 A had no food more. At 17:33 workers started to remove the food from C. 6) A and C deprived of food were oviposited by phorid flies as they remained open for a while without worker attendants.

These cases indicate some unusual traits: 1) Food discharges without queen waiting in I, G, B, C and D′. 2) Discontinuous food discharges in I and A. 3) Discharges in G before those in I, despite queen visits were more frequent with I than G. 4) Repetition of visiting-leaving at G, B, C and D′ after food discharges. 5) Discharges following the order G-I and A-B-C-D′ while ovipositions I-G and B-D′ (A and C not oviposited).

Virtually in all 34 cases, where the beginning of food delivery was confirmed, discharge started without queen waiting. In some cases the temporal sequence before the first discharge was traced for a certain time, though none of them was continuously observed since the appearance of worker excitement. Table 3 presents some numerical data in these cases, and shows that queen waiting is not frequent and each seldom lasts more than 20 sec. Consequently time spent by waiting occupies a very small fraction of the total observed duration.

On arrival at the cell, the queen continuously trembles, constantly shakes antennae, and occasionally inspects the cell with an indecisive manner, i.e. the head is only shallowly inserted in the cell and the body trembles constantly so that inspection does not take a definite behavioral contour (Fig. 13, C). On the arrival of the queen cell attendants sink a little at cell walls but never escape as in *Friesella*. Sometimes workers dart the queen but "turning against" (**1. 3.**) was not observed. Darting is stronger when the queen feeds on the larval food.

Table 3. Some numerical data on predischarge behavior. Observed cases are arranged in the descending order of the duration of observation

Case No.	Figure	Duration of observation (min-sec)	Frequency of queen visits and, in parentheses, their duration (sec)[1]	Ratio of total duration of queen waitings to total duration of observation		Summed time when cell was not occupied by worker body insertions	
				sec/sec	%	sec	% to total obs. duration
23	7, I	108-00	10(about 10×9, 20)	110/6480	1.7	—[2]	
22	7, G	90-30	2(1, 1)	2/5430	0.03	—	
13	11	23-10	5(5, 8, 2, 7, 4)	26/1390	1.9	210	15.1
28		15-00	0		0.0	—	
12″	10	12-53	2(4, 9)	13/773	1.7	165	21.3
9	11	7-30	3(2, 2, 8)	12/450	2.7	59	13.1
32	10	6-21	0		0.0	42	4.1
14	10	5-33	1(7)	7/333	2.1	32	9.6
27		2-40	0		0.0	0	0.0
12		1-43	0		0.0	0	0.0
16	10	1-00	0		0.0	0	0.0

1) "1" means visit lasting one sec or less, all rounded to 1 sec at calculation.
2) Not measured.

The brief queen waiting suggests that this does not serve as the main proximate factor releasing food discharges as in other genera. But waitings may enhance general excitement, hence, indirectly may cause food discharges.

2.3. *Discharges of larval food in cell*

The beginning of food discharges in the cell was frequently overlooked, mostly because of the absence of queen waiting, which have always served as the focus of observations in other genera. Another cause is the inconspicuousness of discharge behavior as compared to other genera. At discharge body trembling ceases and the metasoma is contracted cephalocaudally, but the contraction is less conspicuous than in all other observed genera. The duration of body insertion for each discharge is unusually longer than in any other genera (e.g. *Leurotrigona* and *Trigonisca* ca. 1.0 sec, *Duckeola* 1.5 sec, *Friesella* 1.8 sec, *Melipona* 1.9～2.4 sec, *Tetragona* 3.1 sec, *Scaptotrigona* 3～5 sec, *Cephalotrigona* 4.2 sec):

Duration (sec)	2	3	4	6	7	8	9	10	11	12	13	14	15	16	17	18	19
No. cases	2	2	2	7	8	2	9	5	2	3	6	3	3	3	3	3	2
Duration	20	21	22	23	25	27	28	29	30	31	36	37	57	60			
No. cases	2	1	1	1	2	1	1	1	4	2	1	2	2	1			

The metasomal contraction itself lasts only several seconds. But before or after it, the discharger frequently keeps the inserting posture with body trembling, or often rotates herself along the cell margin (cf. **2. 2.**). The rotation is seen in some other genera, too, e.g. *Tetragona* and frequently in *Friesella*, but the duration of a single discharge insertion was never so long as in *Meliponula*.

There are two facts which might relate to this prolonged discharge posture. First, discharge is performed in the absence of the queen so that it is free from her direct influence. But after discharge each worker escapes from the cell mainly toward the underside of the comb, though more slowly than in other genera except *Melipona* and *Leurotrigona*.[1] Second, the quality of larval food is peculiar. Kerr and Lello (1962) and Kerr and Maule (1964) mentioned the larval food of *Meliponula* as more viscous, somewhat paste-like compared with that of other genera. Soon after discharge, the food is not paste-like but its viscous nature is recognized.

The number of food discharges per cell was accurately counted in only 11 cases: 4 (2 cases), 5 (5), 6 (2), 7 (1), 9 (1), more or less comparable to that in other genera except *Leurotrigona* (mostly 2): *Melipona compressipes* 10.2, *M. seminigra* 14.3 (these two Amazonic species were observed under adverse conditions), *M. quadrifasciata* 6～8, *Cephalotrigona* 5.4, *Tetragona* 5.1, *Duckeola* 3.5, *Friesella* 3～5, *Trigonisca* 4.8.

In all so far observed genera, the first discharge is soon followed by the second and subsequent ones, so that discharge subphase ($\bar{d}_d$) is fairly compact, with a relatively short duration even though sometimes mere body insertions intevene between two later discharges. Exceptions to this rule were observed occasionally in *M. compressipes* and *Friesella*, in which the intervals between two discharges, mostly the later ones, were often quite long. In *Meliponula* some cases were compact as in other genera. But some others took a discontinuous sequence as mentioned in **2. 2.**, reaching occasionally a degree never observed in other genera. Among 12 accurately followed cases, six belonged to the former type (compact) and six to the latter (discontinuous) as given in Table 4.

Beside the cases mentioned, discontinuous sequence is likely in some other cases where the amount of discharged food gradually increased during a considerable time. In discontinuous cases some body insertions between discharges appeared but neither frequently nor successively as in predischarge period.

Visits by the queen with the cell during real discharge sequence, not during prolonged intervals between two discharges in a discontinuous sequence, were observed only twice:

No. 7. The queen have waited by the cell which had already received food about 2/3 of the full capacity. She made a brief inspection between two discharges. After the final discharge she ingested larval food for 90 sec and left the cell. It is unknown whether she had been by the cell since the beginning of discharges. Nos. 4/5 A. The queen made a momentaneous visit during successive discharges.

---

[1] The occurrence of postdischarge escape, a trait common to all genera so far observed, in *Meliponula*, despite the absence of queen waiting, requires the correction of our previous hypothesis on the mechanism of oviposition process as discussed in Section **4**.

Table 4. Numerical data on some oviposition processes accurately observed as to food discharge. M sec. $(D_n \sim D_{n+1})$=nth and $(n+1)$ th discharges separated with a delay of M sec. I=Body insertions by workers. B=Food intake by the queen. V=Queen visits

<table>
<tr><th colspan="5">Compact type</th></tr>
<tr><th>Case No.</th><th>Figure</th><th>Number of food discharges</th><th>Total duration (sec)</th><th>Remarks</th></tr>
<tr><td>22</td><td>7, 8, G</td><td>4</td><td>44</td><td>2 sec·$(D_1 \sim D_2)$, 2·$(D_2 \sim D_3)$</td></tr>
<tr><td>12</td><td>9</td><td>4</td><td>69</td><td></td></tr>
<tr><td>14</td><td>8, 10</td><td>6</td><td>73</td><td></td></tr>
<tr><td>13</td><td>8, 11</td><td>5</td><td>74</td><td></td></tr>
<tr><td>32</td><td>9, 10</td><td>5</td><td>82</td><td></td></tr>
<tr><td>9</td><td>8, 11</td><td>9</td><td>140</td><td>2 sec·$(D_1 \sim D_2)$, 10·$(D_7 \sim D_8)$</td></tr>
<tr><th colspan="5">Discontinuous type</th></tr>
<tr><td>12″</td><td>8, 10</td><td>5</td><td>69</td><td>5 sec·$(D_1 \sim D_2)$, 14·$(D_2 \sim D_3)$</td></tr>
<tr><td>18</td><td>9, 12</td><td>5+2</td><td>177</td><td>$D_5 \sim D_6 \sim D_7$ considerably spaced. $D_5 \sim D_6$ with 2 II</td></tr>
<tr><td>16</td><td>9, 10</td><td>4+2</td><td>201</td><td>$D_4 \sim D_5 \sim D_6$ considerably spaced with II.</td></tr>
<tr><td>23</td><td>7, 8, I</td><td>1+4</td><td>472</td><td>452 sec·$(D_1 \sim D_2)$</td></tr>
<tr><td>1</td><td>9</td><td>1+1+3</td><td>619</td><td>550 sec·$(D_1 \sim D_2)$ with 2 VV (30 and 1 sec, first V with 2 BB) and more than 10 II</td></tr>
<tr><td>11</td><td>9, H</td><td>?</td><td>930</td><td>Divided in three parts, with 1 V</td></tr>
</table>

Thus the queen occasionally takes food from the provisioned cell during $\bar{\mathrm{d}}_\mathrm{d}$ (cf. also No. 1, Table 4 and Fig. 9), which is so far unrecorded in any other genera. In No. 1 a long interval between two discharges, with queen visits and her food intake, shows that such intervals functioned similar to postdischarge period described below.

2.4. *Postdischarge behavior*

Food discharges in the cells forming a batch can be either successive ($\mathrm{D_c}$) or synchronous ($\mathrm{D_y}$). In the genera showing $\mathrm{D_c}$, after the final food discharge in a given cell, the waiting queen oviposits either immediately or with a delay. In the latter case, time between the end of final discharge and the beginning of cell inspection by the queen soon before her oviposition was defined as postdischarge subphase, $\bar{\mathrm{d}}_\mathrm{p}$. In many genera this subphase is virtually absent or brief, even if occurs. In some genera, however, $\bar{\mathrm{d}}_\mathrm{p}$ appears, though facultatively, with a marked prolongation (e.g. *Tetragona*). Such prolonged $\bar{\mathrm{d}}_\mathrm{p}$ also facultatively appears in *Melipona*, though here each batch usually involves only one cell. In *Scaptotrigona*, the integrated oviposition process shows first $\mathrm{D_c}$ later $\mathrm{D_y}$. In this genus the delay is obligatory for many cells. In other genera taking $\mathrm{D_y}$, too, the delay between final discharge and queen oviposition in the second and subsequent cells is obligatory, which is further subdivided into attending and postdischarge waiting phases ($\bar{\mathrm{a}}$ and $\bar{\mathrm{r}}'$), though the latter is virtually absent in most cases (Sakagami and Zucchi 1974). Because the behavior of *Meliponula* is so peculiar, it is difficult to apply the distinction of phases

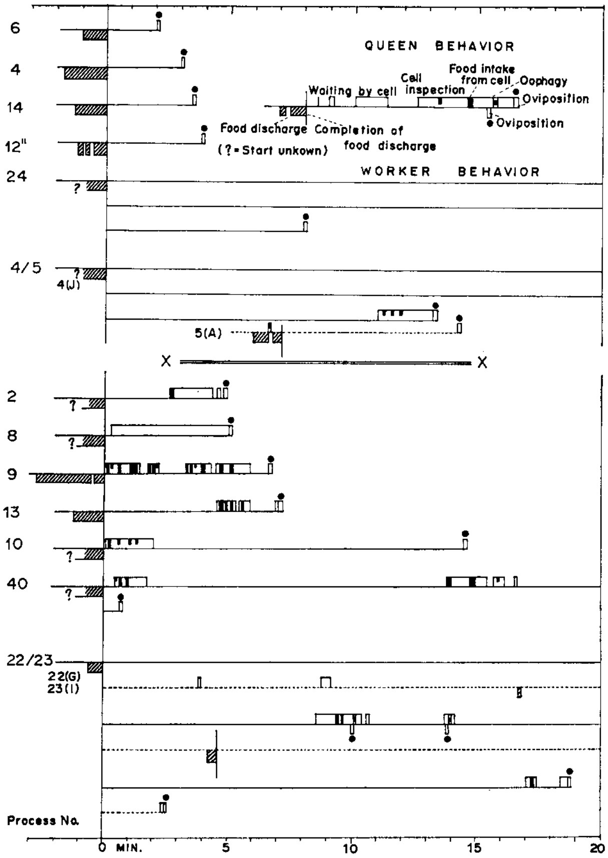

Fig. 8. Temporal sequence of oviposition process after completion of food provisioning in the cell. Cases above line XX=Simple cases without intermediate queen waitings. Cases below XX=Those with intermediate queen waitings. Cell inspection by the queen soon before her oviposition is not shown. When two cells were simultaneously observed (Pr. No. 4/5, 22/23), these are distinguished by solid and broken lines.

given above. Here the time since the end of the final discharge and the beginning of queen oviposition is called postdischarge period, PDP.

The absence of PDP is theoretically possible if the queen persistently waits by the cell and immediately oviposits after the final food discharge. But in all observed cases the queen was absent at the end of the final discharge, so that the delay was obligatory. All accurately observed cases are given in Figs. 8, 9, and some of them more precisely in Figs. 10~12, which show a marked behavioral variation.

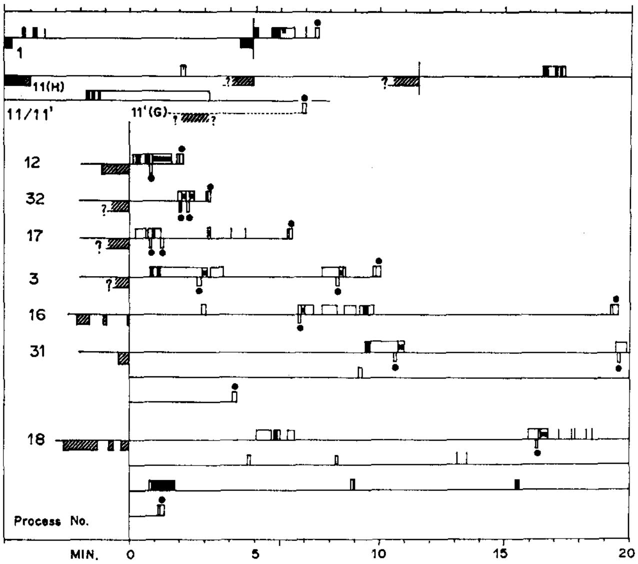

Fig. 9. Continuation of Fig. 8 (Cases with intermediate queen waitings).

Table 5. Some numerical data on postdischarge period (PDP) with queen waitings, arranged in the ascending order of total duration

Case No.	Fig.	Total duration (sec)	No. queen waitings and, in parentheses, their duration (sec), in the order of actual sequence	No. food intake	No. worker oviposition	Total duration of waitings (sec)	% ratio of total duration of waitings to total duration of PDP
12	9	114	1(86)	2	1	86	75.4
32	9, 10	194	1(36)		2	36	18.6
2	8	286	2(99, 10)	1		109	38.2
17	9	378	5(18, 24, 10, 3, 2)		2	57	15.1
9	8, 11	389	4(86, 22, 64, 76)	10		248	63.8
1	9	392	3(15, 45, 1)	2		61	15.5
13	8, 11	416	2(46, 23)	4		69	16.6
3	9	585	3(137, 32, 53)	2	2	222	37.9
10	8	870	1(114)	1		114	13.1
16	9, 10	1180	5(13, 47, 38, 35, 41)	1	1	174	14.7
40	8	1238	4(80, 97, 24, 6)	4		207	16.7
31	9	2646	3(90, 27, 3)	1	2	120	4.5
22	7, 8, G	3504	4(108, 8, 28, 26)	4	2	170	4.9
18	9, 12	3616	16(35, 15, 14, 2, 1, 1, 1, 1, 1, 8, 4, 5, 2, 52, 8, 4)	5	1	153	4.2

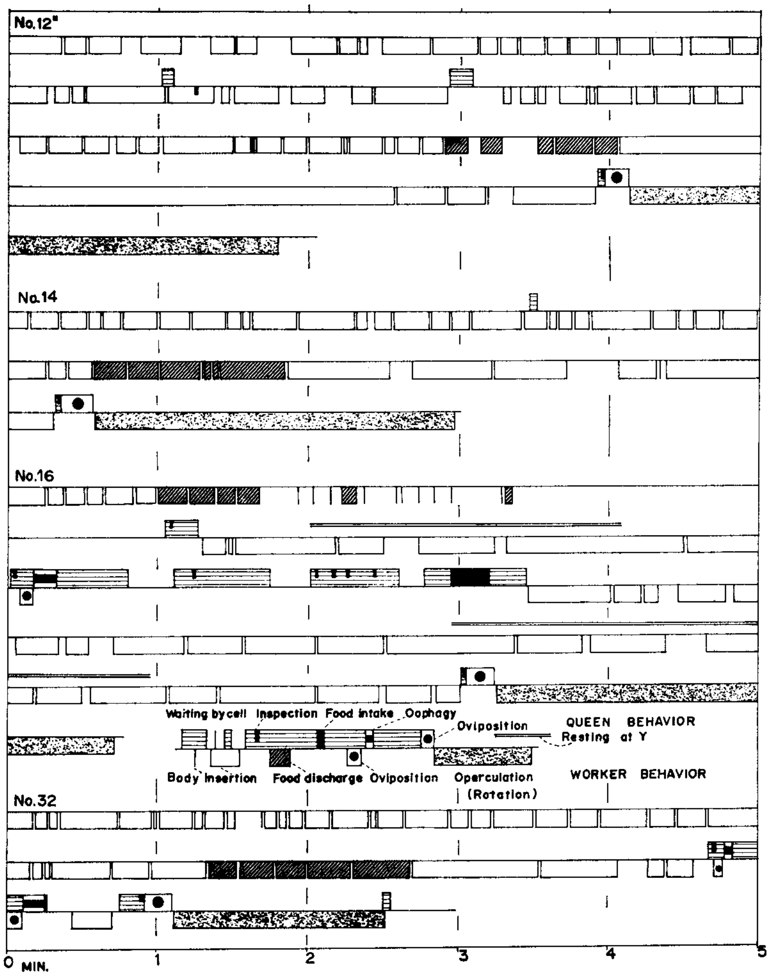

Fig. 10. Temporal sequence of oviposition process in some precisely observed cases. No. 12": Predischarge period relatively long observed, discharge phase discontinuous. No. 14: Relatively simple case. No. 16. Discharge phase discontinuous, postdischarge period long. No. 32: Relatively short case with two worker ovipositions.

In the following eleven cases, the sequence was simple (Fig. 8, above line XX). With a brief or lasting delay after the final food discharge, the queen visited the cell and soon oviposited (except Nos. 8 and 4/5 J) (Duration of PDP in parentheses with min-sec. *=duration of pre-oviposition waiting): No. 6 (ca. 2 min), No. 27/30A (2-10), No. 4 (3-00), No. 14 (3-27), No. 12" (4-47), No. 8 (5-00, *4-42), No. 4/5 A (6-55), No. 23 (17-46), No. 27/30 I (19-10), No. 24 (8-00), No. 4/5 J (51-00,

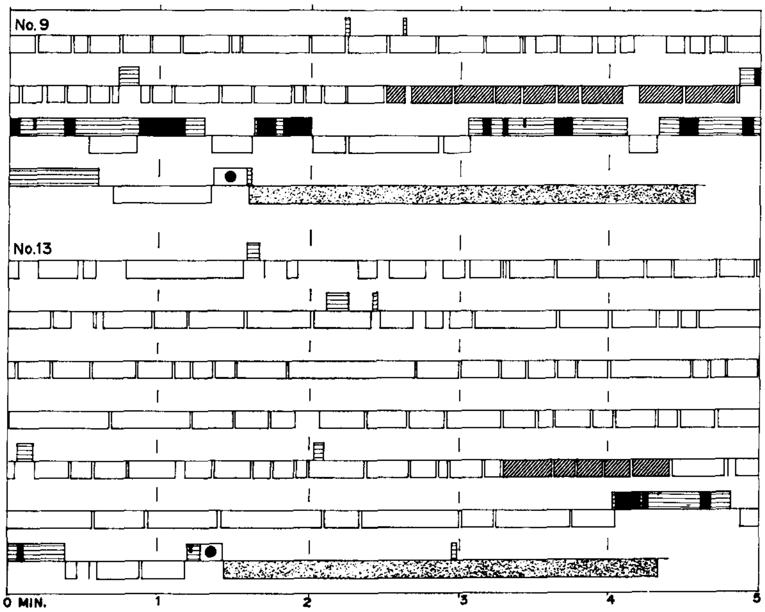

Fig. 11. Temporal sequence of oviposition process in two precisely observed cases. Explanations in Fig. 10. No. 9: Numerous food intakes by the queen. No. 13: Predischarge period relatively long observed.

* 2–13). In these cases, PDP corresponds to ā in D_y type, in two cases, No. 8 and 4/5 J, followed by r̄′.

In other cases (Fig. 8 below and Fig. 9), PDP involved one intermediate waiting or more by the queen, with or without her food intake from the cell. Some numerical data on these cases are given in Table 5, where the total duration is quite variable, occasionally attaining or even exceeding one hour.

On the other hand, queen waiting is mostly brief as shown by the distribution of duration (each one case unless parenthetically mentioned): 1 sec or less (3), 2(4), 3(2), 4(2), 5(4), 6(4), 8(4), 12, 13, 14, 15, 22(2), 23, 24, 26, 27, 28, 32, 35 (2), 36, 38 (2), 41, 46(2), 47, 52, 53, 64(2), 76 (2), 86 (3), 90, 97, 108, 137. Consequently the percentage ratio of total time spent by waitings to total duration of PDP rarely exceeds 50%. As waiting does not increase or lengthen in parallel with total duration of PDP, its ratio falls to 10% or less in prolonged PDP.

During PDP the cell is continuously surrounded by attendants and body insertions are made by some of them. Insertions are not so deep as in predischarge period, obviously because the cell is filled with larval food. One insertion can immediately be followed by another as in predischarge period (Figs. 10∼12), but sometimes insertions are made intermittently (Figs, 10, 12, Nos. 14, 18). Occasionally very long insertions were noted (cf. Fig. 10, No. 12″). Possibly this relates

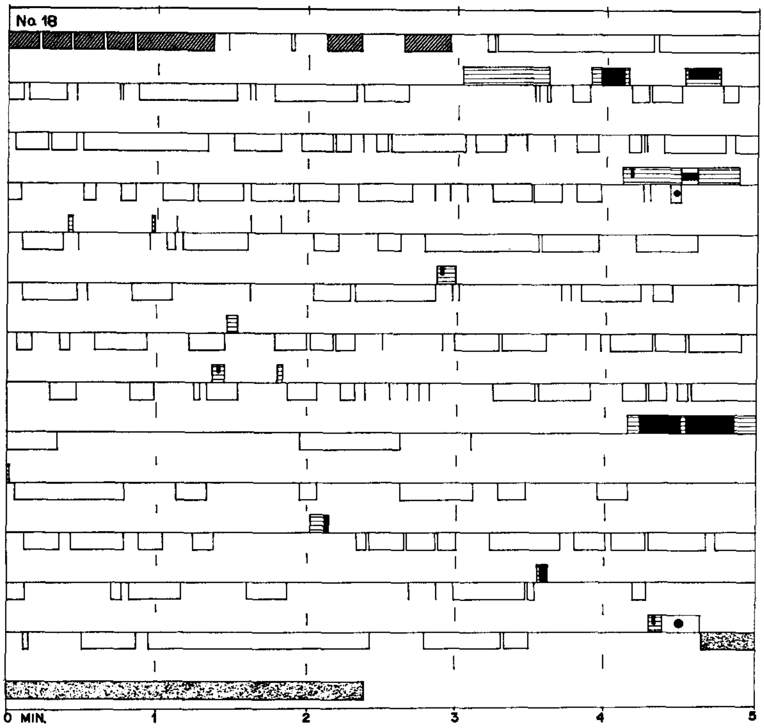

Fig. 12. Temporal sequence of oviposition process No. 18, with prolonged postdischarge period. Explanations in Fig. 10.

to the attending behavior characteristic in ā of the genera with D_y type, but posture peculiar to these genera, a continuous guarding by one worker staying by the cell, was not observed. On this account, however, our observations are yet incomplete.

The queen waiting by the cell behaves as in predischarge period, trembling the body, shaking the antennae and from time to time inspecting the cell with an indecisive manner (Fig. 13, C, cf. **2. 2.**). In the presence of the queen, body insertions by workers distinctly decrease but do not completely disappear. The queen violently taps these workers as in *Melipona*. But she shakes the antennae similarly in any other situations, while *Melipona* only at waiting. Workers tapped by the queen either leave the cell or continue the task for a while. After withdrawal they escape soon or after darting the queen (cf. **1. 3.**).

Frequently the queen takes larval food from the cell (Table 4). Food intake occasionally appears even in discharge phase (**2. 3.**) and is distinguished from a mere inspection by a deeper insertion of the fore body (Fig. 13, D). The duration was measured as follows (each one case unless parenthetically mentioned):

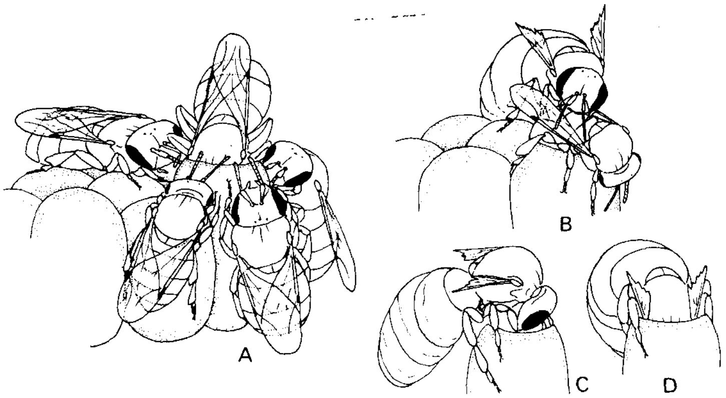

Fig. 13. Worker and queen behavior in oviposition process. A. Predischarge worker excitement. One worker inserting the fore body in the cell. B. Worker oviposition. Queen tapping the worker. C. Queen cell inspection. D. Queen food intake from the cell.

2 sec (6), 3(3), 4(4), 5, 6(5), 7(4), 8(4), 9, 12(2), 11, 12(4), 15, 16, 17, 18, 22, 29. Some short cases might not be real intakes, but the withdrawal of glossa was confirmed in two cases lasting only 2 sec.

Worker oviposition (Fig. 13, B) followed by queen oophagy was not rare (Table 5). It is often predicted by an irritating, brief and shallow cell inspection, occasionally repeated twice. Then the worker rides on the cell by a quick turning and directs against the queen. The metasoma is inserted in the cell and this posture is kept without movements of wings and appendages. The duration was, 3 sec (2 cases), 4(7), 5(2), 6(3), 10(1), 15(1). Eggs are laid vertically on the center of larval food as in *Melipona*, not on the cell margin as in many other genera, and are the same shaped with those laid by the queen but a triffle smaller. The queen violently taps the worker (Fig. 13, B), who hurriedly runs away from the comb after oviposition. No worker oviposition was seen in the absence of the queen. Except three instances, the eggs were soon devoured by the queen, the duration of which fluctuated 3 sec, 4, 6(3 cases), 7, 10, 12(2), 13, 15, 16, 46. Three instances without oophagy were as follows:

No. 17: The queen left the cell when a worker just took the laying posture.

No. 31 (Fig. 9): Egg was laid a little obliquely on larval food. The queen inspected the cell but left it 27 sec after worker oviposition. In both cases worker attendants showed ambiguous responses to the remaining egg, repeating approaches and avoidances until the egg was eaten each by a worker, respectively 103 and 60 sec after oviposition.

No. X: Egg remained attached to the worker and later fell on the comb, where immediately devoured by a nearby worker.

As mentioned at the top of this section, PDP of *Meliponula* is unique among all genera so far observed. As to the temporal position it is closest to $\bar{d}_p$ in *Melipona*, but the queen does not persistently wait by the cell, only intermittently visiting the cell, and leaving after a brief waiting. PDP resembles somewhat $\bar{a}$ in D_y type genera by the absence of the queen at its beginning and through its most part. Yet it differs from $\bar{a}$ by the absence of attending behavior by workers and visit by the queen not necessarily results in oviposition.1) On the latter aspect the sequence resembles again $\bar{d}_p$ in *Melipona* and *Tetragona*, where the waiting queen lays egg after the final food discharge often but not necessarily with a considerable delay. The nature of this facultative delay is unknown. The insufficient amount of food intake by the queen may partly be responsbile. But the queen frequently waits after such intake and ultimately oviposits without additional ingestion. Possibly her oviposition drive increases not monotonously, fluctuating erratically. The peaks and valleys of this fluctuation may cause a facultative delay. On this point further studies are required.

When two ovipositions appear with a brief delay (**2. 1.**), two processes are independent in the sense that worker excitement at both cells is each localized, not spreading the comb (type L). As to the queen behavior, however, it is rather generalized (type G), because she visits two cells alternately. Often one cell is visited preferentially but not exclusively (cf. **2. 3.** cf. Figs. 3, 7).

2. 5. *Oviposition, cell operculation and total length of oviposition process*

Except two cases (cf. **2. 4.**, Nos. 8, and 4/5 J, Fig. 8), the queen oviposition took place soon after her final visit, preceded by a brief inspection of the cell (1 sec or less). During oviposition the wings are beaten, the antennae are constantly shaken and legs are sometimes tapped but not so overtly as in *Duckeola*. The duration was measured as follows: 6 sec (2 cases), 7(4), 8(8), 9(9), 10(4), 11(3), 12(4), 13(1), 14(3), 15(2), 21(1), being distinctly longer than worker oviposition. The mear duration (10.04 sec), is shorter than in *Melipona* (25~32 sec) but longer than in any other genera, the longest of which is in *Duckeola* (6.9 sec).

Cell operculation of *Meliponula* is simple. It appears soon after queen oviposition without delay (=preoperculation subphase $\bar{s}_p$ virtually absent). Operculation is first made by rotating movement of a worker (rotation subphase $\bar{s}_r$), later made by side work (sidework subphase $\bar{s}_r$), usually with participation of other workers or with change of participants. Distinction of $\bar{s}_r$ and $\bar{s}_s$ is clear as in *Melipona*, *Scaptotrigona*, etc., not intervened with transient subphase ($\bar{s}_t$) as in *Tetragona*, *Duckeola*, etc. During $\bar{s}_r$ the queen sometimes visits the cell and taps the rotating worker, but the latter remains rather indifferent. Removal from the cell was never observed in the first half of $\bar{s}_r$ and only rarely in its later half, contrasting to some other species, e.g. *Melipona quadrifasciata*. Duration of $\bar{s}_r$ was measured (min-sec, each one case unless parenthetically mentioned): 1–08, 1–25, 1–26, 1–34, 1–41 ($\bar{s}_s$ finished by the removal caused by queen interference), 1–53, 1–54, 1–55, 2–03, 2–07

---

1) Detailed comparison is postponed until the oviposition behavior of D_y type genera, e.g. *Nannotrigona*, *Plebeia*, etc. is analysed.

(2), 2-15, 2-16, 2-17, 2-18, 2-38, 2-40 (2), 2-54, 3-02. (cf. Figs. 10∼12). Duration is more or less comparable to that in *Melipona* and many other genera, never so prolonged as in *Tetragona* and *Trigonisca*. Duration of total $\bar{s}$ (here $\bar{s}_r+\bar{s}_s$) was measured only in a few cases, adopting the closure of the cell orifice as the end of $\bar{s}_s$: 1-54+0-36, 1-36+0-54, 1-08+1-00, 2-17+0-50.

Total duration of oviposition process, here defined as $\bar{d}_d$ (discharge subphase) +PDP (postidscharge period)+$\bar{o}$ (oviposition phase)+$\bar{s}_r$, was measured only in a few cases as given in Table 6. The result cannot be compared precisely with those in other genera, but a remarkable variation ranging from 5 min to 1 hr is obvious, despite the integrated oviposition process (IOP) of *Meliponula* involves only one unit process (UPO). In *Melipona* with the same pattern (usually IOP=UOP, type B_s), total duration of IOP ($=\bar{r}+\bar{d}+\bar{o}+\bar{s}$) ranges approximately 4∼20 min, never exceeding 30 min, in spite of the occurrence of $\bar{r}$ (=predischarge waiting), which is absent in *Meliponula*. A marked variation range in *Meliponula* is mainly brought by $\bar{d}_d$ and PDP, during most part of which the queen is not by the cell. This curious feature, combined with a high variability in the duration of time since the appearance of worker excitement by the cell to the first food discharge, gives a rather erratic and "diffused" physiognomy to the temporal pattern of the oviposition process, sharply contrasting to that of other genera, which proceeds as a compact and condensed sequence under a high excitement.

Table 6. Duration of oviposition process in some accurately measured cases (min-sec. $\bar{d}_d$=discharge subphase, PDP=postdischarge period, $\bar{o}$=oviposition phase, $\bar{s}$=rotation subphase), arranged in the ascending order

Case No.	Fig.	$\bar{d}_d$	PDP	$\bar{o}$	$\bar{s}$	Total	
						net	excl. $\bar{d}_d$
12	9	1-09	1-54	0-11	2-17	5-31	4-22
14	8, 10	1-13	3-27	16	1-26	6-22	5-09
32	9, 10	1-32	3-14	11	1-41	6-41	5-09
4	8		3-00	6	2-38		5-44
2	8		4-46	9	1-08		6-03
8	8		5-00	9	2-07		7-16
12″	8, 10	1-09	4-47	10	2-40	8-46	7-37
1	9	10-19	6-32	13	1-53	18-57	8-38
9	8, 11	2-20	6-29	11	2-57	11-57	9-37
13	8, 11	1-14	6-56	11	2-54	11-15	10-01
3	9		9-45	8	2-07		12-00
10	8		14-34	12	2-40		17-26
22	7, 8, G	7-52	17-46	7	1-25	27-10	19-18
16	9, 10	5-21	19-40	9	2-16	26-26	23-05
23	7, 8, I	0-44	58-24	12	2-15	61-35	60-51
18	9, 12	2-57	61-16	20	1-35	66-28	63-31

3. Ethological peculiarities of *Meliponula*

The peculiar oviposition behavior of *Meliponula* is compared below with that in other genera so far well studied.

3. 1. *Ethological description of* Meliponula

The oviposition process proceeds as in other genera with a sequence of some phases. Further the following characters are shared with all other genera: 1) Cells are built through successive activities of young comb workers, not as the continuous work of a single individual as in bumblebees. 2) A completed cell attracts some workers, which alternately repeat body insertions in the cell with an excited manner. 3) Worker escapes from the cell after their own food discharges. 4) Queen eggs are laid vertically on larval food.

The responses of workers to the queen also show some patterns common to other genera: 1) Avoidance by workers of the approaching queen in a more exaggerated manner than in honeybees. 2) Gradual formation of royal court around the resting queen. 3) Repetition of a rapid pushing followed by a retreat by each attendant in front of the queen, involving occasional darting. 4) Absence of licking the queen by workers and rarity of food delivery by workers to the queen.

The following features are unique in *Meliponula*, not shared with any other genera (p=more careful comparisons required): 1) Constant and violent shaking of antennae by the queen (only shared with *Axestotrigona*, another African genus, the behavior studies of which are yet not well analysed). 2) Excess cruising by the queen. 3) Frequent "turning against" performed by workers after darting the queen. 4)^{p} Exaggerated rocking movement by workers encircling a completed cell before its provisioning. 5) Food discharges in a cell released without queen waiting (=predischarge waiting phass $\bar{r}$ absent). 6) Food discharges in a cell proceeding without queen waiting. 7) Food discharges in a cell sometimes made quite discontinuously. 8)^{p} Body trembling by the waiting queen. 9)^{p} Inconspicuous metasomal contraction at food discharge. 10) Prolonged body insertion at each food discharge. 11) Obligatory and prolonged postdischarge period with intermittent queen waitings.

Among these features, 1, 3, 4, 8, 9, and 10 are rather "localized", not much affecting the whole sequence of oviposition process. On the other hand, the other features contribute, through their combination, to the realization of a temporal sequence of the process being unique among all other genera observed.

Next, some behavioral features shared with some genera so far ethologically described but not with some others, are enumerated, using the following abbreviations: *Scaptotrigona* (S), *Melipona* (M), *Cephalotrigona* (C), *Leurotrigona* (L), *Tetragona* (Tt), *Duckeola* (D), *Friesella* (F), *Trigonisca* (TS), "all other genera" (X). The following symbols are also used: Similar to ($\fallingdotseq$), different from ($\neq$), partly (t), requiring closer comparisons (p).

Nest architecture: 1) Cells incompletely combed (≒F, D), neither clustered (ǂL, Ts), nor completely combed (ǂS, M, C, Tt). 2) Involucrum present (ǂD, F, L, Ts), though facultatively (ǂX). 3) Differentiation of honey and pollen pots absent (≒X). 4) Queen cells larger than worker cells (ǂM; ≒X, not directly verified but very probable in D, C, Ts). 5) Cells and pots prepared with abundant admixture of resin (ǂL, Ts; ≒X).

Worker behavior: 6) Waste materials carried away on the wing, not thrown down from nest entrance (ǂL, Ts; ≒X).

Queen behavior and queen-worker coactions: 7) Queen tending to rest at definite places (≒L, ǂX^{p}). 8) Queen walking with moderate speed, not too slowly (ǂD, S; ≒X). 9) Rhythmic wing movement of queen present (ǂM^{t}; ≒X) and not rare (ǂTs); consisting of single strokes, not of vibration (≒C, M^{t}, S; ǂD, F, L, Tt). 10) Queen not taking prolonged geopositive resting (ǂD, ≒X). 11) Worker responses to queen out of oviposition process somewhat ritualized (≒M, S; ǂX). 12) Queen not exhibiting actual or ritualized dominance (ǂM, ≒X).

Cell construction: 13) Successive, type $\mathrm{S_c}$ (≒C, M, S, Tt), neither synchronous $\mathrm{S_y}$, ǂL) nor semisynchronous ($\mathrm{S_m}$, ǂD, F, Ts), performed without excitement (ǂL; ≒X).

Oviposition behavior: 15) Ovipositions basically singular ($\mathrm{B_s}$, ≒M^{t}, Ts^{p}), *rarely* loosely batched ($\mathrm{B_f}$) (≒M^{t}, ǂC, Tt, M^{t}), never exclusively batched ($\mathrm{B_e}$, ǂD, F, S, L). 16) Food discharges in cells successive, ($\mathrm{D_s}$), neither synchronous ($\mathrm{D_y}$) nor semisynchronous ($\mathrm{D_m}$) (ǂS; ≒X). 17) Queen more cruising over comb (*C*) than waiting by cell (*W*), i.e. type $\mathrm{C_w}$ (C>W), neither cW (ǂF, M, Ts^{p}), CW′ (ǂC, D, Tt), nor CW (ǂL). 18) Excitement arousal localized (L), not generalized (G) (ǂS; ≒X). 19) Worker excitement before food discharge conspicuous (ǂD^{p}, F; =X). 20) Queen cell inspection before food discharge frequent (≒S, M, ǂF, L, Ts), but never rhythmically repeated (ǂC, D, Tt). 21) Queen violently tapping workers attending cells (≒F, M, Ts, ǂC, D, Tt, S). 22) Before food discharge worker behavior simple, without overt or ritualized responses (≒C, D, M, L, Tt; ǂF, S, Ts). 23) Worker body insertions before food discharge frequent, not much replaced by intention insertions (ǂL, F; ≒X). 24) Number of food discharges per cell not too small (ǂL; ≒X). 25) Postdischarge escape distinct (ǂL) but slow (≒S, D, ǂX). 26) Worker oviposition during oviposition process present (ǂD, F, Ts; ≒X), eggs smaller than queen eggs and laid vertically on larval food, not on cell margin (≒M; ǂC, Tt, S^{t}). 27) Queen oviposition shorter than in M, but longer than in X, made without peculiar leg twitching (ǂD, ≒X). 28) Preoperculation subphase ($\bar{\mathrm{s}}_{\mathrm{p}}$) virtually absent (ǂ$\mathrm{M}^{\mathrm{t}}$, Tt; ≒X). 29) Operculation consisting of rotation and sidework subphases ($\bar{\mathrm{s}}_{\mathrm{r}}$, $\bar{\mathrm{s}}_{\mathrm{s}}$) (ǂF, ≒X). 30) $\bar{\mathrm{s}}_{\mathrm{r}}$ and $\bar{\mathrm{s}}_{\mathrm{s}}$ well separated, without intervention of transient subphase ($\bar{\mathrm{s}}_{\mathrm{t}}$) (≒L, M, S; ǂX). 31) Operculation not too prolonged (ǂTt, Ts; ≒X).

Further considerations of these items are postponed until ethological features of the genera not cited above are described. But some comments are given below on the peculiar temporal sequence of oviposition process in *Meliponula*.

3. 2. *Peculiarity of temporal sequence of oviposition process in* Meliponula

As given in **3. 1.**, the peculiar temporal sequence of oviposition process in *Meliponula* is caused by features 2, 5, 6, 7, 11 among those unique in this genus. The resulting temporal sequence is schematically compared with those of some other genera in Fig. 14, where the pattern in *Plebeia* was drawn based upon our

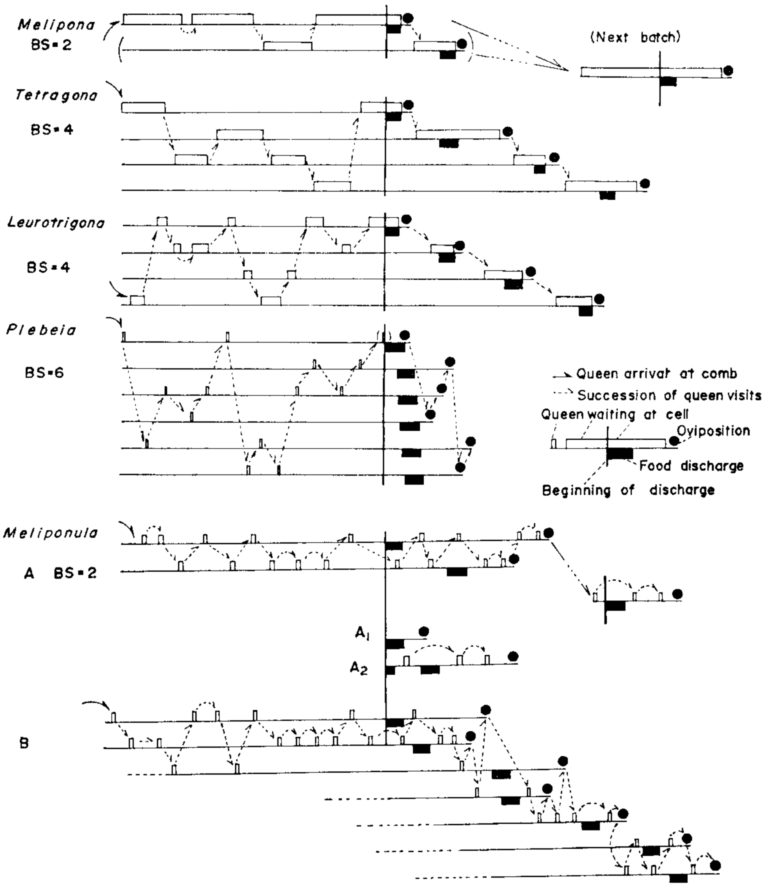

Fig. 14. Simplified temporal articulation of oviposition process in four genera. Batch size (BS) given arbitrarily. Actual size is: *Melipona* (usually 1 in many species, 2–4 in some species), *Tetragona* (usually more than 1, often more than 10, but batch often difficult to delimit), *Leurotrigona* (usually $5\sim6$, occasionally 10 or more), *Plebeia* (usually more than 10, not rarely more than 30), *Meliponula* (usually 1, but difficult to delimit). A. Actually observed case (BS=2). A_1. Simplest case. A_2. Case with discontinuous food discharge and postdischarge queen waitings. B. Imaginary case in a large colony.

unpublished data. As mentioned in introduction, colonies of *Meliponula* are generally large. An imaginary sequence in such a colony was shown at the bottom of the figure, inferred from the results obtained in the present study. Further the types of some behavioral characters in these genera are given below (Symbols cf. **3. 1.**).

Genus	Cell construction	Batch formation	Queen behavior on comb	Excitement arousal	Length of predischarge waiting queen	Food discharges in cells of the same batch
Melipona	S_c	$B_s(B_f)$	cW	L	long	D_s
Tetragona	S_c	B_f	cW′	L	moderate	D_s
Leurotrigona	S_m	B_e	CW	LG	short	D_s
Plebeia	S_y	B_e	C	G	instantaneous	D_y
Meliponula	S_c	$B_s(B_f)$	Cw	L	absent	D_s

From this synopsis and Fig. 14 we can see a gradual integration of oviposition process in the sequence of *Melipona→Tetragona→Leurotrigona→Plebeia*. This does not necessarily mean a phyletic line nor a monotonous evolutionary ascendence, because parallel evolution is always possible and a non-integrated type can be either pre-integrated or dis-integrated. Anyhow, *Meliponula* is quite aberrant from this trend. It is clearly non-integrated concerning the elements mainly determined by workers, whereas the queen does not linked with particular cells.. By her incessant cruising with only brief visits with cells, the whole process goes with a rather amorphous appearance (cf. **2. 5.**), making application of phase succession developed by Sakagami and Zucchi (1974) difficult, or virtually meaningless. At the present it is difficult to discuss how such aberrant sequence evolved. Some suggestions are given in Sections **4** and **5**.

4. Some evolutionary comments on the oviposition behavior of stingless bees

In a previous paper (Sakagami and Zucchi 1974) we gave a preliminary sketch of our hypothesis on the origin of complicated oviposition process in stingless bees, assuming that the process is conducted by a conflict in workers between "flee away the queen" and defense of the cell against her. Subsequent comparisons of various data revealed that the hypothesis is not compatible with some observed facts, including those obtained in *Meliponula*. The final solution will be possible only after closer comparative studies with experimental analyses. A revised interpretation is given below as a tentative to promote further studies. Before dealing with it, however, some general accounts on the behavioral relations between queen and workers must be preceded.

4. 1. *Behavioral relations between queen and workers in social hymenopterans*

In socially less advanced groups, behavioral control of workers by queens seems frequent. Since the confirmation of social dominance in *Polistes* wasps by

Pardi (1942～'48), the same phenomenon has been discovered in many social wasps, e.g. *Polistes* (West Eberhard 1969), *Parischnogaster* (Yoshikawa et al. 1969), *Belonogaster* (Piccioli and Pardi 1970), *Mischocyttarus* (Jeanne 1972, also by us, unpub.), *Ropalidia* and *Parapolybia* (Yamane unpub.). In all these cases dominant and submissive behavior patterns are clear. Food is more frequently transmitted from low-ranked individuals to higher ones, and the queen behaves, in normal situation, as the top-ranked individual. Similar dominace by the queen also exists in bumblebees, but here the behavioral expression is less overt. It does not relate with food transmission, which is in bumblebees practically absent even among workers. And the queen of a normal worker producing colony only rarely expresses her dominance with behavior, which is rather recognized by a unilateral avoidance of the queen by workers (Sakagami and Zucchi 1969) or by the appearance of aggressiveness in the colonies the queens of which are senescent or died (Free 1955).

In socially more advanced groups behavioral control by the queen gives way to chemical or pheromonal control, as is firmly proven in the European honeybee (Butler 1973). Here the queen does not show any overt behavioral dominance. Such would certainly be not only unnecessary but also difficult by her morphoethological specialization developed in parallel with pheromonal control1). However, the transition from behavioral to pheromonal control needs not necessarily be abrupt. Two mechanisms could coexist in the same group. Röseler (1970, cf. also Röseler and Röseler 1974) reported the occurrence of pheromonal inhibition of queen production by the active queen in *Bombus* (*Bombus*) *terrestris* (Linné) but its absence in *B.* (*Pyrobombus*) *hypnorum* (Linné). A mild dominance relation described by Montagner (1966) in *Vespula* (*Paravespula*) *germanica* (Fabricius) and *V.* (*P.*) *vulgaris* (Linné) and the formation of "royal court" with eager licking of the queen by workers of *Vespa orientalis* Fabricius (Ishay and Schwarz 1965), followed by the discovery of an attractant phromone by Ikan et al. (1969), suggest that both types of control are found in the subfamily Vespinae, a summit of vespid social evolution, necessitating a closer interspecific comparison of queen-worker relations in this group.2)

Even in the honeybee with a highly efficient pheromonal control, reminescence of behavioral control is suggested by mortal fight between two queens, appearance of agressive behavior in dequeened nuclei (Sakagami 1954) and by the very nature of "royal court."

---

1) Trivers and Hare (1976) developed an interesting hypothesis on the queen-worker asymmetry in aggressive encounters. But they do not refer to the change to pheromonal control, which lowers aggression by the queen, nevertheless assures the achievement of "the queen's advantage in competition over male-producing workers" predicted by them more efficiently.

2) In another summit, the polygynic Neotropical genus *Stelopolybia* with large colonies, queen-queen and queen-worker relations are characterized by behavioral indifference (Zucchi and Simões, unpub.).

Observing a normal nucleus of the honeybee kept in an observation hive, one can soon perceive that workers in front of the walking queen calmly give her the way. It can also be recognized that a resting queen is encircled by attendants. They frequently touch her with antennae and occasionally lick her, mainly in the metasoma. It is considered that inhibiting pheromone is transmitted to workers through this licking. But if the pheromone is simply attractive to workers, why they do not congest around the queen, forming a ball ? Such balling is indeed observed but not in the normal situation, where the queen and workers are spaced with a shrot distance, resulting in the formation of a beautiful rosette around the queen. A closer observation soon reveals that this distance is kept by rhythmic movements by each worker. Every worker pushes forwards, touches the queen with extended antennae, then suddenly retreats backwards. It is open to question whether this behavior is somewhat comparable to differential reaction exhibited by a *Paramaecium* caught within ring gradient around a CO_2 bubble. But irrespective of its relation to the strength of chemical stimuli, it could also be interpreted in terms of behavior, when we admit a gradual transition from behavioral to chemical control and a compatibility between these two mechanisms. At the present it is difficult to judge whether "pushing forwards" is entirely released by attractive chemical stimuli emitted by the queen, or if it involves a reminescent aggression. On the other hand, "retreating backwards" is, together with afore-mentioned "giving the way", certainly an avoidance response. It could be an avoidance from an excessive chemical stimulus, but it could *simultaneously* be an evolutionary reminescence of a submissive behavior. Keeping this assumption in mind, queen-worker relations in stingless bees are considered below.

4. 2. *Queen-worker coactions in stingless bees*

First an extreme ethological diversity of stingless bees must be stressed as shown by our serial reports. Nevertheless, there exist some traits which are common to most, if not all genera and different from other social hymenopterans.

The queen does not exhibit overt dominant behavior. Her dominance is expressed by eager solicitation of food to workers, when this can be regarded as a dominant behavior (cf. **4. 1.**) or its reminescence (cf. Michener 1974, p. 185). In some group (*Plebeia* s. str., *Hypotrigona* s. str.), solicitation is so violent that it bears an appearance of extortion, where the queen holds the worker down. Another type of dominance is found in all so far observed species of *Melipona*, and only in this genus, in a ritualized manner. The queen gently touches the head of a crouching worker, the behavior patterns of which specifically varies in both dominants and subordinates. A third case is agonistic desposition developing in virgin queens of some genera. But the peculiarity of stingless bees is more conspicuous in the responses of workers to the queen, as enumerated below together with derived inferences.

1) Queen approach invariably evokes escape by workers. This is usually expressed by a hurried run away or even "hiding themselves" between or below

combs, in an exaggerated manner to a degree never found in other social insects (cf. **4. 1.**). This indicates the occurrence of a high drive "flee away the queen" (F̂) in workers, which is probably stronger than in other social insects, in particular, the honeybee.[1]

2) In spite of such avoidance, a royal court is formed around the resting queen. This is a curious paradox: Workers are attracted by the queen, nevertheless they avoid her. It is possible that this gathering around the queen is itself a mild or modified expression of aggressiveness. It is open to question whether the same can be said as to the royal court in the honeybee. Anyhow, it is certain that this attractivness is in both groups not so strong as to release the following response of workers to the leaving queen.

3) Licking the queen by workers has never been observed. This suggests that circulation of pheromone or pheromones in the colony, the presence of which is probable from various anomalies observed in dequeened colonies, is not achieved by direct oral transmission.

4) Workers participating in royal court repeat fore- and backward movements as in the honeybee. But both are often more overt. Moreover, foreward movement is occasionally replaced by darting the queen. The posture of darting, usually with mandibles widely opened, suggests its agonistic nature. Hence the occurrence of a drive "attack on the queen" (Â) is postulated.

5) Frequency and strength of darting is variable among genera, scarce and mild in *Leurotrigona*, *Friesella* and those belonging to *Trigona-Tetragona* complex (*Duckeola*, *Cephalotrigona*, *Tetragona*, etc.), whereas more frequent in some other genera, *Melipona*, *Meliponula*, *Scaptotrigona*, *Scaura*, *Schwarziana*, etc. In any genera, however, darting is never followed by an actual attack but always by a retreat. Moreover, darting seldom appears in workers who can run away freely, but predominantly in those "cornered" by the approaching queen, or "confined" in the royal court, though the queen does not express agonistic disposition. These observations suggest a general relation F̂>Â, in workers to the queen, which is also understood from the results given in **4. 1.**

6) In some genera, e.g. *Scaptotrigona*, a ritualized fanning appears in front of the queen. The behavior called "turning against" in *Meliponula* (**1. 3.**) seems also somewhat ritualized. Further some behavior patterns bear an appearance as if evoked through a conflict between F̂ and Â. For instance, the crouching behavior by *Melipona* workers mentioned above is often preceded by a mild darting, where the fore body is not raised but lowered at the finale of the response, followed by a ritualized dominance behavior by the queen. A curious behavior called "hypnotic turning" in *Plebeia* (cf. Sakagami, Camilo and Zucchi 1973) might be

---

1) In subsequent pages three drives are tentatively postulated to interpret food discharge by stingless bee workers: Food discharge (D̂), flee away the queen (F̂), and attack on the queen (Â). The utility and limit of drive concept were discussed by several authors, notably by Hinde (1966). At the present state of the study of oviposition behavior in stingless bess, applciation of drive concept seems useful to analyse the observed facts.

classified in the same category. On these responses our interpretation is yet premature and could be biased. But the occurrence of these peculiar behavior patterns shows, with darting itself, a peculiarity of stingless bees as to $\hat{A}$. Possibly $\hat{A}$ or its release has not simply been suppressed in parallel with the advance of social organization but has been modified peculiarly in this group.

7) In the honeybee, the royal court is formed distinctly around an inseminated queen, but is obscure or absent around a virigin queen. This may be caused partly but not entirely by higher locomotive activity of the virgin. In many genera of stingless bees the royal court is more distinct around a virgin queen, where workers often exhibit some aggressive disposition.

In *Leurotrigona*, the virgin queen is encircled by a compact and often "two storied" rosette of workers. This rosettle ultimately protects the virgin from the attack by the mother queen. Nevertheless, the responses between the virigin and attendants bear some agonsitic disposition (Terada 1974). In *Melipona quinquefasciata* Lepeletier and *Friesella* spacing between the virgin queen and workers is kept through an overt defensive behavior, e.g. strong beatings by the metasoma by the virigin. It is possible that prisoning of virigin queens within cerumen-made "pots" by some genera (Moure, Nogueira-Neto and Kerr 1956; Juliani 1962) is an expression of overt formation of royal court around a virgin queen.

8) In spite of food solicitation by the queen, food delivery by workers is rare in stingless bees. This strange phenomenon requires further observations. Some comments are given in **4. 6.**

Certainly more information is necessary to understand queen-worker relations in stingless bees. But the following generalization may be permissible from the facts so far known: In spite of their highly evolved social organization, stingless bees have not lost the behavioral expression of $\hat{F}$ and $\hat{A}$ to the queen. These features have survived or even rivived through some modifications, though the general relation ($\hat{F} > \hat{A}$) have been retained.

Here the relation $\hat{F} > \hat{A}$ shows only an average state established between two castes, ignoring possible momentary reversal ($\hat{F} < \hat{A}$) or equilibrium ($\hat{F} \fallingdotseq \hat{A}$). Further we are yet not in a position to determine the relative intensity of $\hat{F}$ and $\hat{A}$. It is postulated that a high $\hat{F}$ (or $\hat{A}$) generally relates with the lower threshold to release the behavior controlled by it. Experimental analyses is indispensable to separate and measure $\hat{F}$ and $\hat{A}$ and the thresholds of corresponding responses. The lack of such evidence inevitably makes the following inferences provisional.

4. 3. *Criticisms against our previous hypothesis on the mechanism of oviposition process*

First we summarize our previous hypothesis with some modification of terms (cf. foot note p. 677):

1) In parallel with cell growth, food discharge drive (henceforth $\hat{D}$) gradually increases, possibily stimulated by the presence of completed cells and in part by some internal conditions, including the crop filled with larval food. 2) But

actual food discharge (henceforth *D*) is not released unless reinforced by further arousal of excitement. Frequent body insertions (henceforth *D'*) is regarded as incipient *D*, unrealized by the lack of sufficient stimuli. 3) Arrival of the queen at the cell activates $\hat{F}$ and $\hat{A}$ in workers. $\hat{F}$ is expressed by replacement of *D'* by intention insertions and in extreme case by predischarge escape from the cell (*Friesella*). 4) Later the presence of the queen raises the defense of the cell by workers, which is controlled by $\hat{A}$, and decreases $\hat{F}$, as shown by gradual recovery of *D'* in C<W type genera (cf. **3.1.**, **2.2**) except *Friesella* and *Leurotrigona*. 5) In C>W type genera decrease of $\hat{F}$ is achieved by a generalized arousal of excitement instead of localized one. 6) In some genera $\hat{A}$ is expressed by aggressive responses of workers to the queen (*Scaptotrigona*, *Schwarziana*, *Scaura*, etc.). 7) Gradual prevalence of cell defense against flee away (higher $\hat{A}$ relative to $\hat{F}$) liberates *D*, which brings a momentary recovery of $\hat{F}$, shown by postdischarge escape (henceforth *E*) known in all genera. 8) The first *D* by an attending worker removes the final obstacle for *D* in others, resulting in a rapid succession of *D*, which is also known in all genera.

Main errors in this hypothesis were brought by an overestimation of aggressive behavior and a confusion of responses controlled by $\hat{A}$ and $\hat{D}$: 1) As aggressive responses to the queen by workers (henceforth *A*) were so violent in some genera, e.g. *Scaptotrigona* and *Schwarziana*, that the influence of $\hat{A}$ was prematurely generalized and applied to other genera, which seldom exhibit such overt responses. 2) Even in *Scaptotrigona* and *Schwarziana* gradual increase of $\hat{A}$ cannot be asserted. What distinct is only the realization of *D* after successive *A*. If *E* is inhibited by $\hat{D}$, general increase of $(\hat{F}>\hat{A})$ is possible but a selective increase of $\hat{A}$ cannot be concluded. Rather the opposite possibility, intermittent decrease of $\hat{A}$ through *A* is more likely. 3) If *D* is released by $(\hat{A}+\hat{D})>\hat{F}$ as in our hypothesis, we obtain $\hat{A}>(\hat{F}-\hat{D})$ and, at lim $\hat{D}\rightarrow 0$ by the release of *D*, $\hat{A}>\hat{F}$. Obviously the situation may be not so simple, but $\hat{A}$ must be fairly high relative to $\hat{F}$ by our previous inference. Nevertheless, *D* is nearly invariably followed by *E*, which is indubitably released by $\hat{F}$. 4) Realization of *E* under a high $\hat{A}$ would be possible only when *D* brings not only $\hat{D}\rightarrow 0$ but also $\hat{A}\rightarrow 0$. This is unlikely unless postulating an amalgamation of $\hat{A}$ and $\hat{D}$. It is difficult to assume that *D* is not controlled by its own drive $\hat{D}$, even though deeply affected by $\hat{F}$ and $\hat{A}$, because brood rearing is a firmly established habit in Aculeata. Of course, it is very unlikely to deny the autonomy of $\hat{A}$, one of the strongest drives in many animals. 5) An alternative possibility, *D* causes $\hat{D}\rightarrow 0$ and the latter secondarily decreases $\hat{A}$, is not impossible. But a selective decrease of $\hat{A}$ by $\hat{D}$ is less likely, even though $\hat{D}$ affects $(\hat{F}>\hat{A})$ (cf. 4.5.).

Therefore, we postulate that $\hat{A}$ is not sufficiently high relative to $\hat{F}$ soon after *D*. Further the following two criticisms are added to: 6) The terms generalized and localized arousals of excitement were used without appropriate explanations. These were used to mean the increase of $\hat{D}$ affected by $(\hat{F}, \hat{A})$. 7) The hypothesis cannot explain the occurrence of *D* without queen waiting *W*, and *E* soon after *D* without *W* in *Meliponula.* Certainly we deal with a highly complciated process. A revised working hypothesis is tentatively presented below.

4. 4. *A revised hypothesis*

It is obvious that completed cells affect $\hat{\mathrm{D}}$ of nurse age workers. It is also very probable that D' represents an intention movement of D judging from its posture, excitement accompanied with, and its temporal position in the context of oviposition process. Further, the appearance of D in *Meliponula* without W and some anomalous cases cited in **4. 5.** show that alternate repetition of D' gradually increases $\hat{\mathrm{D}}$ and reciprocal stimulation $\hat{\mathrm{D}} \leftrightarrow \hat{\mathrm{D}}\hat{\mathrm{D}}$ (=coaction of $\hat{\mathrm{D}}$ among cell attendants) "can" circumstancially release D without W. However, an unbearable delay of D in such anomalous cases (e.g. queenless colonies of *Leurotrigona*) suggests that D is usually not soon released unless accelerated by a *catalyst.* That the queen plays such role is certain from the behavioral sequence of oviposition process in queenright colonies of all genera except *Meliponula.*

Arrival of the queen at the cell may evoke a conflict of two drives, $\hat{\mathrm{F}}$ and $\hat{\mathrm{A}}$, where in general $\hat{\mathrm{F}} > \hat{\mathrm{A}}$ (cf. **4. 2.**). Or, more likely, $(\hat{\mathrm{F}} > \hat{\mathrm{A}})$ is already evoked through repeated D' to a certain level, and reinforced by queen arrival (cf. **4. 5.**). The relation $(\hat{\mathrm{F}} > \hat{\mathrm{A}})$ may release predischarge escape, as is occasionally observed in *Friesella.* But under the influence of ever increasing $\hat{\mathrm{D}}$, workers are forced to remain by the cell and subsequently governed by $\hat{\mathrm{D}}$, $\hat{\mathrm{F}}$, $\hat{\mathrm{A}}$, or, $(\hat{\mathrm{F}} > \hat{\mathrm{A}}) \leftrightarrow (\hat{\mathrm{D}}\upharpoonleft \rightarrow \hat{\mathrm{D}}\hat{\mathrm{D}}\upharpoonleft)$.

First we put some possibilities out of consideration, simply because we are yet not in the position to incorporate them in our hypothesis: 1) Some drives other than $\hat{\mathrm{D}}$, $\hat{\mathrm{F}}$ and $\hat{\mathrm{A}}$ might affect the process. 2) Absolute and relative levels of drives at queen arrival may be variable among genera. 3) Levels of these drives may vary during the process not necessarily *linearly and monotonously,* as suggested by partial predischarge E at queen arrival followed by re-gathering in *Friesella,* and suppression of D' followed by its recovery in some genera. Putting these possibilities aside, we postulate: 1) Linear change of drive levels. 2) Monotonous increase of $\hat{\mathrm{D}}$ during the process. 3) The same levels of $\hat{\mathrm{F}}$ and $\hat{\mathrm{A}}$ at queen arrival in various genera. 4) Monotonous increase or decrease of $\hat{\mathrm{F}}$ and $\hat{\mathrm{A}}$ from queen arrival till D. Under these limitations some simplified model situations were constructed (Fig. 15). We will examine which of these models are more compatible with actual behavioral sequence:

Models FA: $\hat{\mathrm{F}}/\hat{\mathrm{A}}$ constant. Selective increase or decrease of $\hat{\mathrm{F}}$ or $\hat{\mathrm{A}}$ is needless. The level of $\hat{\mathrm{F}}$ relative to $\hat{\mathrm{A}}$ is high enough to release E. Among three models, FA-I is most probable, because it can evoke $(\hat{\mathrm{F}} > \hat{\mathrm{A}})$ and consequently $(\hat{\mathrm{D}} \rightarrow \hat{\mathrm{D}}\hat{\mathrm{D}}) \rightarrow D$ simply by the presence of the queen. FA-II, i.e. increase of $\hat{\mathrm{D}}$ through a lasting effect of $(\hat{\mathrm{F}} > \hat{\mathrm{A}})$, which remains itself at the same level, is less probable. Such situation, even if occurs, will be counter-selected by it inefficiency. FA-III is least probable. Intervention of habituation is here unlikely.

Models F: Gradual increase of $\hat{\mathrm{F}}/\hat{\mathrm{A}}$. $\hat{\mathrm{F}}$ must selectively be reinforced against $\hat{\mathrm{A}}$. The relative level of $\hat{\mathrm{F}}$ after D is sufficient to release E. Here $\hat{\mathrm{F}}$ should be

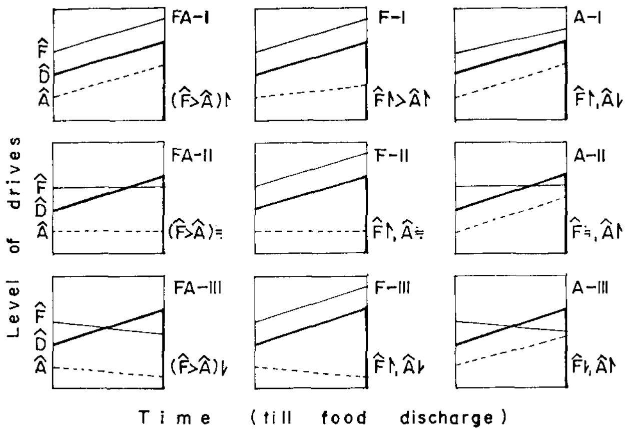

Fig. 15. Models showing the relation among three drives, "flee away the queen" (F̂), "attack on the queen" (Â) and "food discharge" (D̂). Level of D̂ given arbitrarily in relation to F̂ and Â, and assumed to reach 0 by food discharge.

adjusted to avoid the release of predischarge *E*. Weaker increase or decrease of Â before *D* might be brought by suppression by the queen or by intermittent release of *A* by workers. The first case is possibly represented by F-I and the second case by F-III.

Models A: Gradual decrease of F̂/Â. Â is selectively reinforced against F̂. The relative level of F̂ after *D* seems insufficient to release *E* so that all three models are not probable, especially A-III, which corresponds to our previous hypothesis.

Therefore Models FA-I and F-I~III are regarded as compatible with actual behavioral sequence, all assuming the influence of high F̂, which realizes *D* through (F̂>Â)↔(D̂↾→D̂D̂↾), or more simply the release of *D* is reinforced by F̂, not by Â as proposed previously. How the revised hypothesis fits to particular cases must be tested through further comparative studies and experimental analyses. Here are given some preliminary comments.

1) Localized and generalized arousals of excitement (L and G types, cf. Sakagami and Zucchi 1974): In L type genera activation of (D̂↾↔D̂D̂↾)→*D* by (F̂>Â) proceeds for each cell rather separately and the spread of higher D̂ throughout the comb is weak and slow. In G type genera (*Plebeia*, *Nannotrigona*, etc.) D̂↾ spreads quickly through the comb since the earlier stages of oviposition process.

2) *Scaptotrigona* and *Schwarziana*: These and some other genera are characterized by violent aggressive responses to the queen by workers until *D*. The precise considerations are postponed. Possibly here Â is high relative to F̂ and is gradually lowered during predischarge stages through intermittent release of *A* as represented by model F-III.

3) *Friesella*: Here F̂ seems high relative to Â, which results in frequent

predischarge *E*. Workers gradually return to the cell forced by a high $\hat{\mathrm{D}}$, but a high $\hat{\mathrm{F}}$, which is suggested by intention movement of *D*, i.e. unrealized *D*, makes $\hat{\mathrm{D}}\upharpoonright \rightarrow D \rightarrow E$ possible without exhibiting overt *A*. Further, violent tapping by the queen of cell attendants may contrubite to increase $\hat{\mathrm{F}}$.

4) *Melipona*, *Trigonisca*, *Tetragona* and allied genera. In these genera, the queen exhibits particular behavior by the cell, which probably reinfroces $\hat{\mathrm{D}}\upharpoonright$. But it is uncertain whether $\hat{\mathrm{D}}$ is brought by $(\hat{\mathrm{F}}>\hat{\mathrm{A}})\upharpoonright$ or $\hat{\mathrm{F}}\upharpoonright$ or $\hat{\mathrm{A}}\upharpoonright$. Darting is occasionally found in *Melipona* but rare in others. It is unknown whether $\hat{\mathrm{A}}$ is high but not emptied through *A*, or the level of $(\hat{\mathrm{F}}>\hat{\mathrm{A}})$ is low. Behavior coactions are summarized:

Melipona: Queen (Violent tapping of workers as in *Friesella*)/Worker (Frequent *D'*); *Trigonisca*: Queen (Violent tapping)/Worker (*D'* with ritualized "presentation"); *Tetragona*: Queen (Ritualized cell inspection and mild tapping)/worker (D' made alternately with queen cell inspection). It is impressive that this elaborate behavioral coaction between queen and workers is found in five genera belonging to *Trigona-Tetragona* complex nearly in the same manner (*Cephalotrigona*, *Trigona* s.str., *Tetragona*, *Friseomelitta*, *Duckeola*), probably indicating their phyletic affinity.

4. 5. *Some anomalies in postdischarge escape and an inquiry in the evolution of oviposition process in stingless bees*

Beside the instances presented above, there are some particular cases concerning the release of *E*. First, a comment is given as to the mechanism underlying *E*. We have so far interpreted *E* as released by a combined effect of high $\hat{\mathrm{F}}$ and minimized $\hat{\mathrm{D}}$. But isn't *E* released simply as an avoidance reaction to the odor of discharged larval food, which acts as a repellent? Certainly the role of such odor is not excluded. But *E* is released only in the discharger herself. In other workers, *D* by a worker releases a high excitement and subsequent *D*, probably caused by rapid increase of $\hat{\mathrm{D}}$. In G type genera, excitement spreads violently through the comb but none of participants exhibits escape reaction before their own *D*. Moreover, each cell is attended by a worker who usually stays by the cell with food till the arrival of the queen. These facts suggest that *E* is intimately linked with $\hat{\mathrm{D}}$, not simply representing an avoidance reaction controlled by a lower level mechanism.

Second, it must be mentioned that a marked reduction of $\hat{\mathrm{D}}$ soon after *E* can recover within a relatively short time. This is suggested by the fact that some dischargers return to the comb or near the cell waited by the queen and attended by other workers (cf. Sakagami, Montenegro and Kerr 1965, Fig. 3, H).

Judging from distinct metasomal contraction at food discharge, the discharger seems to empty most if not all of her crop content. Her renewed participation in the excitement on the comb implies some yet untested possibilities, e.g. 1) Such worker returns to the comb after filling up her crop with food. 2) Crop condition is an important, but not exclusive factor to determine $\hat{\mathrm{D}}$. 3) Renewed participation is governed by drives other than $\hat{\mathrm{D}}$. 4) Drive constellation in each participant changes in the course of oviposition process, etc.

Third, some anomalies in *E* are enumerated and commented.

1) Slow or inconspicuous *E*: *E* is slow in *Melipona*, *Meliponula* and *Duckeola* and rather inconspicuous in *Leurotrigona*. In three first genera, slow *E* might reflect merely their general motor pattern. This is especially likely in *Duckeola*, possessing a sluggish disposition. On the other hand, inconspicuous *E* in *Leurotrigona* is difficult to explain. This genus seems to have a high $\hat{F}$ relative to $\hat{A}$ as in *Friesella*, suggested by frequent appearance of unrealized *D′* in front of the queen. But its $\hat{D}$ seems much higher than in other genera. This is indicated by excited trembling developed already during, not after, cell building. Further, nurse age workers have so swollen crops that most cells are filled with food by only two *D*.

2) *E* released without *W*: In the following cases, *E* is released without queen waiting by the cell.

2a) At *DD* in the next and subsequent cells of a batch of G type genera. *D* in the first cell evokes a high excitement and *DD* are released at other cells not waited by the queen. Here $\hat{D}$ may be reinforced first by $(\hat{F}>\hat{A})$ in the presence of the queen, but later $(\hat{F}>\hat{A})\leftrightarrow(\hat{D}\upharpoonleft\rightarrow\hat{D}\hat{D})$ may raise $\hat{D}$ and $\hat{F}$ without the queen nearby.

2b) *D* in a queenless colony of *Partamona testacea*. Laying workers appeared in the colony. The oviposition process was markedly disorganized but postdischarge *E* was distinct, each made by a jump or by a short flight, just as observed in a queenright colony of a congeneric species, *P. cupira*. Unfortunately, our observations with queenless colonies of other species are yet poor. We overlooked to observe *E* in a queenless colony of *Cephalotrigona*. In *Leurotrigona* *E* was absent in queenless colonies. But in this genus *E* is inconspicuous even in queenright colonies (cf. above) and oviposition process in queenless colonies was much more organized than in other genera.

2c) *D* in pollen pots: Another curious fact is *D* made in pollen pots, observed in *Scaptotrigona tubiba* and more frequently in *Melipona marginata*. Occasionally the orifice of a pollen pot is vertically elongated, becoming neatly circular with a knife-edged margin just as in a completed cell of the same diameter. After endless repetition of *D′* by several workers, one of them discharges larval food in it and escapes from the pot. This performance, *D* and *E*, is followed by other workers. Although this curious behavior is observed in queenright colonies the queen seldom visits the pots. This reveals also the importance of a neatly circular orifice with a knife-edged margin to release *D′*.

2d) *D* in *Meliponula*. In this case it is possible that $(\hat{F}>\hat{A})$ affects $\hat{D}$ by occasional visits of the queen with the cell. But as mentioned in **2.3.** and **2.4.** *W* is infrequent and time spent by it is too brief to assume its effective control upon $\hat{D}$.

The cases mentioned above imply in combination the presence of occasional release of *D* not preceded by *W*, i.e. not reinforced by the actual presence of the queen. However, the appearance of *E* after *D* in these instances shows a high $\hat{F}$

soon after D, again indicating the relation between $(\hat{F}>\hat{A})$ and $\hat{D}$ being not unilateral but reciprocal, i.e. $(\hat{F}>\hat{A})\leftrightarrow\hat{D}$, which is masked in normal oviposition process of all genera except *Meliponula*. If this assumption is correct, a curious reciprocal relation between $(\hat{F}>\hat{A})$ and $\hat{D}$ cannot be explained by the behavior sequence in the colonies of recent groups alone, necessitating an inquiry into its evolution. We know well that our present knowledge is too limited to trace the past of stingless bee behavior. But it may be permitted to have a conjecture for further exploration, provided its incompleteness is kept in mind.

We imagine a following stage in the evolution of proto-meliponines. They had already formed a small colony. They adhered to mass provisioning but already produced caste differentiation. The monogynic queen controlled workers by behavioral dominance. Under these conditions, workers built the cells, filled them with food and the queen laid in them. This sequence proceeded without much friction, unless oviposition rate was low. The sequence could go still smoothly with an increased oviposition rate, if they switched to *progressive provisioning*, as practiced in all recent highly eusocial insects *except* stingless bees, because provisioning was made there *after* oviposition. But adhesion to mass provisioning and increased oviposition rate brought an unbalance of performance between queen and workers, resulting in waiting of the queen by the cell before being provisioned. Workers filling such cells with larval food confronted with behavioral dominance by the queen. This evoked a conflcit between $\hat{F}$ and $\hat{A}$ in workers. But $\hat{F}$ was obviously higher than $\hat{A}$, so that workers escaped from the cell after food provisioning, i.e. after being liberated from $\hat{D}$. This brought out a linkage of $\hat{D}$ and $(\hat{F}>\hat{A})$. The outcome was release of D only at a high level of $(\hat{F}>\hat{A})$ realized by W. But circumstantially D and E without W developed by an intimate linkage of $(\hat{F}>\hat{A})$ and $\hat{D}$. This sequence, occuring in recent genera only under anomalous situations, was adopted by *Meliponula* as normal pattern.

Probably the primordium of complicated oviposition behavior was established by the linkage of $\hat{D}$ and $(\hat{F}>\hat{A})$. Later, in parallel with change of behavioral to pheromonal control (cf. **4. 1.**), aggressive behavior by workers revived and was sometimes modified. The queen behavior also diversified. In some group, W was localized at particular cells while in others the queen tended to visit several cells successively. Two tendencies (L and G types) would have been reversible at earlier stages, but gradually took some definite patterns as represented by recent genera. Through this evolutionary course, some ethological features were retained in most if not all genera: Mass provisioning, excited D' in completed cells, queen waiting (anomalous in *Meliponula*), postdischarge escape E (inconspicuous in *Leutrotigona*), rapid succession of DD in a cell (occasionally irregular in *Meliponula*), cell operculation first by rotation, later by sidework (exception *Friesella*). If the assumptions given in this sketch are correct, we can infer that the origin of postdischarge E and mutual influence of $(\hat{F}>\hat{A})$ and $\hat{D}$ was very old, much earlier than elaboration of oviposition behavior in recent genera.

4. 6. *Food intake by stingless bee queens*

Some comments are added to food intake by the queen, which involves some unsolved problems. The presence or absence of food intake by the queen during oviposition process is tabulated below as to the genera ethologically analysed:

Genus	Food delivery to gravid queen by workers	Postdischarge delay	Intake of larval food	Intake of worker born eggs
Melipona	Very rare	Facultative, often long	+	+
Scaptotrigona	〃	Obligatory	+	+
Tetragona	Not confirmed	Facultative, often long	Not confirmed	+
Cephalotrigona	〃	Facultative, not well analysed	〃	+
Meliponula	Very rare	Virtually obligatory	+	+
Duckeola	Not confirmed	Virtually absent	〃	–
Leurotrigona	Very rare	Very rare	〃	–
Friesella	〃	〃	〃	–
Trigonisca	〃	〃	〃	–

The rarity of food delivery to the queen by workers must be pointed out. Possibly this depends in part on the lack of sufficient data. By concentration of our efforts on the oviposition process, we have made few continuous observations of queen behavior. It is likely that the queen receives much food than assumed from the second column of the table. We and our students observed direct food delivery in *Melipona quadrifaciata*, *M. subnitida*, *M. quinquefasciata*, *M. marginata* and *Paratrigona lineata* in other occasions. It is also not impossible that in some genera the queen is fed when she rests below the combs, hidden from the observers. However, it is certain that the frequency of food delivery is incomparably lower than in honeybees. This is curious because food regurgitation among workers is frequent but workers rarely give the food to the queen in spite of her eager solicitation. In short, the solicitation-delivery behavioral system is well equipped but actual devliery is rare. Apparently in some genera this is intimately linked with food intake by the queen during oviposition process. But the table shows the absence of observations of food intake in other genera during the process. Closer observations are required for these genera. As far as the results in the table concern, food intake during the process is confined to the genera with worker oviposition during the process. This is valid for most but not all observed genera yet not well analysed. Concerning the genera with food intake during the process, it is noteworthy that all of them possess postdischarge delay, either obligatorily or facultatively. This implies the occurrence of this phase, or subphase $\bar{d}_p$, is intimately linked with food intake by the queen, even though actual sequence is often erratic and difficult to interpret (cf. **2. 4.**).

5. Evolutionary and phyletic position of *Meliponula*

Finally some comments are added to the evolutionary and phyletic position of *Meliponula*, with special reference to ethological characters.

Kerr and Lello (1962) and Kerr and Maule (1964) regarded *Meliponula* as primitive based upon three items of evidence: 1) Larval food viscous; 2) Poison sac of workers the largest among stingless bees, occupying about 25% of body weight (only 5% in *Apis*); 3) Morphological habitus closely resembling a fossil species, *Meliponorytes succini* Tosi. Wille (1963) considered the unusually large poison sac as a specialization rather than a primitive condition. He pointed out two other primitive conditions, metasomal ganglion III remaining in the metasoma and the sting least degenerated among stingless bees. On the other hand, he enumerated eleven characters showing specialized conditions and concluded that *Meliponula* is in general more primitive than *Melipona* but more specialized than any other stingless bee groups (=*Trigona* s. lat. in his usage).

Here, another certainly specialized condition is added to. In *Meliponula* the ovaries of workers are tightly fused one another, forming a complete ring around the ventriculus (Fig. 16). For this peculiarity, the ventriculus must be cut in order to exmaine the ovarian conditions. This fusion, already detected in the pupal stage, was never found in all other genera ovarially examined.

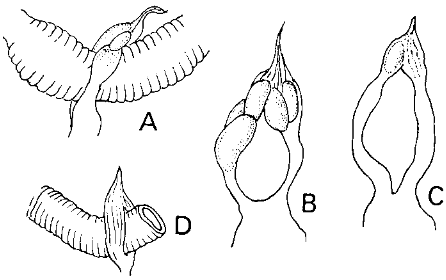

Fig. 16. Worker ovaries. A. Tightly fused, forming a ring surrounding ventriculus. B. Ditto, with some developing oocytes, ventriculus removed. C. Ditto, oocytes less developed. D. Ovaries of a pupa 36 days after hatching.

It is still premature to discuss the problem from the ethological point of view, as evolutionary and phyletic evaluations of ethological characters are yet not established. However, the available information seems to favor specialization than primitiveness of *Meliponula*. From the inference given in **4. 5.**, (F̂>Â)↔D̂ and *E* are regarded as of very old origin. The absence of *W* at *D* could be either primitive or specialized. But if primitive, it is difficult to explain the presence of *E* and (F̂>Â) in workers, the latter being inferred from avoidance of the queen, frequent darting and somewhat ritualized "turning against" (cf. **1. 3**). Aberrant temporal structure of oviposition process is also regarded as secondary. It seems basically non-integrated type or type L, judging from the features mainly determined by worker behavior. The curious oviposition sequence is possibly an outcome of a deviation appeared in queen behavior. Instead of adhering particular cells, the queen developed incessant cruising (**1. 2.**) accompanied with short *W*. This brought *D* without *W* and subsequently an unusually amorphous temporal pattern, being unique among all stingless bees. Although not in a strict sense, the behavior sequence of the queen resembles somewhat that of the queens of honeybees and monogynic social wasps.

Concerning the phyletic position, Kerr et al. (op. cit.) gave *Meliponula* the place closest to *Melipona* among all other stingless bees. Wille (1963) analysed further. He adopted four characters to judge the phyletic ramification (P=primitive condition, S=specialized condition): I. Small and sparsely hairly (P) or large and more densely hairy (S), II. Mesosomal portion of dorsal vessel straight (P) or arched (S), III. Metasomal ganglion 3 in metasoma (P) or in mesosoma (S), IV. Caste determined trophogenetically (P) or genotypically (S). These conditions are distributed:

Character	*Melipona*	*Meliponula*	Other groups
I, II	S	S	P
III, IV	S	P	P

Wille concluded that *Melipona* and *Meliponula* separated from other groups with specialization in I and II. Later *Melipona* specialized in III and IV1), but *Meliponula* retained primitive conditions in these characters.

Ethological information is yet ineffective to judge the phyletic ramification. There is no definite ethological features shared by *Melipona* and *Meliponula* alone. Singular oviposition (type B_s=rarity of batch formation) could be one of such. But this feature can take different conditions according to colony state. In weak colonies, type B_f may change to B_s (e.g. *Trigonisca*). On the other hand, type B_f might appear in large colonies of *Meliponula* (Fig. 14). Some prolific species of *Melipona*, e.g. *M. rufiventris*, show B_f than B_s. Even if a strong tendency to B_s was confirmed in *Melipona* and *Meliponula*, it does not always favor their phyletic affinity, because B_s could be either pre- or distintegrated condition (cf. 3.2.), just as in case of clustered and combed cell arrangements (Michener 1961, Wille 1969). At the present it is premature to determine the phyletic relation of two genera from this character. Another character condition, worker eggs laid vertically on the larval food, not on cell margin, was first regarded as common to *Meliponula* and *Melipona* alone. But later this condition was found in some, presumably not much related genera, *Paratrigona, Celetrigona* and *Scaura,* suggesting its parallel evolution or prevalence in the past.

What is ethologically certain is the isolated position of *Meliponula* within stingless bees. The number of character conditions unique in this genus is probably

---

1) An alternate opinion supporting trophogenic caste determination in *Melipona* was recently presented by Darchan and Delage-Darchan (1975).

highest among stingless bees. Certainly some of them (cf. **3. 1.**) evolved intimately linked one another rather than independently. Nevertheless, they prove as a whole the singularity of this genus as concluded by Wille (1963), who regarded it as an independent monobasic genus, in spite of his lumping tendency (cf. also Wille and Michener 1973) against the splitting system by Moure (1951, 1961).

Summary

The present paper analyses the oviposition behavior of *Meliponula bocandei* (Spinola), an aberrant African stingless bee. The most important features disclosed are: Constant and violent antennal shaking by the queen, excess cruising by the queen, frequent "turning against" by workers after darting the queen, food discharges released without queen waiting, food discharges proceeding without queen waiting and sometimes performed quite discontinuously, and postdischarge escape by workers without queen waiting. The last three items are incompatible with our previous hypothesis on the mechanism of oviposition process in stingless bees. A revised hypothesis was presented, together with some discussions on the evolution of oviposition behavior in stingless bees.

Acknowledgements

The work was developed at Faculdade de Filosofia, Ciências e Letras de Rio Claro, Rio Claro, State of São Paulo, aided by grants from Fundacão de Amparo à Pesquisa do Estado de São Paulo, Conselho Nacional de Pesquisas and Campanha Nacional de Aperfeiçoamento de Pessoal de Nível Superior. In particular our thanks are due to Dr. Warwick E. Kerr for his generous allowance for the use of facilitites in Departamento de Biologia Geral, Rio Claro.

References

Alfonsus, E.R. 1932. The rocking movements of bees. J. econ. Entom. **25**: 815–820.

Araújo, V. de Portugal 1955. Notas sôbre colônias de meliponíeos de Ángola, África. Dusenia **6**: 97–114.

——— 1956. La culture des Melipones et son introduction en Europe. Rapp. presenté au XVI Congr. Internat. Apicult. 11 pp.

Beig, D. and Sh. F. Sakagami 1964. Behavior studies of the stingless bees, with special reference to the oviposition process. II. *Melipona seminigra merrillae* Cockerell. Annot. Zool. Japon. **37**: 112–119.

Butler, C.G. 1973. The queen and the sprit of the hive. President. Address Roy. Entom. Soc. London, Ann. Meet. 7 March 1973. Proc. 55–65.

Camilo-Atique, C. 1974. Variabilidade do comportamento de *Melipona rufiventris rufiventris* (Hymen., Apidae). Tese de Mestrado, 147 pp., Ribeirão Prêto.

Free, J.B. 1955. Behaviour of egg-laying workers of bumblebees. Brit. J. Anim. Behav. **3**: 147–153.

Hinde, R.A. 1966. Animal behaviour. A synthesis of ethology and comparative psychology, x+534 pp., MacGraw-Hill.

Ikan, R., Gottlieb, R., Bergman, E.D. and J. Ishay 1969. The pheromone of the queen of the Oriental hornet, *Vespa orientalis*. J. Insect Physiol. **15**: 1709–1712.

Ishay, J. and J. Schwarz 1965. On the nature of the sounds produced within the nest of the Oriental hornet, *Vespa orientalis* F. Insectes Sociaux **12**: 383–387.

Jeanne, R.L. 1972. Social biology of the Neotropical wasp *Mischocyttarus drewseni*. Bull. Mus. Comp. Zool. Harvard Univ. **144**: 63–150.

Juliani, L. 1962. O aprisionamento de rainhas virgens em colônias de Trigonini (Hymenoptera, Apoidea). Bol. Univ. Feder. Paraná, Zool. **20**: 1–11.

Kerr, W.E. and Lello, E. de 1962. Sting glands in stingless bees – a vestigeal character. J. N. York Entom. Soc. **70**: 190–214.

——— and Maule, V. 1964. Geographical distribution of stingless bees and its implications. *Ibid.* **72**: 2–17.

———, Sakagami, Sh. F., Zucchi, R., Araûjo, V. de Portugal e J.M.F. de Camargo 1966. Observações sôbre a arquitetura dos ninhos e comportamento de algumas espécies de abelhas sem ferrão das vizinhanças de Manaus, Amazônas (Hymenoptera, Apoidea). Atas Simp. Biota Amazônia **5**: 255–309.

Michener, C.D. 1961. Observations on the nests and behavior of *Trigona* in Australia and New Guinea (Hymenoptera, Apidae). Amer. Mus. Novit. **2026**: 1–46.

——— 1974. The social behavior of the bees. A comparative study, xii+404 pp., Harvard Univ. Press, Cambridge, Mass.

Montagner, H. 1966. Le mécanisme et les conséquences des comportements trophallactiques chez les guêpes du genre *Vespa*. Thèses, Faculté des Sci. Univ. Nancy, 143 pp.

Moure, J.S. 1951. Notas sôbre Meliponinae (Hymen.-Apoidea). Dusenia **2**: 25–70.

——— 1961. A preliminary supra-specific classification of the Old World meliponine bees (Hym., Apoidea). Studia Entom. **4**: 181–242.

———, Nogueira-Neto, P. and W.E. Kerr 1956. Evolutionary problems among Meliponinae (Hymen. Apidae). Proc. 10. Internat. Congr. Entom. Montreal **2**: 481–494.

Pardi, L. 1942. Ricerche sui Polistini. V. La poliginia iniziale di *Polistes gallicus* (L.). Bol. Ist. Entom. R. Univ. Bologna **14**: 1–106.

——— 1946. Ricerche sui Polistini. VII. La "dominazione" e il ciclo ovarico annuale in *Polistes gallicus* (L.). *Ibid.* **15**: 25–84.

——— 1948. Dominance order in *Polistes* wasps. Physiol. Zool. **21**: 1–13.

Piccioli, M.T.M. e L. Pardi 1970. Studi della biologia di *Belonogaster* (Hym. Vespidae). 1. Sull'etogramma di *Belonogaster griseus* (Fab.). Monit. Zool. Ital. **3**: 197–225.

Röseler, P.-F. 1970. Unterschiede in der Kastendetermination zwischen den Hummelarten *Bombus hypnorum* und *Bombus terrestris*. Zs. Naturforsch. **25**: 543–548.

——— und I. Röseler 1974. Morphologische und physiologische Differenzierung der Kasten bei den Hummelarten *Bombus hypnorum* (L.) und *Bombus terrestris* (L.). Zool. Jahrb. Physiol. **78**: 175–198.

Sakagami, Sh. F. 1953. Untersuchungen über die Arbeitsteilung in einem Zwergvolk der Honigbiene. Beiträge zur Biologie des Bienenvolkes, *Apis mellifera* L. I. Jap. J. Zool. **11**: 117–185.

——— 1954. Occurrence of an aggressive behaviour in queenless hives, with considerations on the social organization of honeybee. Insectes Sociaux **1**: 331–343.

——— 1966. Techniques for the observation of behavior and social organization of stingless bees by using a special hive. Pap. Avuls. Depto. Zool. Secret. Agric. São Paulo **19**: 151–162.

——— 1971. Ethosoziologischer Vergleich zwischen Honigbienen und stachellosen Bienen. Zs. Tierpsychol. **28**: 337–350.

——— 1975. Stingless bees (excl. *Tetragonula*) from the continental Southeast Asia in the collection of Bernice P. Bishop Museum, Honolulu (Hymenoptera, Apidae). J. Fac. Sci., Hokkaido Univ., VI, Zool. **20**: 49–76.

————, Beig, D. and Y. Akahira 1965. Behavior studies of the stingless bees, with special reference to the oviposition process. III. Appearance of laying workers in an orphan colony of *Partamona* (*Partamona*) *testacea testacea* (Klug). Jap. J. Ecol. **14**: 50–57.

————, ———— and C. Kyan 1964. Behavior studies of the stingless bees, with special reference to the oviposition process. IV. *Cephalotrigona femorata* (Smith). Kontyû **32**: 464–471.

————, ————, Zucchi, R. and Y. Akahira 1963. Occurrence of ovary developed workers in queenright colonies of stingless bees. Rev. Brasil. Biol. **23**: 115–129.

————, Camilo, C. and R. Zucchi, 1973. Oviposition behavior of a Brazilian stingless bee, *Plebeia* (*Friesella*) *schrottkyi*, with some remarks on the behavioral evolution in stingless bees. J. Fac. Sci., Hokkaido Univ., VI, Zool. **19**: 163–189.

————, Montenegro, M.J. and W.E. Kerr 1965. Behavior studies of the stingless bees, with special reference to the oviposition process. V. *Melipona quadrifasciata anthidioides* Lepeletier. *Ibid.* **15**: 578–607.

———— and Y. Oniki 1963. Behavior studies of the stingless bees, with special reference to the oviposition process. I. *Melipona compressipes manaosensis* Schwarz. *Ibid.*, **15**: 300–318.

———— and R. Zucchi 1963. The oviposition process in a stingless bee, *Trigona* (*Scaptotrigona*) *postica* Latreille. Studia Entom. **6**: 490–510.

———— und ———— 1965. Winterverhalten einer neotropischen Hummel, *Bombus atratus*, innerhalb des Beobachtungskastens. Ein Beitrag zur Biologie der Hummeln. J. Fac. Sci., Hokkaido Univ., VI, Zool. **15**: 712–762.

———— e ———— 1966. Estudo comparativo do comportamento de várias espécies de abelhas sem ferrão, com especial referências ao processo de aprovisionamento e postura das células. Ciência e Cultura **18**: 283–296.

———— and ———— 1967. Behavior studies of the stingless bees, with special reference to the oviposition process. VI. *Trigona* (*Tetragona*) *clavipes*. J. Fac. Sci., Hokkaido Univ., VI, Zool. **16**: 292–313.

———— and ———— 1968. Oviposition behavior of an Amazonic stingless bee, *Trigona* (*Duckeola*) *ghilianii*. *Ibid.* **16**: 564–581.

———— and ———— 1974. Oviposition behavior of two dwarf stingless bees, *Hypotrigona* (*Leurotrigona*) *muelleri* and *H.* (*Trigonisca*) *duckei*, with notes on the temporal articulation of oviposition process in stingless bees. *Ibid.* **19**: 361–421.

Terada, Y. 1974. Contribuicão ao estudo de regulacão social em *Leurotrigona muelleri* e *Frieseomelitta varia* (Hym., Apidae). Tese de Mestrado, 96 pp., Ribeirão Prêto.

Trivers, R.L. and H. Hare 1976. Haplodiploidy and the evolution of the social insects. Science **191**: 249–263.

West Eberhard, M.J. 1969. The social biology of polistine wasps. Mus. Zool. Univ. Michigan **140**: 1–101.

Wille, A. 1963. Phylogenetic significance of an unusual stingless bee, *Meliponula bocandei* (Spinola). Rev. Biol. Trop. **11**: 25–45.

———— 1969. A new species of stingless bee, *Trigona* (*Plebeia*) from Costa Rica, with descriptions of its general behavior and cluster-type nest. *Ibid.* **15**: 299–313.

———— and C.D. Michener 1973. The nest architecture of stingless bees with special reference to those of Costa Rica. *Ibid.* **21**: Suppl. 1, 278 pp.

Yoshikawa, K., Ohgushi, R. and Sh. F. Sakagami 1969. Preliminary report on entomology of the Osaka City University 5th Scientific Expedition to Southeast Asia 1966, with descriptions of two new genera of stenogasterine wasps by J. van der Vecht. Nature and Life S.E. Asia **6**: 153–201.