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Author(s)	SAKAGAMI, Shôichi F.; ZUCCHI, Ronaldo
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Behavior Studies of the Stingless Bees, with Special Reference to the Oviposition Process VI. *Trigona (Tetragona) clavipes.*[1)]

By

Shôichi F. Sakagami and **Ronaldo Zucchi**

Zoological Institute, Hokkaido University, Sapporo, Japan and Departamênto de Genética, Faculdade de Filosofia, Ciências e Letras de Ribeirão Prêto, Brazil

(With 6 Text-figures and 2 Tables)

In our previous papers, the interesting behavioral sequence of the proviisoning and oviposition process (*POP*) of stingless bees has been reported. A comparsion of various species as to this aspect was tentatively given (Sakagami & Zucchi, 1966) and more detailed descriptions of behavior in particular species were successively reported as follows (each form is hencefoth abbreviated as given parenthetically):

Nannotrigona (Scaptotrigona) postica (Latreille) (Sakagami & Zucchi, 1963;=*Scaptotrigona*), *Melipona compressipes manaosensis* Schwarz (Sakagami & Oniki, 1963;=*M. compressipes, or Melipona* when cited together with the two following forms), *M. seminigra merrillae* Cockerell (Beig & Sakagami, 1964;=*M. seminigra*), *M. quadrifasciata anthidioides* Lepeletier (Sakagami, Montenegro & Kerr, 1965;=*M. quadrifasciata*), *Cephalotrigona femorata* (Smith) (Sakagami, Beig & Kyan, 1964;=*Cephalotrigona*).

As the sixth report of our serial work on the aspect mentioned, the present paper deals with *Trigona (Tetragona) clavipes* (Fabricius), the type species of the subgenus *Tetragona*, either in the wide sense in Schwarz (1932, '48) or in the restricted usage in Moure (1961), whose system is followed in our serial work. Some general remarks on our serial work were already given in the first report (Sakagami & Oniki, 1963).

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1. Observations of non-oviposition phase

1.1. Nest structure: At the end of March, 1963, a hollow tree trunk containing a large nest of *T. clavipes* was kindly sent to us at Rio Claro by Dr. Paulo Nogueira-Neto from his fazenda in Usina Ester, near Cosmópolis, S.P. The nest, opened on April 9, showed an architecture common to the subgenus (in the definition by Moure, 1961), possessing successively arranged horizontal combs covered with involucrum which separates brood chamber and storage area, and no structural differentiation of pollen and honey pots. The remarkable feature is its enormous size. As seen in Fig. 1, the nest occupied a hollow nearly 1.5 m in length. The length of each area is approximately as follows: Upper batumen 10cm, upper storage area 27cm, brood chamber 35cm, lower storage area 70cm, lower batumen 5cm. Although not counted exactly, at least 20 brood combs were observed. Most storage pots were fully loaded. Probably the nest is an example of how certain stingless bee species can make huge colonies under favorable circumstances. The outermost sheets of involucrum were gradually hardened, being transitional to surrounding laminate batumen. The storage pots were mostly 3cm high and 2cm wide, but many were very large, reaching 5cm high and 3cm wide, though not as large as recorded by v. Ihering(1903)for *Melipona nigra* Lepeletier (55×48 mm).

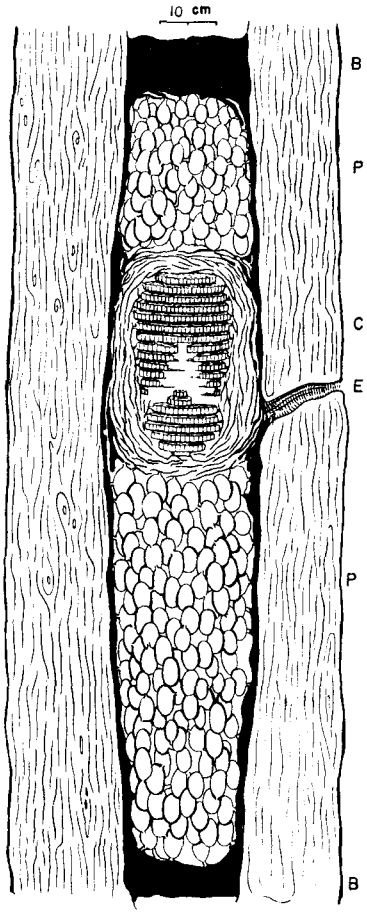

Fig. 1. Structure of the nest observed. Diameters of trunk and hollow and lengths of each area (B, Batumen; P, Storage pot area ; C, Brood chamber; E, Entrance) are reduced in the correct proportions, but minor architecture is given semischematically.

Only a few storage pots and combs were transferred into the observation hive (Sakagami, 1966) on the same day, together with the queen and a fraction of the original worker poupulation, which apparently exceeded several tens of thousands. The rest of the nest material was left outdoors. The interesting behavior of *Scaptotrigona* visiting these remains for collection of nest material was described elsewhere (Sakagami & Camargo, 1964). The colony was kept for a month in the hive but the following observations were mostly made on April 10 to 18.

1.2. Behavior of queen and workers in general: The queen is slightly larger

than workers in head and mesosomal widths. Her behavior in non-oviposition phase consists, as in other species, of alternate wandering and resting. Some places, as on old combs or between involucrum, are used for resting, but there are no definite preferences for particular places. When resting on a vertical wall, the queen takes a negative geotactic position. The speed at wandering is slower than in some species such as *Trigona* (*Geotrigona*) *mombuca* (Smith) or *T.* (*Tetragonisca*) *jaty* (Smith), but distinctly faster than in *T.* (*Duckeola*) *ghilianii* (Spinola), and does not markedly alter in the oviposition phases. Either in resting or in wandering, antennae are constantly shaken and wings are continuously vibrated. In contrast to the other species so far recorded, in which the queen either does not make wing movements (*M. quadrifasciata*) or rhythmically beats wings (all other species), the queen of *T. clavipes* is characterized by prolonged vibration of wings, lasting, for some measured intervals, 4.5 sec. (4 times), 5 (3), 5.5(2), 6(1). The duration does not markedly differ either while resting or while wandering. The pauses between these vibrating phases are usually momentary, in which the wings are rather asymmetrically folded.

No particular observations were made on worker and male behavior, except for the following notes: 1) Workers make frequent characteristic wing flutterings, either while resting or while wandering, opening the wings and soon closing them rapidly. The fluttering is often repeated in rapid succesion. 2) Workers ventilating in the entrace corridor direct the head toward the outside, the metasoma is slightly raised and the apex is bent downward. 3) Regurgitation between workers is frequently seen. In this occasion, both feeder and recipient, or at least one of them, slightly to distinctly twist their heads, as in other species. (This posture, together with the outward orientation during ventilation, is common to all stingless bees observed by us, so that henceforth it will not be mentioned in subsequent papers).4)Defecation by workers is made at several definite places within the hive. Excrement, at least of household workers, is semisolid, not fluid as in bumblebees (Sakagami & Zucchi, 1965, p. 719). These fecal deposits are, together with dead bodies and other waste material, accumulated near the entrance every night, on which invading phorid flies oviposit. But the deposits are daily removed by bees flying from the hive, so that no serious damage by phorid flies is caused, as long as the colony is not too weak. 5) As in other stingless bees, the full pigmentation of adults is considerably delayed following emergence. Approximately five stages are distinguished: A.Completely pale (Soon after emergence); B.Mesosoma yellowish, metasoma below with pale bands; C. Metasomal bands becoming darker; D. Mesosoma darkened, with yellow scutellum, metasomal bands blackish; and E. Completely colored. All foragers are in stage E. Workers participating in *POP* are mostly D, partly C. Workers acting as royal attendants are seen in all stages from B to E. 6) Males also emerge without full pigmentation. No participation of males in any task was noticed. Young males are tolerated by workers, but occasionally molested. Once a worker detached the metasoma of a living male with her mandibles.

The queen-worker relationship is common to that in the other species observed in the following points: 1) Gradual formation of a royal court when the queen stays quiet for a while (number of attendants usually 7–10, once 16); 2) Repeated rapid advances and retreats by attendants in front of the queen; 3) Escape by

workers at a sudden encounter with the approaching queen; and 4) Gradual retreat by workers which perceive the approaching queen. Besides these patterns basic to stingless bees, the intercaste behavior is relatively simple. No exaggerated or rather ritualized behavior pattern as in *Melipona* and *Scaptotrigona* was observed, resembling in this aspect *Cephalotrigona*. The queen often touches the workers with her antennae, and buccal contacts were occasionally seen. But no definite food delivery of workers to the queen was confirmed.

1.3. Cell construction:

Cell construction began on April 12, at night. There were 22 cells in which oviposition occurred and 6 in preparation at 8:30, April 13. On the same day, another comb, and on April 15, a third comb were started. But the rates of cell construction and oviposition decreased after April 14, since food deficiency and ingestion of contents of cells previously oviposited in increased in parallel, for instance, at 11:13, (28 cells ingested of 46 cells in total) in comb A, 45/117 in B and 1/53 in C. This trait, also known in the other species observed, seems to be an adjustment commonly taken by stingless bees during food deficiency. The colony was fed at 14:30, April 15. The rate of cell construction increased and ingestion of cell contents disappeared. But the former dropped again on April 18. No close observations were made thereafter, but fluctuations of cell construction and oviposition rates were repeatedly seen during one month.

Each cell is constructed by successive activities of a number of workers. The observations given for *M. quadrifasciata* can be applied here without much modification. The cells are succesively, not synchronously, built, so that there are cells in various stages of growth at a given time as follows:

Date	Hour	Stages						Total
		Started	1/4-built	1/2-built	3/4-built	4/4-built	Completed	
IV13	10:15	3	1	2	1	3	1	11
	11:25	1		3		2	1	7
IV17	9:58		1	14	1	3	4	23
	10:40	3			4	10	9	26
	15:05	2	8	7	3	7	2	29

(Values 1/4, 3/4, 4/4 mean the height of growing cell relative to that of egg-containing cell)

The difference between April 13 and 17 shows the effect of artificial feeding. At any rate, the number of cells before oviposition is far greater than in *Melipona*, in which the number rarely exceeds 4, even at the optimal colony condition (*M. quadrifasciata*). In *Tetragona*, as in *Cephalotrigona*, the number was 4 even at the peak of food shortage (April 15, 11:13) and usually more than 10.

The time required for the completion of one cell was measured for some examples (all on April 17):

Hour of start	Approximate duration since start (in min.)			
	1/2-built	3/4-4-built	Completed	Oviposited
14: 35	30		107	127
〃 〃	〃		〃	126
14: 10		35-40		71
〃 〃		35	50	73
〃 〃	35			64
〃 〃	〃	50	75	109
15: 00		25	70	75
〃 〃	25		〃	72

In general, the cell is completed, that is, the cell collar is formed, 1–1.5 hours, and oviposited in 1–2 hours after the beginning of construction. A remarkable trait in *T. clavipes*, the occasional fixation of the queen to a cell still without a complete collar is referred to later.

The combs taken from the original nest were mostly circular in contour. This seems to be conditioned by spatial limitations. Within the observation hive, where the growth of a single comb was not limited spatially, the workers tend to keep the growing margin of comb linear, resulting in a rather geometric contour (Fig. 3, left).

2. Observations of provisioning and oviposition

The previous papers revealed that the *POP* of stingless bees showed a definite behavioral sequence, consisting of the following phases: Queen fixation, worker provisioning, queen oviposition and worker operculation. Before describing the event after this order, the rhythm of *POP* in *T. clavipes* is commented.

2.1. Oviposition rhythm: The rhythm and pattern of *POP* are variable among stingless bees. In all *Melipona* so far studied, each *POP* is singular and discrete, that is, only one cell is provisioned and oviposited in at one time, and the interval between two *POP* is usually long, though occasionally two *POP* are observed during a short time, within 10 min., especially in *M. quadrifasciata*. In *Cephalotrigona*, a rapid succession of 2–3 ovipositions is rather the rule. In *Scaptotrigona*, 10 or more cells are provisioned and oviposited in with rapid succession, and in the later phase of this integral process, food discharges into several cells are synchronously performed by many workers. The *POP* of *T. clavipes* is closest to that of *Cephalotrigona*. The number of successively oviposited cells is often larger, approaching the formation of a batch as in *Scaptotrigona* though each cell is always successively, never synchronously, provisioned. This batch formation is greatly affected by colony conditions. In Fig. 2, the rates of oviposition during food deficiency (right) and after artificial feeding (left) are given. After feeding, not only did oviposition rate increase but also batch formation became more distinct. Probably the smaller size of batch observed in *Cephalotrigona* is partly an outcome of such colony conditions, because this Amazonian species was observed under the

unfavorable conditions of South Brazil. On the other hand, it cannot be generalized that unfavorable conditions always obscure batch formation. In *Scaptotrigona* and some other groups, food deficiency causes a longer interval between batches, but no marked decrease in the batch size.

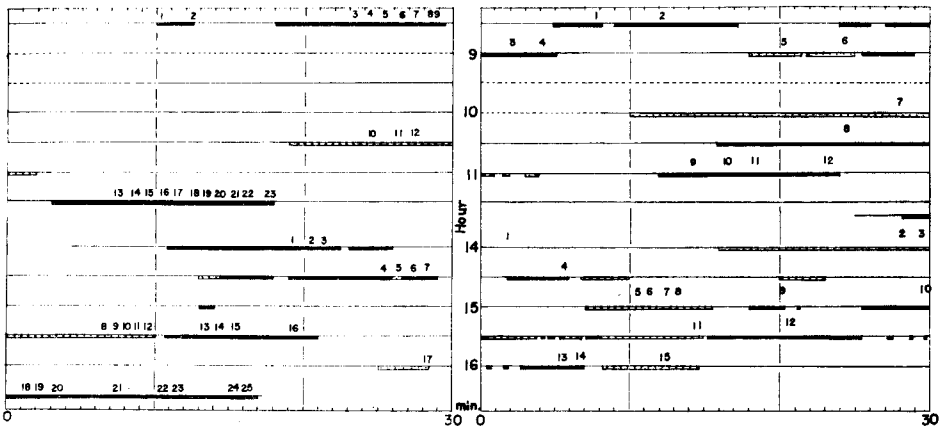

Fig. 2. Oviposition rate before (right, April 13) and after (left, April 17) feeding. Numerals show successive ovipositions (fore- and afternoon separately numbered). Black and dotted bars indicate the presence of the queen on two different combs. Broken lines indicate unobserved hours.

The oviposition rate per hour is given for several cases:

April 13,	a.m. 4.0,	p.m. 6.0.	Food deficiency
〃 16,		p.m. 14.6.	Soon after feeding
〃 17,	a.m. 6.9,	p.m. 8.3.	

Taking the rate on April 17 as a standard, so the rate is approximately 7–8 eggs per hour or 150–200 per day, which decreased during food deficiency to less than 100 (April 13, 8:30, 22 oviposited cells and April 15, 14:36, 216 ovipoisted cells. Hence, about 65 per day). Even under adverse condition in the artificial hive with a fraction of the original population, the oviposition rate is distinctly higher than in *Melipona*, whose so far known maximum rate does not exceed 50 per day. It is assumed that in the original population with several tens of thousands of workers, the rate exceeded 500 per day, probably with distinct batch formation.

The interval between two successive ovipositions fluctuates as follows:

Interval	<1	1	2	3	4	5	6	7	8	11-15	16-20	21-30	31-60 (in min.)
Cases obs.	6	36	10	6	8	1	3	1	1	2	4	3	8

The concentration of the intervals in the range 1–4 min. is caused by batch formation, and the wide fluctuation of the intervals from 5 to 60 min. shows an irregular oviposition rhythm not observed in *Scaptotrigona*. Because of this ir-

regularity, the size of batch is difficult to determine definitely. Two sets of frequency distributions are given below. In the first set, two successive oviposition made within 10 min. were regarded as belonging to the same batch. In the second set, this interval was extended to 30 min.

	Size of batch										
	1	2	3	4	5	6	7	8	9	10	11
10 min. cluster	5	6	2	1	3	2		2		1	
30 min. cluster	1	4	2	2		2		2		1	2

Fig. 3 shows the spatial distribution of successively oviposited cells on April 17, afternoon. The spatial order is apparently irregular. Occasionally the cells of different combs are successively oviposited.

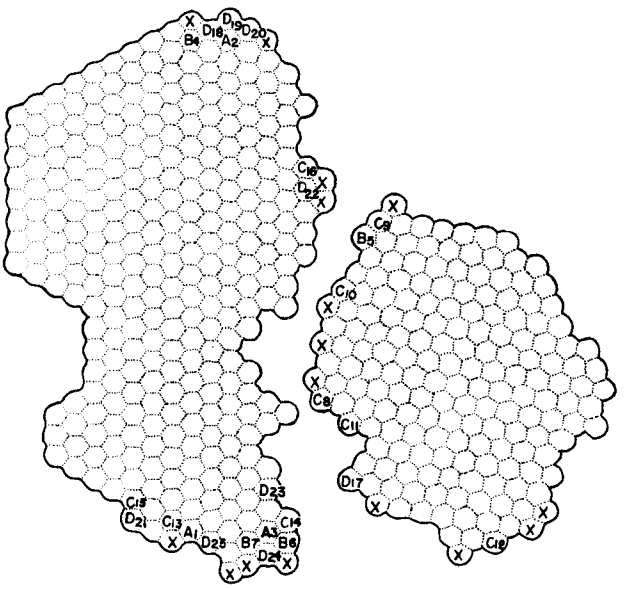

Fig. 3. Spatial distribution of cells successively oviposited in. $A_1 \sim A_3$, $B_4 \sim B_7$, $C_8 \sim C_{16}$ and $D_{17} \sim D_{25}$ represent succesive batches (Numerals are same as those in Fig. 2, left, afternoon) and X, the cells oviposited in during 16:50–18:00, probably consisting of two or three batches.

2.2. *Queen fixation*:

The *POP* begins at the arrival of the queen at the cell ready to be provisioned and is finished by the operculation of the egg containing cell. To facilitate understanding of the following descriptions, two continuous records, taking the queen behavior as the focus of observation and covering a number of *POP*, are given in Figs. 4 and 5, which show the temporal sequence of the process excluding the operculation phase.

Table 1. Some numerical data on primary fixation of the queen

Case Number	A	B	C	D	E	F	G	H	I	J
1	933	652	70.0	20	3.1	7(2)	4	73.6	61	14+13
2	822	470	57.1	83	17.7	27(9)	4	39.1	65	30+ 2
3	368	328	89.2	60	18.3	8	4	19.8	67	24
4	495	314	63.2	125	39.8	9	2	39.8	55	25+ 1
5	427	298	70.0	47	16.7	7	1		42	21
6	404	285	70.5	42	14.8	11(6)	5	?	52	13
7	275	270	98.5	270	100.0	1	1		50	29
8	355	266	75.0	27	10.2	4	2	90.5	57	20+ 7
9	244	244	65.5	2	1.2	6	3	22.5	28	12
10	239	195	81.4	1	0.5	4(1)	2	6.7	55	22+ 1
11	301	180	60.0	61	33.9	8	3	36.6	47	32+ 2
12	274	180	65.7	8	4.4	16(5)	5	40.0	63	18
13	143	143	100.0	66	46.2	3	1		26	15
14	363	137	37.7	1	0.7	5(2)	4	0.7	25	12
15	185	116	62.8	47	40.5	5	2	90.5	24	15+ 1
16	117	115	98.2	115	100.0	1	1		23	4+ 4
17	100	100	100.0	1	1.0	9(1)	3	1.0	28	5
18	105	90	86.0	40	44.5	2	2	44.5	14	5+ 1
19	86	86	99.1	15	17.7	2	1		17	12
20	110	85	77.3	85	100.0	2	2	100.0	23	12
21	183	63	34.5	60	95.3	5(2)	3	96.9	23	6
22	50	50	100.0	50	100.0	1	1		14	10
23	123	3	2.4	3	100.0	1	1		1	1

A. Total duration of fixation phase (ΣW+Σf+F) in sec.; B. Total duration. (Σf+F) in sec.; C. % ratio of B/A; D. Duration of final fixation (F) in sec.; E. % ratio of D/B; F. Number of fixations (short visits in parentheses); G. Number of cells fixed or visited; H. % Ratio of duration of fixation to the oviposited cells to total duration of fixation phase (A); I. Number of queen cell inspections. J. Number of worker body insertions (short insertions+ long (more than 5 sec.) insertions).

Queen fixation means a persistent wait by the queen at the side of a cell in which provisioning by workers later occurs. In *T. clavipes* ovipositions tend to be made in rapid succession, forming a batch. In such cases, fixation to the second and subsequent cells is often very short. We distinguish, therefore, *primary fixation* to the first cell oviposited in a batch and *secondary fixations* to the second and subsequent cells. The distinction of batches is arbitrarily defined by 10 min. clusters given in the preceding section. First the description deals with primary fixation.

When the queen resting outside of a comb is stimulated by increased laying motivation, she begins wandering (W) to and fro. Sooner or later she arrives at the comb. Immediately or after wandering on the comb, she fixes to one cell. But this incipient fixation (f) rarely develops into final fixation (F=fixation immediately before provisioning). Usually she abandons the cell, begins wandering or fixes to another cell. The alternation of wandering and fixing is seen in *Melipona*, too.

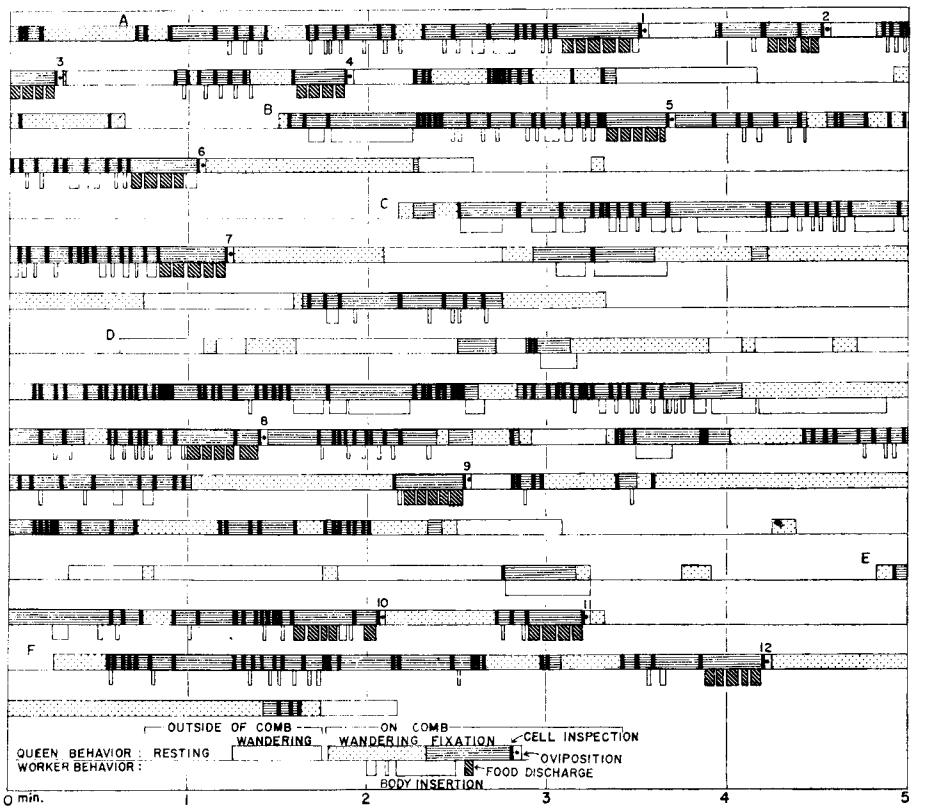

Fig. 4. Temproal sequence of provisioning and oviposition on April 13 (15:08-16:25, with 12 ovipositions). Numerals show the successive ovipositions and alphabets the series of successive ovipositions arbitrarily clustered for the sake of explanation. Short-term behavior lasting less than 1 sec. is rounded to 1 sec. Corresponding intervals between two immediately successive acts are not always exactly scaled.

But the repetition is very frequent in *T. clavipes* as in *Scaptotrigona* and probably *Cephalotrigona*, though no close observations were made in the latter. Therefore, following the start of wandering, the fixation phase occurs with diverse temporal patterns such as WF (very rare), WfWfWf, etc. Some instances are given in Figs. 4 and 5. In contrast to *Melipona*, and similar to *Scapto-* and *Cephalotrigona*, there are usually several cells ready to be provisioned at one time. Correspondingly the queen visits and fixes to different cells during primary fixation, for instance, $Wf_1\ Wf_2\ Wf_3\ Wf_2\ WF_1$, etc.

Table 1 gives some numerical data concerning primary fixation. The cases are arranged in descending order of the total time spent for fixation ($\Sigma f+F$, Column B). The total duration of primary fixation from the beginning of wandering to the

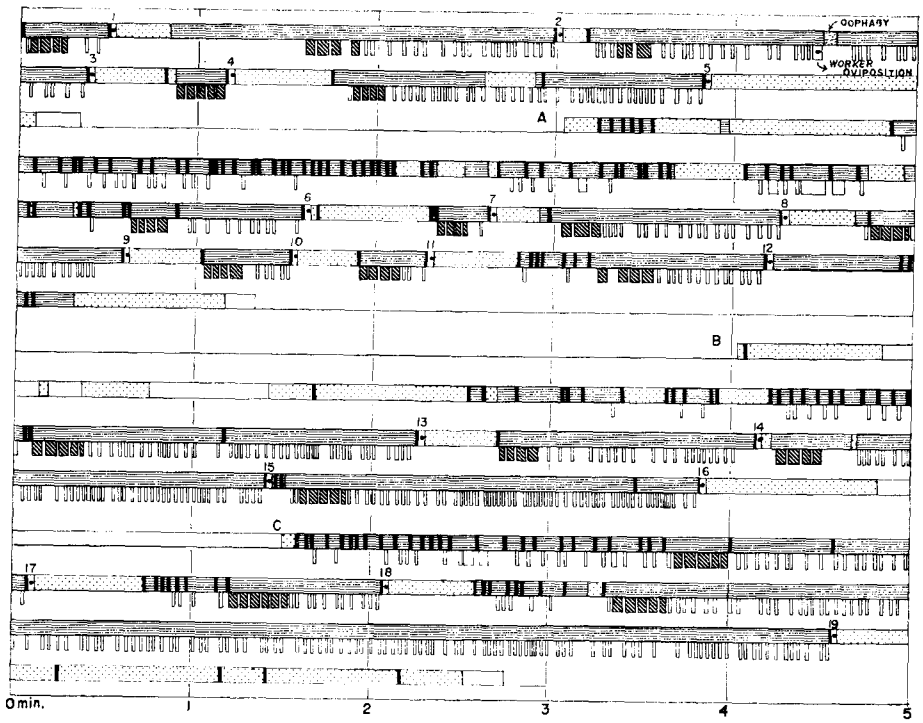

Fig. 5. Temporal sequence of provisioning on April 16 (16:24–17:36, with 19 ovipositions). For further explanations see Fig. 4.

first discharge of larval food to the cell ($=\sum W+\sum f+F$) varies considerably, from 50 to 933 sec. (Column A, mean 4 min. 26 sec.). The duration spent for fixation, that is, total duration minus duration spent for wandering, ranges from 3 to 652 sec. (Column B, mean 3 min. 22 sec.), occupying an average 72.6% of the total duration (Column C). During wandering and fixing, the queen continues wing vibration. Fixation is usually made horizontally, that is, sitting on the comb, only rarely vertically, clinging to the outer wall of the cell. As shown by the black vertical bars in Figs. 4 and 5, the most characteristic feature of the queen during fixation is her repeated, rather ritualized cell inspections, as referred to later.

In the other species so far studied, the structurally completed cell with collar evoked an excited concentration of workers, which alternately performed rapid insertions of fore-body to the cell. The queen usually fixed only to such cells with worker attendants. This phase was closely studied in *M. quadrifasciata* and defined as prefixation phase. In *T. clavipes*, however, the prefixation phase is often indistinct. The concentrations of workers can appear at some completed cells. But,

the queen often visits cells not attended by workers and repeats wandering and fixing. For instance, in the cases shown in Figs. 4, D, and 5, A, the queen fixed and repeatedly inspected a cell without workers. Of course, frequently she abandons the cell after prolonged fixation, but it is also not rare that such solitary fixation (Fig. 6, A) gradually attracts workers. The queen may either stay at the cell without workers or abandon it according to her motivation state.

That the alternation of wandering and fixing, *n*(Wf), and shifting to other cells is basic to the fixation phase of *T. clavipes*, is recognizable from the following items of evidence: 1) The number of fixations (including short visits) is more than 2, that is, there is at least one alternation of wandering and fixing, in 19/23 cases (Table 1, Column F). 2) The ratio, expressed as %, *duration of final fixation/total duration of fixation* is 35.5% as a mean, but often very low, only 0.5–1.2%(Column E). This indicates the release of food discharge without prolonged final fixation, when worker excitation increases by *n*(Wf). 3) Mean duration of final fixation is 53.4 sec. (Column D), about similar to that in *Cephalotrigona*, distinctly shorter than in *M. quadrifasciata* (=116 sec.), suggesting a different nature of the fixation phase. 4) In 16/23 cases, more than 2 cells are visited during primary fixation (Column G). Through this shifting, worker excitation spreads out over the comb. Under the influence of this general excitation, the *POP* to the second and subsequent cells proceeds with queen fixation shorter than that to the first cell. 5) When several cells are alternately visited and fixed by the queen, the total duration of fixation to the cell in which oviposition first occurs (=final fixation plus some earlier fixations to the same cell) is not always maximum among these cells. The fixation to the cell in which oviposition first occurs is longer than that to other cells only in 8/15 cases and occasionally take only 0.7–1.0% of the total duration of the fixation phase (Column H). This also shows that the cell in which oviposition first occurs is not pre-determined until immediately before the final fixation. From these items of evidence, it is certain that primary fixation is not only directed to the particular cell to be oviposited in first, but evokes a general excitement which makes possible, according to colony conditions, successive ovipositions to several cells.

Another characteristic in the fixation phase of *T. clavipes* is fixation to a cell still without a complete collar. The cases were not exactly recorded but appeared quite frequently, even in the presence of well-collared cells nearby (Fig. 6, A). The result of this fixation is variable. Sometimes the fixation attracts a concentration of workers, which complete the collar rapidly. Occasionally even provisioning and oviposition appear in such incomplete cells. Among 105 ovipositions observed in detail, there are 8 cases in which the cells were not provided with complete collars at queen oviposition, 4 after primary fixation and 4 after secondary fixation. This discrepancy between cell structure and release of *POP* was never seen in the other stingless bee species observed.

As already mentioned, the most characteristic feature of the fixation phase of *T. clavipes* is the alternation of queen cell inspection and worker body insertion. At inspection, the queen slowly inserts the lower half of her head, or often only the mouthparts, with mandibles closed. Wing vibration either continues or momentarily ceases. The duration of a single inspection is about 1 sec. or shorter, except for a few cases lasting several seconds (for instance, Fig. 4, D). The

interval between two successive inspections is obviously variable, frequently 2–5 sec., but not rarely 1 sec. or even shorter (*cf.* Figs. 4 and 5). The frequency of inspection in primary fixation is given in Tab. 1, Column I. Excluding Case 23, the frequency per minute ranges from 5.6 to 21.9 (mean 13.0). It is obvious that such frequent inspections are unnecessary to *know* whether or not the cell is ready to be provisioned and oviposited in. It is assumed that this repetition has the significance of attracting workers, increasing their excitement, thus leading them to the food discharge. The repeated inspection are also characteristic of *Cephalotrigona*, but were not observed in *Melipona* and *Scaptotrigona*.

Once the queen fixes to a particular cell and repeats her ritualized inspections, some workers gradually come to the cell and attend it. They appear excited, their bodies trembling violently, touching the cell, and alternately insert their fore-bodies into the cell. This body insertion is often made by one and the same individual 2–3 times in succession, then another one replaces it and does the same act. The queen often touches the inserting worker with her antennae, but not as violently as in *Melipona*. The duration of each insertion is usually about 1 sec. In 23 primary fixations observed, the insertions longer than 5 sec. were only 32/357, namely less than 1/10, whereas in *M. quadrifasciata* insertion is longer, freqently lasting 3–5 sec. As in the other species, the inserting worker often rapidly moves away from the cell after withdrawal, though not as constantly as after food discharge. The number of body insertion per primary fixation is given in Table 1, Column J. The ratio *queen inspection / worker insertion* was about 1.0 in *Cephalotrigona*, but higher in *T. clavipes*, ranged 1.00–5.68 (mean 2.34). This is partly caused by fixation of the queen to cells still not attended by workers. But as seen in Figs. 4 and 5, the ratio is higher even after workers begin inserting into the cell.

It is interesting that frequent body insertions by workers before provisioning are commonly seen in all stingless bees so far observed. Apparently such frequent repetition of brief insertions, each lasting only one second or so, is not important for further work on the cell. Probably this repetition is necessary to increase general excitement up to the level at which food discharge is released. In *T. clavipes* and *Cephalotrigona* this general excitement is seemingly conditioned by the frequent queen inspections, too. Besides queen inspection and worker insertion, there appears to be no exaggerated queen-worker behavior patterns in the fixation phase as seen in *Melipona* and *Scaptotrigona*.

The data on secondary fixation are given in Table 2. All cases were classified into five classes according to the total duration of the fixation phase. Among 84 cases, the duration was less than 10 sec. (Class I) in 23.7% (20 cases), less than 50 sec. (Class I and II) in 67.9% (57 cases) (Tab. 2, Column B). In these cases, the pattern is mostly WF, that is, without alternation of wandering and fixing (Columns C,D,E), and total duration of fixation is equal to that of final fixation ($\Sigma f = F$) (Column G). The difference between I and II mainly depends on the duration of wandering (Column F). The numbers of both queen inspections and worker body insertions are low (Column H-K). In these cases, the main stimulus

Table 2. Some numerical data on secondary fixation by the queen

Class	A	B	C	D	E	F	G	H	I	J	K
I	0-10	20	1-4	90.0	1.20	91.7	100.0	1.5	0-4	0.4	0-2
II	11-50	37	1-3	94.6	1.08	41.7	100.0	3.1	0-14	1.2	0-10
III	51-100	6	1-4	50.0	1.83	45.7	87.6	7.5	2-12	3.0	0-6
IV	101-200	11	1-8	36.4	2.46	67.7	61.0	16.3	6-27	8.1	0-14
V	200-	10	1-10	10.0	4.80	81.6	61.5	42.9	22-89	22.0	5-38

A. Range of total duration in sec.; B. Number of observed cases; C.Number of fixations. D. % of cases with only one fixation (=Type WF); E.Mean number of cells fixed or visited; F. mean ratio of *fixation duration/total duration* expressed as %; G. Mean ratio of *duration of final fixation/total duration* expressed as %; H. Mean number of queen cell inspections; I. Ditto, range; J. Mean number of worker body insertions, K. Ditto, range.

necessary to release food discharge was already given by the increased excitation during previous *POP*. In about 21.2% (Classes III-V), however, total duration of the fixation phase lengthens considerably, with corresponding increase of queen inspection and worker insertion and decrease of the relative length of final fixation. The difference in the ratio *fixation duration/total duration* between II+III and IV+V corresponds to the gradual appearance of alternate wandering and fixing, thus the latter classes approach the pattern of primary fixation, WF→ *n*(WF)WF.

The chronological order of fixation phases of different lengths in each batch is irregular as follows (primary fixation in gothic):

April 13 **V**-I-I, **V**-V, **IV**-IV-IV-IV-V, **IV**-II-II-III-III-IV-**IV**-V, **V**-V, **IV**-II-V
16 **IV**-III-II-I-II-V-II-II-II-II-II-II-II-II-III, **V**-II-I-I-V-III-III
17 **IV**-I-II-II-I-II-II, **IV**-II-I-I-II-I-II-II-I-III, **III**-II-I **V**-II-II-I,
V-I-II-II-IV-II-IV, **III**-I-II-III-IV-V-I-I-I-II-II
18 **V**-II-V, **V**-V-IV-II-V, **IV**-I-II

Short secondary fixations appeared more frequently on April 16–17 (ratio I+II/III~V, 46/12) than on April 13 and 18 (9/15). This corresponds to the rate of cell construction, the increase of which results in larger size of batch, consequently the frequent appearance of short secondary fixations.

As far as provisioning is concerned, each *POP* is successive in *T. clavipes*, as in *Melipona* and *Cephalotrigona*, not semisynchronous as in *Scaptotrigona*. But the appearance of short secondary fixations, together with some items of information given in reference to primary fixation, suggests a tendency toward the integration of behavioral sequences in a number of *POP* belonging to the same batch. The fixation and resulting general excitement in each *POP* affect succesively the subsequent ones of the same batch. In *M. quadrifasciata*, occasionally two ovipositions occur within a short interval. In such cases, fixation to the second cell is not markedly shortened or simplified, as far as can be judged by the data available to us. In *T. clavipes*, parallel to the tendency toward batch formation, all *POP* of the

same batch are apparently not strictly succesive but are integrated to some degree.

2.3. Provisioning, worker oviposition and queen oophagy: The provisioning is defined as the period from the beginning of first food discharge to the beginning of the queen oviposition. During this phase, workers perform food discharges, body insertions and occasionally ovipositions into the cell; and the queen, occasional inspections and, after worker oviposition, oophagy.

As in other species, larval food is discharged with a distinct contraction of the metasoma. After the contraction the worker often rotates herself about 1/2–2/3 circumferences along the cell margin. Again as in other species, the discharger hurriedly "escapes" after withdrawal from the cell, often to 3–4 cm from the cell. The duration of a single discharge ranges as follows:

Duration (sec.)	1	2	3	4	5	6	7
No. cases	2	58	99	56	11	1	1

The mean is 3.1 sec., slightly longer than in *Melipona* (1.9–2.4 sec.), about as long as in *Scapto-* and *Cephalotrigona*. The first discharge is soon followed by a second, third and so on. The number of discharges in a single process ranges as follows (number of discharges/number of cases observed): 3/3, 4/30, 5/49, 6/25, 7/2, 8/1, 15/1. The mean is 5.1, slightly less than in the other species so far observed (*M. quadrifasciata*, 6–8, *Cephalotrigona*, 5.4, *Scaptotrigona*, 4–8). The duration from the beginning of the first discharge to the end of the last one fluctuates as follows (duration in sec./number of cases): 10/1, 11/1, 12/5, 13/5, 14/2, 15/8, 16/3, 17/3, 18/7, 19/4, 20/1, 22/1, 23/3, 24/1, 25/2, 26/2, 28/1. The mean duration, 17.2 sec., is slightly shorter than in the other species (*Cephalotrigona* ca. 25 sec., *Scaptotrigona* ca. 30 sec., *M. quadrifasciata* mostly 15–40 sec.).

In the other species, especially in *Melipona*, queen oviposition occurs either soon after the last food discharge or after the postdischarge subphase (*pds*) of variable length. The *pds* was not noticed in *Cephalotrigona* but observations were insufficient in this species. In *Scaptotrigona*, the prolonged *pds* to the initial several cells of the same batch appears to be characteristic of the species. The *pds* was closely observed in *M. quadrifasciata*. It was confirmed that the appearance of this subphase showed a two-peaked distribution, either absent or quite prolonged when it appears. In *M. seminigra* and *M. compressipes*. *pds* appeared but very irregularly, probably caused by unfavorable rearing conditions for these Amazonian forms. In *T. clavipes*, too, the two-peaked distribution of *pds* is evident from the following distribution (duration of *pds* in sec./number of cases): 0～1/28, 2～5/2, 5～10/2, 11～60/8, 61～/9. The longest *pds* was observed on April 16, lasting 353 sec. (Fig. 5, No. 19). There is no correlation between appearance or not of *pds* and duration of fixation, either primary or secondary. On the other hand, a high correlation with the date of observation is revealed:

	Length of *pds* (P) relative to discharge subphase (D)			
	P=0	P≤D	D<P≤3D	3D<P
April 13	26	1		
15	1	1		
16	1	7	5	10
17	14	12	8	10
18	8	2		1

As already mentioned, cell construction and oviposition were more intense on April 16～17, than on the other days. The difference is obvious when Figs. 4 and 5 are compared with each other, but we cannot explain how colony conditions relate to the facultative appearance of *pds*.

The queen stays at the cell throughout the provisioning phase, not wandering to other cells as in *Scaptotrigona*, or retreating from the cell after food discharge, which is very characteristic in *Melipona*. Among 111 cases observed, the queen left the cell for a while only in 4 cases during the provisioning phase. One case is noteworthy: April 17, 8: 42, (D, food discharge; I, worker body insertion; Ip, queen inspection) 4D-5I-6D-Ip-brief wandering of the queen-5D-I-Ip-3I-Ip-queen oviposition. The case is abnormal in the high number of food discharges and their irregular distribution.

Cell inspection by the queen always appears immediately before her oviposition (*cf.* Figs. 4 & 5). Excluding this final inspection, queen inspection during the provisioning phase appeared in 33/111 cases as follows:

Duration of *pds*	Number of cases	Remarks (D, discharge subphase; P, Postdischarge subphase, *pds*; *ip*, inspection)
P=0	9	In 7 case 1*ip*, in 2 cases 2 *ip*, obviously all in D
P≤D	11	In 2 cases 1 *ip* in D; in 1 case 1 *ip* in D and 2 *ip* in P; in 8 cases 1 *ip* in P; in 1 case 4 *ip* in P
D<P	19	In 9 cases 1 *ip*; in 5, 3 and 2 cases respectively 2, 3 and 5 *ip*, all in P

It is interesting to note that, but difficult to explain why, all queen inspections appeared in *pds* when this was very long. Anyhow, it is clear that queen inspections, so frequent during the fixation phase, do not appear often in the provisioning phase. Fig. 5, B shows well the contrast between fixation and provisioning phases in this aspect. It was previously assumed that frequent queen inspections induce general excitement of workers. The fact mentioned above suggests that these inspections are especially important in order to release food discharges.

Body insertions by workers also frequently occur in the provisioning phase. Among 111 cases observed, insertions appeared during the discharge subphase only 5 times (*cf.* Fig. 4, No. 10). On the other hand, insertions are very frequent in *pds* as follows:

Number of insertions	Cases observed in different durations of *pds* (*cf.*the table given above)			
	P=0	P≤D	D<P≤3D	3D<P
0	42	8		
1-5	5	11	5	2
6-10		8	4	1
11-20		1	4	5
21-40				8
41-				5
Total	47	28	13	21

The number of insertions increases when the *pds* lengthens. As seen in Fig. 5,a prolonged *pds* is virtually filled by frequent insertions, most of which are less than 1 sec. and usually not deep insertions. Successive insertions by one and the same worker are also frequent, and many workers "escape" from the cell after such repeated insertions. By frequent repetitions of insertions, the *pds* of *T. clavipes* is distinguished from that of *M. quadrifasciata*, in which insertions appear but not as intensively. It is assumed that in their excited state after food discharges, workers repeat insertions until the queen oviposits. If this is true, the occurrence or not of *pds* must be explained by some conditions of the queen, not of the workers. But we have, for the time being, no sufficient explanation concerning the facultative appearance of *pds*.

The occurrence of workers with developed ovaries in queenright colonies (Sakagami *et al.*, 1963) and worker oviposition and subsequent queen oophagy in *POP* is a very peculiar event in stingless bees. On April 13 and 15, it was observed that some workers climbed on the cells and applied their metasomal tips to the cell margins during the provisioning phase. But the actual oviposition appeared after the feeding of the colony on April 15: April 16, 1/16 case (*cf.* Fig. 5, No. 3); April 1, 79/50; April 18, 2/11. In most cases, only one oviposition was obseed within the same unit process, but on April 17, in 4 cases, 2, 3, 3, 4 oviposi-tions were observed within the same process. Duration of oviposition ranges as follows: 2 sec.(2 cases), 2.5(1), 3(10), 4(1), and the mean, 2.8 sec., is longer than in *Cephalotrigona* (instantaneous), about as long as in *Scaptotrigona* (3 sec.) and longer than in *Melipona* (4.8–7.0 sec.). After a brief inspection of the cell, the worker climbs on the cell and deposits the egg at the upper margin of the cell (Fig. 6, B), as in *Cephalo-* and *Scaptotrigona*, not on the larval food as in *Melipona*. The worker eggs are, in contrast to *Melipona* and as in *Cephalotrigona*, slightly larger and more oval than the queen eggs, though not as enermous and rounded as in *Scaptotrigona*.

In all cases observed, worker oviposition was soon followed by queen oophagy, which is characterized by a violent trembling of the body. The duration of oophagy ranges as follows: 2 sec. (5 cases), 3 sec. (7), 3.5 ,4, 4.5, 5 sec. (each one case). The mean 2.99 sec., is shorter than in any other species. All cases of worker oviposition and queen oophagy appeared in *pds*. In one case soon after the final

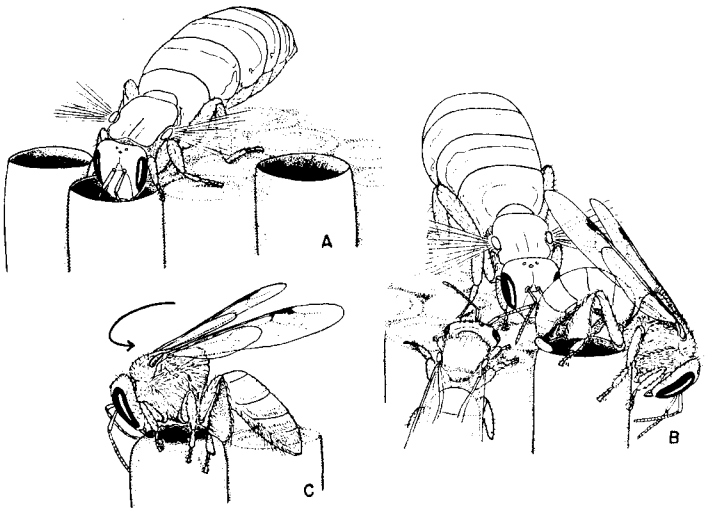

Fig. 6. A. Queen cell inspection. The queen vibrating wings, fixing to an incomplete cell without worker attendants in the presence of well-collared, cells nearby. B. Worker oviposition in front of the queen. C. Cell operculation by a worker. Rotation without inserting metasomal tip.

food discharge, in two others soon before the queen cell inspection before her own oviposition, and in all others between body insertions by workers. In the cases in which more than one worker oviposition was observed in the same *POP*, the sequence was recorded as follows (D, food discharge; I, worker body insertion; Ip, queen inspection; 00, worker oviposition followed by queen oophagy; V, queen oviposition):

5D-1p-15I-00-11I-00-9I-00-5I-Ip-V; 4D-00-10I-00-3I-Ip-2I-00-3I-00-Ip-V
5D-8I-00-Ip-7I-00-8I-00-Ip-V; 5D-7I-00-00-7I-Ip-12II-Ip-V

Intake of discharged larval food by the queen during the provisioning phase, observed in the other species except *Cephalotrigona*, was not confirmed.

2.4. Queen oviposition and cell operculation: Queen oviposition is simple and does not differ from that of the other species. After a brief cell inspection, she climbs on it, inserts her metasomal tip and oviposits with metasomal contraction. Wing vibration continues during this act. The duration of oviposition ranges as follows: 1 sec. (13 cases), 2 (13), 3(4), 5(2). The mean, 1.9 sec., is longer than in *Cephalotrigona* (instantaneous), shorter than in *Scaptotrigona* (4–5 sec.) and distinctly shorter than in *Melipona* (25–35 sec.). It is also shorter than in worker oviposition. After oviposition, the queen rapidly moves away from the cell and usually goes to the center of the comb, then she either leaves the comb or again starts wandering and fixing to another cell.

Operculation of the egg-containing cell begins either soon after the queen leaves (7/17 cases) or after a delay (10/17) ranging as follows: 3, 8, 8, 16, 40, 53, 55, 66, 78, and 95 sec. Even after beginning operculation, the performer often abandons the work due to the approach of the wandering queen. In this aspect, *T. clavipes* resembles *Melipona*, especially, *M. quadrifasciata*, and differs from *Scapto-* and *Cephalotrigona*, which start operculation soon after the queen oviposits in and performs the task with a high consistency, without abandoning it when the queen approaches.

In all species so far observed, the operculation phase is divided into rotation and sidework subphases. At first one worker climbs on the cell, inserting her metasomal tip into the cell. By rotating her body on the cell, the cell collar is bent inward with her mandibles and the cell orifice is gradually reduced. After the orifice becomes quite narrow, she works from the side of the cell. In *Melipona* and *Scaptotrigona*, these two subphases are clearly distinguished, and the metasoma is not withdrawn during the rotation subphase. In *Cephalotrigona* the metasoma is withdrawn earlier, when the orifice is still large, and rotation continues even after withdrawal. *T. clavipes* resembles *Cephalotrigona* in this aspect, but the poor differentiation of the two subphases is more conspicuous: 1) Each rotation often ceases without making a complete turn, interrupted by mandibular manipulation of the orifice without rotation; 2) The metasomal tip is withdrawn very rapidly, and rotation without metasomal insertion appears rather regularly (Fig. 6, C); 3) Often side work appears when the orifice is still very large.

In connection with these items, the operculation technique appears very slow and inefficient. The difference between operculation in this species and *Scaptotrigona* is, if an anthropomorphic expression is permitted, comparable to the difference between child's play and master work by a specialist. Probably as an outcome of this inefficiency, the duration of operculation is very long. The time from the start to the disappearance of the orifice was measured in 19 cases as follows (duration in min./number of cases): 7.0/1; 9.5/1; 10.0/1; 10.5/2; 11.0/1; 11.5/2; 12.0/3; 12.5/1; 13.0/2; 13.5/2; 13.5/2; 15.5/1; 16.0/1. The mean, ca. 11.5 min., is distinctly longer than in *Scaptotrigona* (ca. 3.5 min.), *Cephalotrigona* (ca. 5.5 min.) and *Melipona* (2–5 min.). As in the other species, operculation is made by one and the same worker until or just before the orifice is completely closed. Thereafter, one or two other workers can participate in the work or the work is taken over by them. The final moving away of workers was measured only in one instance: Complete closure of cell orifice at 10 min. 55 sec. and the final departure at 14 min. following the beginning of operculation. As already mentioned, the *POP* of this species occasionally appears at a cell without a complete collar, but the prolonged operculation is not caused by this fact. In one instance dealing with such cell, the orifice was closed at 10 min. 59 sec. As given above, there are many normal cases with longer duration.

Besides the normal operculation described above, there are two records of abnormal cases worth mentioning. In two instances, the queen took the ovi-

postion posture on the cell, but for an unknown reason, no egg was laid. In both cases, after "hesitation", the orifice was closed not by rotation of a single worker, but by side work by two workers:

Case 1: Workers successively inspected the orifice (12 times) but all retreated with avoidance reactions. At 71 sec. after the queen left, 2 workers participated in the work. Once a worker took a piece of cerumen from the collar of a cell nearby and applied it to the orifice. No rotation work was observed throughout the observation. *Case* 2: Repeatedly inspecting and retreating, at 93 sec. after the queen left, two workers began work at the cell side. In this case, however, one worker started rotation at 140 sec. after the queen left. Then side work appeared again. Observations were not continued thereafter.

Closure of a cell without an egg by side work from the beginning is also observed in *Cephalotrigona*, after an egg laid by a laying worker is removed by another worker. On the other hand, a cell with a worker-laid egg was normally closed with rotation in an orphan colony of *Partamona* (*Partamona*) *testacea testacea* (Klug) (Sakagami, Beig & Akahira, 1964). These facts indicates that rotation of a worker is released by the presence of an egg in the cell. The later appearance of rotation in Case 2 might be explained by the threshold to release this response being attained during the side work performance. It is open to question, however, whether or not such secondary rotation appears in *Melipona* and *Scaptotrigona*, too, which are characterized by a sharp differentiation of rotation and side work subphases.

Concluding remarks

The behavioral sequence in the provisioning and oviposition process of *T. clavipes* agrees with that in the other species so far observed in the succession of several phases, queen fixation, provisioning, oviposition and operculation. Furthermore, there are some minor characteristics which are common to all species so far reported:

1) Frequent body insertions by workers in the fixation phase; 2) Rapid succession of larval food discharges by workers; 3) Avoidance reaction of workers after each food discharge; 4) Worker oviposition and subsequent queen oophagy during provisioning phase; 5) Differentiation of operculation into rotation and sidework subphases; 6) Performance of operculation by one and the same worker, except for the final stage of side work subphase. Although not involved in the provisioning and oviposition process, the following features are also common to all species so far observed: 7) Absence of frequent food delivery by workers to the queen, in comparison with the honeybees; 8) Intimate relation between cell construction and provisioning, in contrast to the honeybees; 9) Successive, not synchronous, construction of cells, and resulting occurrence of cells of various stages at a given time (Some items, for instance, 4, 5, 9 are not common to certain groups subsequently reported in this serial work).

Excluding these common features, the diagnosis of ethological characteristics of *T. clavipes* with special reference to the oviposition process is given as follows (S=*Scaptotrigona*, C=*Cephalotrigona*, M=*Melipona*):

1) Queen vibrating wings continuously, not beating (≒CSM); 2) Absence of exaggerated or ritualized behavioral interactions between queen and workers (=C, ≒SM); 3) Interval between successive ovipositions irregular, sometimes discrete, sometimes forming a batch (=C, ≒M), in the latter case, provisioning of each cell successive, not synchronous (=C, ≒S); 4) Primary fixation of queen consisting of frequent alternation of wandering and fixing (=S, ?=C, ≒M); 5) Queen fixation occasionally directed to cell without worker attendants (≒CSM); 6) Queen often fixing to cell without complete collar and occasionally even ovipositing in it (≒CSM); 7) Frequent, rather ritualized cell inspections by queen during fixation phase (=C, ≒SM), 8) Queen not touching violently with antennae workers inserting bodies into cell (=CS, ≒M); 9) During provisioning phases, queen not wandering to other cells (≒S), nor making peculiar retreat from cell (=C, ≒M); 10) Worker oviposition at upper margin of cell (=CS), not on center of larval food (≒M); 11) Worker egg larger than queen egg (=CS, ≒M), though not enormous (=C, ≒S) 12) Queen oophagy characterized by peculiar body trembling (≒CSM). 13) Queen oviposition lasting longer than in C, shorter than in S, and distinctly shorter than in M: 14) Start of operculation often delayed after queen oviposition (=M, ≒CS); 15) Rotation and side work subphases in operculation phase poorly differentiated (=C, ≒SM); 16) Operculation inefficient and very long, often lasting more than 10 min. (≒CSM).

Some of these features might be variable according to colony conditions. We cannot definitely conclude relative stability of these features from the observations made with a single colony. Nevertheless, we believe many of them are characteristic of species or supraspecific taxa, judging from observations on the other species. Comparing the behavioral characteristics of *T. clavipes* to those of other species observed, coincidence is the highest with *Cephalotrigona* and lowest with *Melipona*. Further discussion of the relation between ethological results and the classification system based upon adult morphology will be given elsewhere. But the results seem to coincide with the morphological classification to some degree. *Tetragona* and *Cephalotrigona* belong to the same group (Group a, in Moure, 1961, p. 185), which could be named the *Trigona-Tetragona* complex, and involves the following "subgroups", irrespective of whether they are regarded as genera, subgenera or species groups: Neotropical — *Cephalotrigona*, *Oxytigona*, *Trigona*, *Tetragona*, *Frieseomelitta*, *Geotrigona*, *Duckeola*, *Tetragonisca*, *Ptilotrigona*; Ethiopean — *Dactylurina*; Oriental and Australian — *Lepidotrigona*, *Homotrigona*, *Heterotrigona*, *Platytrigona*, *Lophotrigona*, *Tetragonula*, *Tetragonilla*, *Geniotrigona*, *Odontotrigona* and *Tetrigona* (Moure, 1961). The separation of *Melipona* form the other stingless bee groups is universally accepted in all classification systems (Schwarz, 1932, '48; Moure, 1951, '61; Wille, 1963). Of course we have no idea to establish a classificatory system based upon ethological characteristics alone. But these characteristics must be considered for the construction of a more reasonable system.

On the other hand, we must not ignore the influence of colony conditions upon the provisioning and oviposition process. After the artificial feeding to the colony, the following changes were observed: 1) Disappearance of ingestion of contents of cells previously oviposited in; 2) Increase of cell construction and

queen oviposition; 3) Formation of batches and appearance of short secondary fixations; 4) Appearance of worker oviposition; 5) Appearance of prolonged postdischarge subphase. Except for the last item, all these features can be explained by improved nutritional conditions both for queen and workers. The provisioning and oviposition process of *T. clavipes* and other stingless bee species involves therefore two aspects, those variable according to colony conditions and those characteristic of species or higher taxa.

Summary

The brood cell provisioning and oviposition process of *Trigona* (*Tetragona*) *clavipes* (Fabricius), a comb-building stingless bee species, was observed in an artificial hive. This species resembles *Cephalotigona femorata* (Smith) in many behavioral characteristics, especially in the succession of several queen ovipositions at brief intervals, frequent, nearly ritualized cell inspections by the queen during the fixation phase, worker egg larger than queen egg and laid at upper margin of cell, and poor differentiation of rotation and sidework subphases in cell operculation. It differs from *Cephalotrigona* in several features, for instance, continuous wing vibration by the queen instead of rhythmic wing beats, fixation by the queen and even subsequent oviposition to a cell without a complete collar, body trembling by the queen at oophagy, and inefficient operculation lasting often more than 10 min.

Also, the influence of colony conditions upon cell construction, queen oviposition, worker oviposition, etc. was confirmed through artificial feeding.

References

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. Jap. **37**: 112–199.

Ihering, H.v. 1903. Biologie der stachellosen Honigbienen Brasiliens. Zool. Jb., Systematik **19** (1904): 179–287, pls. 10–22.

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). Stud. Entom. **4**:181–242.

Sakagami, Sh. F. 1966. Techniques for the observation of behaviour and social organization of stingless bees by using a special hive. Pap. Avuls. Dept. Zool. Secret. Agric. São Paulo. **19**: 151–162.

————, Beig, D., and Y. Akahira 1964. Behavior studies of the stingless bees, with special reference to the oviposition process. III. Appearance of laying worker in an orphan colony of *Partamona* (*Partamona*) *testacea testacea* (Klug.) Jap. J. Ecol. **14**: 50–57.

————, ———— and Ch. Kyan 1964. Behavior studies of the stingless bees, with special reference to the oviposition process. IV. *Cephalotrigona femorata* (Smith). Kontyu **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.

———, and J.F.M. Camargo 1964. Cerumen collection accompanied by thieving and attacking in a stingless bee, *Nannotrigona* (*Scaptotrigona*) *postica* (Latreille), with a consideration on territoriality in social insects. Rev. Biol. Trop. **12**: 197–207.

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

———, and Y. Oniki 1963. Behavior studies on 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**: 497–510.

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

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

Schwarz, H.F. 1932. The genus *Melipona*: the type genus of the Meliponidae or stingless bees. Bull. Amer. Mus. Nat. Hist. **63**: 231–461, pls. 1–10.

——— 1948. Stingless bees (Meliponidae) of the Western Hemisphere. Ibid. **90**: 1–546.

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