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Oviposition behavior of an Amazonic Stingless Bee, *Trigona* (*Duckeola*) *ghilianii*[1][2]

By

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(*With 3 Text-figures and 2 Tables*)

As the seventh report of our serial work on the oviposition behavior of the stingless bees, the present paper deals with *Trigona* (*Duckeola*) *ghilianii* Spinola, 1853, an aberrant species representing a monospecific offshoot within the *Trigona-Tetragona* Complex. An enormous nest of this species was discovered and taken at Ponta Negra near Manaus, Amazonas, on January 28, 1963, by the members of the Faculdade de Filosofia, Ciências e Letras de Rio Claro Expedition to Amazonas. The colony was transported by air to Rio Claro, State of São Paulo, and introduced on Feburary 15, into an observation hive of the type used in our previous work (Sakagami, 1966). The colony lost a considerable part of the original population in the course of extraction and transport, but the remaining inhabitants well adapted themselves to the artificial conditions. The first oviposition was observed on February 19 and the observations described below were made intermittently until June 30, 1963.

Hitherto the oviposition behavior of several forms has been described in detail (Beig and Sakagami, 1964; Buschinelli e Stort, 1965; Sakagami, Beig and Akahira, 1964;

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

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Sakagami, Beig and Kyan, 1964; Sakagami, Montenegro and Kerr, 1965; Sakagami and Oniki, 1963; Sakagami and Zucchi, 1963, 1967. (The scientific names of the forms observed are given in the references), and a preliminary comparison among various groups was given (Sakagami e Zucchi, 1966). In the following descriptions, the species or forms so far studied are cited for the comparison, unless necessary, only with their generic or subgeneric names.

1. Behavior in inter-oviposition periods

As in the other species so far studied, the ovipositions of *Duckeola* appear rhythmically with definite intervals. Because our attentions were focussed to the oviposition behavior, no systematic records were taken as to the behavior seen between oviposition processes, except the following notes.

1.1. Nest architecture: The peculiar nest architecture (Kerr *et al.*, 1967) was retained in the observation hive, notably, 1) Absence of involucrum, 2) Formation of an incomplete comb system, with irregular expansions both in horizontal and vertical directions, 3) Construction of thick pillars and thick and rough cell walls, and 4) Abundant use of resins. On the other hand, the following two records seem to suggest adjustive tendencies: 1) Decreased size of storage pots (3～4 cm high and 2～4 cm wide under natural condition whereas 1.5～2.0 cm h and 1.2～1.5 cm w in the hive), 2) Temporary use of the broken brood cells for honey storage.

1.2. Worker behavior: In general the worker behavior is characterized by a marked sluggish disposition. Although mild defensive attacks were noticed when the nest was taken near Manaus, guard bees at the entrance of the observation hive were timid, showing no positive defense, apparently in part caused by the diminished population size. The ventilating posture is as in other species: Head lowered, directing against nest entrance, antennae hung down, metasoma conspicuously raised, often up to 40～45° to the substratum, with the tip slightly curved down. This fanning posture also appeared when the hive was artificially disturbed, nevertheless raise of hive temperature was not recorded. At walking, workers often vibrate their wings briefly but strongly, with irregular intervals, probably representing the pattern homologous to the wing fluttering in *Tetragona.* Another peculiar behavior is the momentaneous stop of the walk, followed by raise of forebody together with fore legs (Fig. 1, A).

Further a characteristic behavior pattern appears in a worker sitting on an already operculated cell. She suddenly makes a short but very sharp dashing movement, through which she turns about the half to two thirds of the contour of the cell. This movement is often repeated two to three times successively, resulting in a complete revolution around the cell. The behavior sequence is rapid and abruptly ends by sharp opening and closing of wings. The performer seems to be very alert, as if taking a warning posture, although the behavior appears without noticeable disturbance.

1.3. Queen behavior: The queen of this species is seemingly still unknown. A brief morphological note taken besides observations of behavior is given as follows:

Physogastric; not too conspicuously larger than worker as in *Trigona* or *Tetragonisca*, with mesosoma distinctly but not remarkably wider than in workers. Head conspicuously elongate (Fig. 3, A and C), narrower than, but distinctly longer than in workers. Head pale yellowish brown; stematicum, surroundings of antennal sockets, and ill-defined obliquely longitudinal patches along upper margin of clypeus darker. Antennae dark brown, with scape pale yellowish brown. Mesosoma and legs yellowish brown; mesoscutum, except lateral margins, and metanotum blackish; pleura and hind tibiae with ill-defined transverse stripes. Terga dark brown, paler pregladularly; sterna paler, each with ill-defined transverse band.

Further morphological detail could not be obtained because the unique specimen was alive. The elongate head is the most characteristic feature among those described above.

The queen is also very sluggish. At walking antennae are shaken constantly but not so violently as in, for instance, *Meliponula bocandei* (Spinola) (unpubl.). As in *Tetragona* the queen is characterized by continuous vibration of wings, instead of making rhythmical wing stroking. In *Tetragona* vibration continues 4~5 sec., but in *Duckeola*, separated by momentaneous pauses, 5~15 sec. or more, not rarely more than 30 sec., though more intermittent at walking.

Another peculiarity is the geopositive orientation frequently taken at resting. At prolonged resting on lateral walls of peripheral brood cells or on storage pots, she always directs downwards, with prolonged wing vibration (Fig. 1, B).

During an inter-oviposition period, the queen alternates moving to and fro and resting. No particular resting place occurs. A 30 min. continuous observation taken at an inter-oviposition period is summarized below (R. Resting without wing vibration; Rv. Resting with wing vibration; Rg. Resting with geopositive posture and wing vibration; W. Walking without wing vibration; Wv. Walking with wing vibration; C. Cell inspection; "?". The queen under combs, and her behavior not traced. Figures parenthetically given are durations in min. Hyphens mean the continuation of the same behavior pattern):

Min.	Behavior
1.	—R(29),W (5), R(12),W(4),R(10)—
2.	—R(4), Rg(56)————
3.~5.	——— Rg ————
6.	—Rg(49), Wv(5), Rg(6)————
7.~8.	——— Rg ————
9.	—Rg(40), R (20)————
10.	—R (19), W(4), Rg (37)————
11.~16.	———Rg————
17.	—Rg(8), Wv (52)————
18.	—Wv(6),C (4), Wv(8),C(3), Wv(28), C(3),Rg(8) ————
19.	—Rg(6), Wv(37), Rg(17) ————
20.~21.	———Rg————
22.	—Rg(58), Wv(2) ————
23.	—Wv(8), W(3), Wv(25), ?(24)———
24.	——— ? ————
25.	—?(6), W(4), Rg(50) ————
26.	——— Rg ————
27.	—Rg(4),?(9),Wv(4),W(24), Wv(19)-
28.	—Wv(4), Rv(56)————
29.~30.	———Rv————

1.4. Queen-Worker interactions: The behavioral interactions between queen and workers are relatively simple as in *Tetragona.* Four patterns basic to all stingless bees are recognized also in *Duckeola*: 1) Gradual formation of a royal court around the resting queen. 2) Repetition of rapid advance followed by retreat by attendants in front of the queen. 3) Escape by workers at sudden encounter with the queen. 4) Gradual retreat by workers perceiving the apporaching queen. All these responses are less exaggerated than in the other species studied, probably in part in connection with the sluggish disposition of both castes. No elaborated responses by workers to the queen, as seen in *Melipona* and *Scaptotrigona,* are noticed. The queen frequently touches workers with antennae, and short buccal contacts were occasionally observed. But no definite food delivery of workers to the queen was confirmed within the observation period.1)

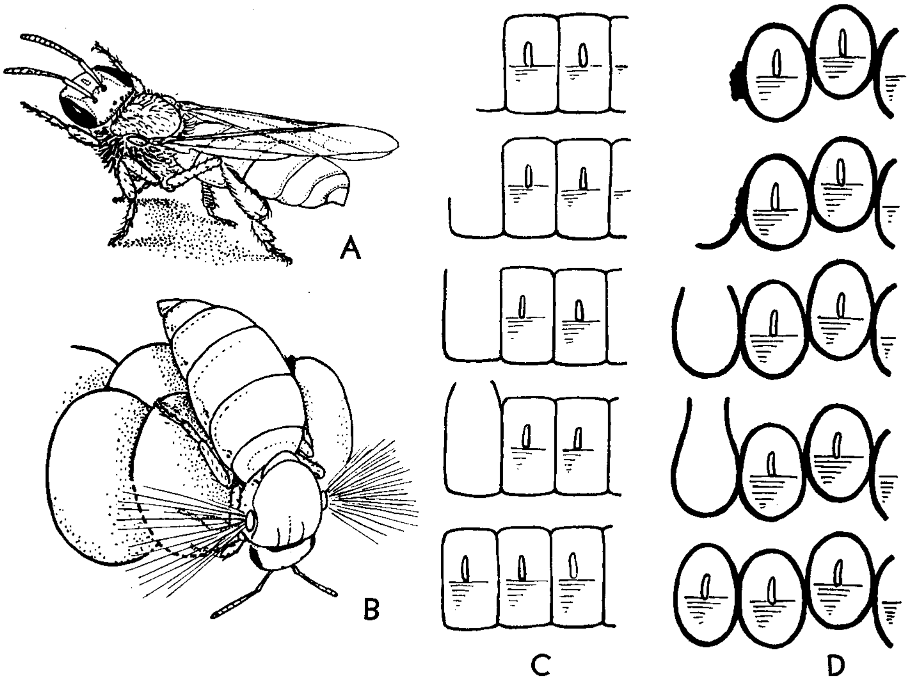

Fig. 1. A.Raise of forebody by a worker. B. Continuous wing vibration by the queen with geopositive orientation. C. Cell construction in stingless bees with typical combs. D. Ditto in *Duckeola,* Both in C and D, the process of cell construction is shown from top to bottom by the lighthand cell.

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1) In these earlier studies of our serial work, we failed to observe the worker food delivery to the queen in most species. Later, one of us (R.Z.) found it in several species (unpublished).

2. Pattern of oviposition rhythm and cell construction

In our previous papers, several patterns of the oviposition rhythm in stingless bees were mentioned. The pattern in *Duckeola* is intermediate between *Tetragona* and *Scaptotrigona*. In *Tetragona*, as in *Cephalotrigona*, each queen oviposition is made either discrete, as in *Melipona*, or in batch. In *Scaptotrigona*, the batch formation is exclusive, and, in the later part of a batched *POP*[1], food discharges to several cells are made synchronously. In *Duckeola* the batch formation is exclusive, but unit *POP* to each cell is successive. Food discharges to each cell are always made after the queen oviposition to the preceding cell.

The cell construction observed in the hive of this species may be called semisynchronous. Cells are begun to build successively after most structurally completed cells were oviposited in during a batched *POP*. At first cells of various stages of growth are found at a given time. But no start of new cell occurs at the later part of an interoviposition period, and the latter was usually very long. Consequently, soon before the start of a batched *POP*, no cells of younger stages were present and most empty cells were already structurally completed.

As in the other species studied, each cell is built by the summation of successive activities by a number of workers (Sagakami, Montenegro and Kerr, 1965). Corresponding to the architectural peculiarities, the cell construction in this species differs from that in other typical comb builders in the following points: 1) In other species, cell construction is started from the bottom and the outer side walls of ready made neighbouring cells are simply used as inner walls of the new cell, though presumably smoothed later. In *Duckeola* at first an irregular mass of cerumen is deposited on the outer lateral wall of a ready made cell. The mass is gradually expanded and transformed to the bottom and side walls of the new cell (Compare Fig. 1, C and D, top stage). 2) In contrast to the other species, each brood cell of *Duckeola* is not tightly united at top and bottom, and the collar made at the final stage is more conspicuously lengthened upward, taking the appearance of an electric lamp (Fig. 1, C and D, bottom).

The time required for the completion of one cell was not exactly measured caused by prolonged inter-oviposition periods. A few incomplete records are cited as follows:

1. June 14, 16:00. Four cells at start, five at 1/4 stage (=height of the cell one fourth of that of an oviposited cell), one at 1/2 stage. June 15, 10:30. All mentioned cells completed (collared) with two other later collared cells. Between 12:00～14:00. All oviposited in.
2. June 15, 17:20. Three at 1/4 stage, two at 1/2 stage, one collared. June 16, 11:20. All collared with three later cells collared. 16:20. Unchanged. 18:00. All oviposited in.
3. June 18, 9:20. Eleven cells all at 4/5 stage, 14:00. Unchanged. 17:30. Seven cells oviposited in. June 19, 9:05. Four cells remained since the preceding day and seven others collared. 10:15. All oviposited in. 17:33. Only four cells at start.

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1) The provisioning and oviposition process.

As already mentioned, the interval between two batched *POP* was very long, as shown by several records (in hours): 22, 24, 27, 27, 28.5. In two other instances, the intervals were continuously recorded through four and five successive processes as follows: 19.5–27.5–27.5 hrs and 19–26–ca.25–23 hrs. The intervals are relatively regular, with the mean 25.5 hrs, or, approximately one per day. A few records were taken as to the interval between structural completion of cells and start of oviposition process (in hrs): 1.25, 1.5, ca.2.0, 2.2, 3.5, 5.5, 5.5, more than 6.0. No such prolonged delay was observed in the other species so far observed.

The number of cells oviposited in a batched *POP* is distributed as follows: 9 (3 cases), 11(4), 12(3), 15(1), 16(1), 17(2). Further there were several instances, where only certain cells of structurally completed cells were provisioned and oviposited in: 5 out of 8, 5/10, 9/10, 9/13, 10/11, 12/13 and 15/17. The mean number of cells oviposited in a batched *POP* is 10.8, and the mean oviposition rate per day is 11.5, even lower than in *Melipona.* Obviously the oviposition rate must be higher under natural condition, judging from the enormous size of the nest (Kerr *et al.*, 1967). Prolonged inter-oviposition periods and delayed start of *POP* after structural completion of cells observed in our hive might not always be realized under natural condition. On the other hand, the exclusive batch formation is certainly characteristic to the species.

3. Behavior in provisioning-oviposition process

Several successive phase distinguished in *POP* of the other species studied are also recognized in *Duckeola*, in the order of *queen fixation*, *provisioning*, *queen oviposition* and *operculation.* The chronological sequence of four accurately observed cases are given in Fig. 2, where the queen behavior is taken as the foccus of observations. All workers performing cell construction, provisioning and operculation possessed black banded sterna, while still pale brownish terga, not blackish as in foragers, indicating their relatively young ages.

3.1. *Queen fixation*: In all so far observed species, *POP* is preceded by the concentration of workers around structurally completed cells and their excited alternation of body insertions into these cells. This prefixation phase, very conspicuous in *Scaptotrigona* and *Melipona*, is less remarkable in *Duckeola.* As in *Tetragona*, the queen occasionally "fixes" to a cell still with no or only a few worker attendants. This premature fixation often results in the arousal of worker excitement. The queen fixation to cells still with incomplete collars, observed in *Tetragona*, was not seen in *Duckeola*, because such cells were, as already noted, virtually absent soon before *POP.* In general worker excitement was seen less exaggerated than in the other species.

As in the other species, the queen, presumably stimulated by increased laying motivation, visitis the comb, wanders to and fro there (*W*), and stands near one cell with complete collar. This incipient fixation (*f*) rarely develops into the final fixation (*F*). She abandons the cell, makes wandering and again fixes to this

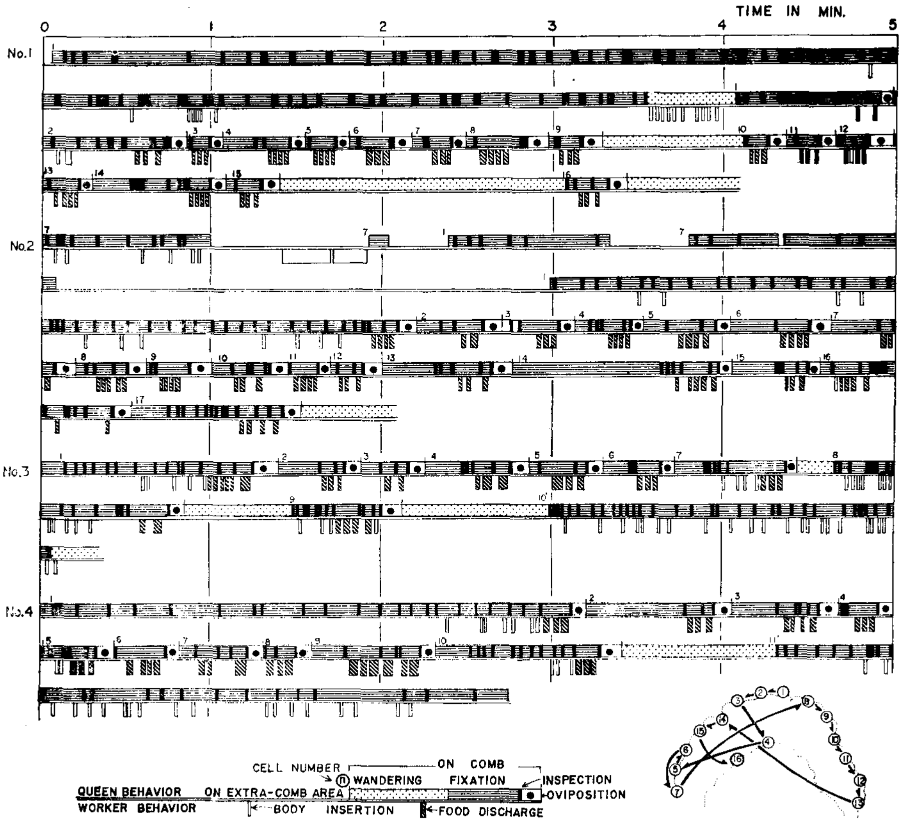

Fig. 2. Chronological sequence of provisioning-oviposition process. No. 1, March 9, 15:25; No. 2, March 12, 18:15; No. 3, March 18, 12:30, No. 4, March 19, 16:44. In Nos. 1 and 2, all completed cells were oviposited, while in Nos. 3, 4, some cells remained without ovipositions. Cell number means the sequence of oviposition. In No. 2., Cell 7 was first "fixed" by the queen, but the oviposition to this cell was made later. In Nos. 3 and 4, Cells 10′ and 11′ were not provisioned, in spite of prolgoned queen fixation. Spatial arrangement of cells oviposited in No. 1 is given at bottom righthand.

or another cell, resulting in repetition of *Wf* until *F*. This initial part of the fixation phase was not closely analysed. But the general sequence is more or less similar to that in *Tetragona*.

As in *Tetragona*, *primary fixation* before the first oviposition is distinctly longer than *secondary fixations* before the second, third and subsequent ovipositions. Table 1 presents some numerical data on primary fixation in several precisely observed cases. The mean duration of primary fixation is 121.5 sec. But it can become quite short, when the worker excitement was already strengthened in the course of *Wf* before *F* (Table 1, No. 9).

Table 1. Some numerical data on primary fixation by the queen in several selected cases

Case No.	Duration (in sec.)			Behavior				Behavior per minute			
	A	B	C	D	E	F	G	H	I	J	K
1	70	66	4	12	0	12	2	11.3	18.0	0	2.1
2	199	168	31	7	35	42	11	12.7	13.8	12.6	4.2
3	93	46	47	10	7	17	4	10.9	12.6	18.0	10.2
4	71	57	14	6	14	20	8	16.9	25.8	15.0	8.4
5	237	206	31	4	37	41	11	10.4	7.8	10.8	3.0
6	57	21	36	7	5	12	4	12.6	11.4	19.8	11.4
7	177	34	143	17	6	23	7	7.8	7.2	10.2	12.0
8	175	28	147	20	10	30	2	10.3	8.4	21.6	4.2
9	15	0	15	2	0	2	0	8.0	8.0	0	0

A. B+C; B. Duration since the start of primary fixation to the start of first body insertion by a worker; C. Duration since the start of first body insertion by a worker to the start of food discharge; D,E,F. Queen inspections performed during A, B, C; G, Worker body insertions performed during C; H,I,J. Queen inspections per minute during A, B, C; K. Worker body insertions per minute during C.

As in *Tetragona*, the fixation phase is characterized by repeated cell inspections by the queen and body insertions by workers. At inspection the queen inserts the fore body deeper than in *Tetragona.* Usually the whole head, often also the anterior half of the mesosoma including fore legs is slowly inserted (Fig. 3, B). Wing vibration usually ceases during the inspection and reappears soon after the characteristically slow withdrawal. The attending workers repeat advance-retreat responses during queen inspection. The duration of each inspection ranges as follows (in sec.):

Duration	~1.0	~2.0	~3.0	~4.0	~5.0	~6.0	~7.0
Cases observed	108	124	59	26	5	1	2

The mean, 1.9 sec., is distinctly longer than in *Tetragona,* in which inspections rarely exceed 1.0 sec.

The interval between successive inspections was (in sec.):

Interval	~1.0	~2.0	~3.0	~4.0	~5.0	~6.0	~7.0	~8.0	~9.0	~1.1
Cases observed	19	32	39	40	41	15	15	15	5	23

The mean frequency of inspections per minute in selected cases (Table 1, Column H) ranges 7.8~16.9, generally more frequent after the first body insertion by a worker. As in *Tetragona* and *Cephalotrigona* such frequent and brief "inspections" are certainly unnecessary to *confirm* whether or not cell is ready to be provisioned and oviposited in. Presumably repeated inspections play a role to increase the motivation of food discharges by workers.

The body insertions by workers (Fig. 3, A) appear at the later half of the fixation phase (*cf.* Fig. 2), but, as in *Tetragona,* in most cases, less frequent than queen inspections (Table 1, F versus G, or J versus K). The pattern of this behavior is similar to that in *Tetragona*, with insertion of head and anterior mesosoma. The queen often touches the inserting worker with her antennae, but not so violently as in *Melipona* (Fig. 3, A). The duration of each insertion is about 1 sec. As in the other species, the inserting worker usually moves away from the cell after withdrawal, though not so rapidly as after food discharges.

In *Melipona quadrifasciata anthidioides*, it was confirmed that the frequency of worker insertions increases at prefixation phase, and decreases at the earlier part of fixation phase. This change was explained by the avoidance component of the response by workers to the queen. The same tendency is seen in *Tetragona*, but less conspicuously, partly caused by the irregular start of fixation phase in this species, the queen of which often fixes to cells still not attended by workers. In *Duckeola* the decreased body insertions at the earlier part of the fixation phase is more distinct; though not precisely analysed as in *Melipona*. As seen in Table 1, the earlier part of the fixation phase is "filled" with queen inspections but not by worker insertions (*cf.* also Fig. 2). At this stage, attending workers attempt to insert the forebody in the cell but retreat without actual insertions, which appear at the later part of the fixation phase. In a previous paper (Sakagami, Montenergro and Kerr, 1965), the worker insertion was interpreted as a modified cell building behavior, in which the original function of smoothing cell walls was secondarily changed to a signal response informing for the queen the readiness of the cell to be oviposited in.

However, now we are inclining to the opinion to regard this behavior as an incomplete food discharge reaction, somewhat comparable to *intention movement* in Lorenz and Tinbergen, although its significance as a signal response is not always excluded. If this interpretation would be correct the food discharge would be elicited through the following process: Body insertion or intentional food discharge reaction becomes intense at prefixation phase, but it is inhibited at the arrival of the queen, by the workers' avoidance to the latter. The fixation by the queen, with (*Tetragona* and *Cephalotrigona*) or without ritualized inspections (*Melipona*) gradually removes the avoidance by workers and lowers the threshold to elicit the reaction, resulting in the reappearance of insertions and finally actual food discharges. In this case, the "escape" response after withdrawal from the cell, which appears more distinctly after food discharges than after mere body insertions, may indicate the recovery of the avoidance to the queen.

In *Tetragona*, when several ovipositions are "batched", the secondary fixations after the first oviposition become shorter and the numbers of queen inspections and worker insertions decrease. This means that the excitement released by the primary fixation and the first queen oviposition is maintained in subsequent ovipositions, resulting in the simplification of secondary fixations, in contrast to the instance in *Melipona*, where each oviposition process is independent, always preceded by the fixation phase of the similar temporal pattern. In *Duckeola* with exclusive batch formation, the secondary fixations are much more simplified (*cf.* Fig. 2). The alternation of wandering and fixing was observed only in five out of 135 cases observed. The frequency distribution of duration, numbers of queen inspections and of worker insertions are respectively as follows:

Duration (in sec.): 0~10 (34 cases), 11~20 (26), 21~30 (11), 31~50(7), 56 and 57 (each one case), with the mean 14.3 sec. (72.9 sec. in *Tetragona*).

Frequency of queen inspections 1 (41 cases), 2 (20), 3 (12), 4 (5), 5, 6, 7, 11, 14 (each one case), 8(2), with the mean 2.3 (8.9 in *Tetragona*).

Frequency of worker insertions: 0 (64 cases), 1 (14), 2 (1), 3 (3), 4 (2), 12(1), with the mean 0.53 (2.2 in *Tetragona*).

The integration of ovipositions belonging to the same batch is obviously stronger than in *Tetragona.* In several batches, duration and numbers of queen inspections and worker insertions were precisely recorded as given in Table 2. It is noticed that some later ovipositions are often preceded by prolonged fixations. This indicates the deficiency of larval food. After successive food discharges, the number of workers on comb, with abundant larval food in their crops, gradually decreases. The final fixation in Nos. 3 and 4 (*cf.* Fig. 2 and Table 2) shows the complete deficiency. In spite of lasting fixation by the queen and repeated body insertions by workers, the food discharges were not elicited and the queen abandoned the cells.

3.2. Provisioning: The provisioning phase starts by the beginning of the first food discharge and ends by the beginning of oviposition by the queen. The behavior seen in this phase is food discharges by workers and occasional inspections

Table 2. Some numerical data on secondary

Batch No.	Order of successive							
	1	2	3	4	5	6	7	8
1	14+4	5+2	1	2	1	1	1	1
	93	*36*	*2*	*16*	*2*	*6*	*7*	*5*
2	41+11	2	2	3	1	1	1	2
	237	*16*	*12*	*12*	*12*	*12*	*12*	*7*
3	12+4	1	1	3	1	2	5+4	14+12
	57	*15*	*8*	*18*	*8*	*12*	*30*	*56*
4	23+7	1	1	2	3+2	1	1	1
	177	*36*	*18*	*6*	*10*	*4*	*7*	*1*
5	20+8	2	1	4	2	2	2	1+1
	17	*13*	*6*	*23*	*8*	*8*	*27*	*9*
6	2	2+1	1+1	1+1	1	2+1	3	1
	15	*21*	*21*	*12*	*14*	*12*	*18*	*2*
7	30+2	1	3+1	4+1	1	2	1	1
	145	*17*	*19*	*10*	*21*	*13*	*15*	*13*

Figures mean the number of queen inspections and, if present, with "+", that of given in italics. (The first case in each batch is the primary fixation) by the queen. The prolonged post-discharge subphase since final food discharge to queen oviposition, frequently seen in *Melipona* and *Tetragona*, was not observed. The body insertions by workers, occasionally seen in the other species were observed only once (*cf.* Fig. 2, No. 1–12). During the provisioning phase, the queen does not make wandering as in *Scaptotrigona* or characteristic retreat after the first discharge as in *Melipona*. Furthermore, the remarkable events seen in the other species so far studied, worker oviposition and subsequent queen oophagy, were also absent. Consequently the provisioning phase in *Duckeola* is simpler than in the other species so far studied.

The larval food is discharged with a strong metasomal contraction (Fig. 3, C). which is very conspicuous in *Duckeola*, possessing relatively elongate metasoma. The duration of a single discharge is distributed: 0~1 sec. (11 cases), ~2 sec. (107), ~3 (20), ~4 and ~5 (each 1), with the mean 1.6 sec., distinctly shorter than in the other species so far studied (*Tetragona* 3.1 sec.; *Meliponas*, 1.9~2.4 sec.). After discharge, the worker "escapes" from the cell, but not so hurriedly as in the other species, reflecting the sluggish disposition characterisitic to the species. The first discharge evokes a general excitement of workers on combs, though less exaggerated as in the other species, notably, *Scaptotrigona*, and is soon followed by a second, third, etc. The number of discharges before each oviposition is distributed: 2 (9 cases), 3 (54), 4 (46), 5 (5), 6 (5), 7 (1). The mean, 3.55, is the lowest value among the species so far studied (*Tetragona*, 5.1; *Cephalotrigona*, 5.4; *Scaptotrigona*, 4~8; *Meliponas* 6~8). The duration of discharge subphase (from the beginning of the first discharge to the end of the last one) and post-discharge subphase (from the end of the last discharge to the beginning of queen oviposition)

fixaion by the queen in several selected cases

oviposition in each batch								
9	10	11	12	13	14	15	16	17
1	1	1	1	1	4	1		
4	*2*	*5*	*3*	*5*	*35*	*5*		
2+1	1	1	1	3	3	3	1+1	10
6	*8*	*2*	*3*	*26*	*57*	*19*	*7*	*38*
8+3	32+23*							
15	*123*							
1	11+4	24+19*						
13	*50*	*200*						
4	2	1	2	2+1	1	3	4	
39	*22*	*4*	*3*	*7*	*4*	*13*	*25*	
2+1	1	1	1					
11	*13*	*6*	*21*					
2								
23								

worker insertions in each secondary fixation, together with its duration (in sec.)

fluctuate as follows: Discharge. 3 sec. (4 cases), 4 (2), 5(4), 6(4), 7(7), 8(8), 9(10), 10(11), 11(1), 12(4), 13, 14, 15, 16, 17, 24, 27, 41 (each one case), 19(2), the mean, 11.4 sec. Postdischarge. Less than 1 sec. (3), 1 sec. (14), 2(34), 3(8), 4(2), 5(2), 6(1), 7(1), the mean 2.0 sec. The mean duration of discharge subphase is the shrotest among the species so far studied (*Tetragona,* 17.2 sec., longer in the other species). There is no correlation between the lengths of both subphases.

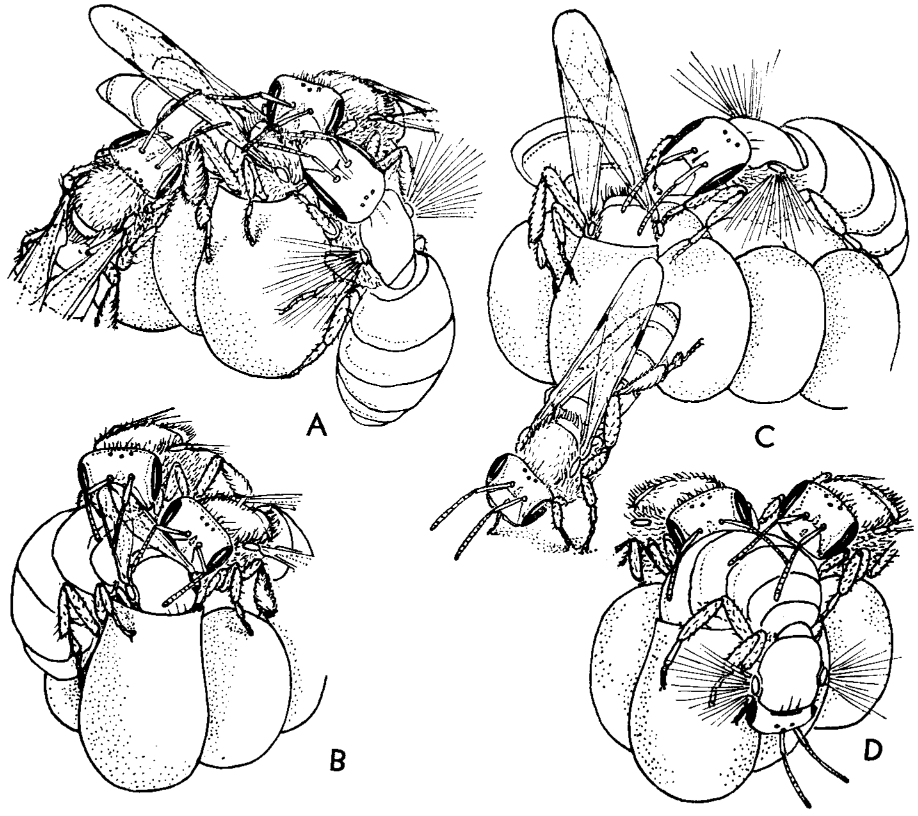

Fig. 3. A. Body insertion by a worker. Quen touching her with antennae. B. Cell inspection by the queen. C. Food discharge by a worker. Another worker (bottom) *escapes* from the cell after discharge. D. Queen oviposition.

During the provisioning phase, the queen inspection always appears soon before her oviposition. Excluding this final inspection, queen inspections appear occasionally during the provisioning phase. The appearance does not correlate to the order of successive ovipositions in a batch. As to food discharges, the higher number of discharges seems to be linked with the appearance of inspections,

Number of discharges	With inspection	Without inspection
2	3	11
3	17	19
4	7	13
5	3	1
6	2	
7	2	

Similarly the prolonged duration of provisioning phase seems to be correlated to the frequent appearance of queen inspections

Duration (in sec.)	With inspection	Without inspection
~ 5		1
6~10	13	30
11~15	11	4
16~20	3	
21~25	2	
26~	3	

In general *POP* in *Duckeola* is very simple and once started, proceeds rapidly, although the duration between batches is very long. The duration since the beginning of the primary fixation to the end of the final oviposition was measured in several batches as follows (number of ovipositions in parentheses, "+" means the presence of cells remained without ovipositions: 4 min. 30 sec. (6+), 8 m 15 s(10), 10 m 20 s (9+), 11 m 25 s (17), 12 m 45 s (10+), 14 m 06 s (17), 14 m 57 s (15), 16 m 23 s (15).

3.3. Queen oviposition and operculation: The behavior pattern of oviposition in *Duckeola* is quite characteristic. After a brief inspection, the queen climbs on the cell, and inserts her metasomal tip (Fig. 3, D). The duration of oviposition is relatively long: 3 sec. (2 cases), 4(10), 5(23), 6(20), 7(21), 8(4), 9(3), 10(1). The mean, 6.9 sec. is distinctly longer than in *Tetragona* (1.9 sec.) and *Cephalotrigona* (instantaneous), slightly longer than in *Scaptotrigona* (4~5 sec.) but shorter than in *Meliponas* (25~32 sec.). During the oviposition the antennae make peculiar movements, consisted of strong upholding followed by gentle lowering. Furthermore, each pair of legs makes characteristic reciprocal twitchings. This twitching, never observed in the other stingless bees, so resembles that made by bumblebee queens at ovipositions (*cf.* Free and Butler, 1959; Sakagami und Zucchi, 1965), that we nearly fell into temptation to guess the phyletic affinity between *Duckeola* and bumblebees.

Operculation of the cell was not observed in many instances. In all 12 cases relatively well recorded, it began soon after the queen oviposition, without delay as frequently observed in *Tetragona* and *Melipona quadrifasciata*. As in the other species, the operculation phase is divided into rotation subphase (a worker inserts the metasomal tip in the cell and work by rotating her body on the cell) and sidework subphase (working at the side of the cell without body insertion and rotation), and the most work is made continuously by a single worker. As in *Tetragona* and *Cephalotrigona*, however, the separation of two subphases is less distinct than in *Melipona* and *Scaptotrigona*. The metasomal tip is often withdrawn already during rotation and the latter often reappears during the side work subphase. One instance is cited (Numerals mean the time in sec. since the end of queen oviposition):

> 0. Beginning of rotation: 35. Metasoma withdrawn but still rotating: 45. Taking sidework posture followed by immediate return to rotating: 60. Side work; 70. Again rotating without metasomal insertion: 80. Side work; 89. Again rotating followed by immediate return to side work: 98. Rotation without metasomal insertion; 106. Sidework; 174. Orifice completely closed.

The end of the initial rotation, irrespective of its later reappearance, was measured: 35, 45, and 48 sec., and the closure of cell orifice in several cases was: 2 min. 31 sec., 2 m 38 s, 2 m 45 s, 3 m 18 s, 3 m 23 s, 5 m 44 s, 6 m 57 s and 7 m 48 s, comparable to the other species except *Tetragona*, the cell closure of which is very inefficient, lasting 11.5 min in average.

After the closure the worker still remains on the cell, making further elaboration. At this stage, one or two other workers participate in the work, or the work is taken over by them. The final moving away of workers was measured in three cases: 12 min. 03 sec., 13 m 31 s, 17 m 48 s.

> In two instances, the queen took the laying posture, but failed to oviposit in the cells, which were made too close to the glass lid of the observation hive. In both cases, the excited alternation of body insertions by workers was observed but the operculation did not take place until the second arrival of the queen and her successful oviposition. Related cases were observed in *Cephalotrigona, Tetragona* and *Partamona.*

Concluding remarks

The behavioral sequence in the provisioning-oviposition process of *Trigona* (*Duckeola*) *ghilianii* agrees with that in the other species so far studied in the succession of several phases, queen fixation, provisioning, oviposition and operculation. A brief comparison of the behavior of *Duckeola* to that of the other species is given here. The characteristics common to the other species are: 1) Frequent body insertions by workers in fixation phase; 2) Rapid succession of larval food discharges by workers; 3) Avoidance reaction of workers after each food discharge; 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. Excluding these items, an ethological description of *Duckeola* with special reference to the oviposition process is given as follows (S, *Scaptotrigona*; *M*, *Melipona*; C, *Cephalotrigona*; T, *Tetragona*; "=", Similar to; "≠" Different from; "≧", Similar but much exaggerated; "≦", Similar but less exaggerated):

1) Queen vibrating wings continuously, not beating (≧T, ≠CSM); 2) Queen often resting with geopositive orientation (≠TCSM); 3) No exaggerated behavioral interactions between queen and workers (=TC, ≠SM); 4) Cell building started by accumulating cerumen on side wall of another cell (≠TCSM); 5) Cell building semisynchronous (≠TCSM); 6) Oviposition process always batched (=S, ≠TCM), but food discharges to each cell strictly successive (≠S); 7) Primary fiaxtion by queen consisted of alternation of wandering and fixing (≦TS, ≠M); 8) Prefixation phase not conspicuous (=T, ≠CSM); 9) Queen not fixing to cell without collar (≠T, =CSM); 10) Frequent, rather ritualized cell inspections by queen during fixation phase (=TC, ≠SM); 11) Queen touching but not violently with antennae workers inserting body into cell (=TCS, ≠M); 12) During provisioning phase, queen not wandering to other cells (≠S), nor making peculiar retreat from cell (≠M, =TC); 13) Worker oviposition and queen oophagy during oviposition process absent (≠TCSM); 14) Queen oviposition shorter than in M, but longer than in STC, accompanied with peculiar twitching of legs (≠TCSM); 15) Operculation begun soon after queen oviposition (=CS, ≠TM); 16) Rotation and sidework subphases in operculation poorly differentiated (=TC, ≠SM).

As already referred to in previous papers, some of these features might vary according to colony conditions. However, many of them are seemingly species-specific. The most characteristic features of *Duckeola* are prolonged wing vibration of the queen with geopositive posture, deposition of cerumen before cell building and leg twitching at queen oviposition. As to the relation to the other species, or groups, common (+) and different (−) characters are enumerated as follows:

	Tetragona	*Cephalotrigona*	*Scaptotrigona*	*Melipona*
+	8	7	3	2
−	8	10	14	15

These figures are still provisional because the distinction of different ethological characters are yet incomplete. But the affinity of *Duckeola* to *Cephalotrigona* and *Tetragona* than to *Scaptorigona* and *Melipona* is undeniable.

The peculiarity of *T.* (*Ducekola*) *ghilianii* among *Trigona-Tetragona* Complex was stated either morphologically (Moure, 1951, 1963) or cytologically (Number of chromosomes is in *Duckeola* $n=15$, whereas $n=18$ in all other stingless bees so far studied, except *Frieseomelitta* with $n=9$, *cf.* Kerr, 1963, Kerr and Lello, 1964, Kerr and Maule, 1964). The observations given in the present study confirm, together with the peculiar nest architecture of this species (Kerr *et al.* 1957), that *T.*(*D.*) *ghilianii* forms an aberrant monospecfiic offshoot within *Trigona-Tetragona* Complex.

A few words must be given as to an important outcome from the present study, the absence of worker oviposition during the oviposition process, in contrast to many other species so far studied (cf. Sakagami *et al.*, 1963, Sakagami e Zucchi, 1966). It might be suspected that this negative evidence merely indicated the condition particular to the colony studied. But we believe this character is species-specific. Throughout the observation period no worker with developed ovaries was discovered in the colony, in contrast to many other stingless bee groups, nevertheless the colony continued without marked dwindling. Moreover, in several species of the subgenus *Frieseomelitta*, which is close to *Duckeola*, we observed no worker oviposition and no ovarially developed worker under queenright condition (unpublished). Therefore, it is certain that some groups of *Trigona-Tetragona* Complex behave just as in the honeybees, in which worker ovaries are inhibited by the queen (reviewed by Butler, 1967). The inhibition of worker ovaries by the queen in the honeybees and the intake of worker born eggs by the queen in many stingless bees are regarded two different ways of the control of the colony organization. In a recent paper, one of us (S.F.S.) held an opinion that the honeybee type control was more primitive than the stingless bee type control, naturally in the sense of the chronological sequence, not of biological "elaboration", because the queen inhibition was confimred in socially more primitive halictine bees, just at the dawn of the caste system (Sakagami and Hayashida, 1968). If this opinion is correct, *Duckeola* and *Frieseomelitta*, with honeybee type social control, are regarded to represent the stage more primitive than that shown by other stingless bees, with stingless bee type social control. This assumption favors to the opinion by Kerr, who regards *Frieseomelitta* as the most primitive group in the stingless bees. However, the secondary change from stingless bee type to honeybee type control is not absolutely excluded, however very improbable. Based upon various morphological characters, Wille (1963) regards small *Plebeia*-like stingless bees as the type closest to the ancestors of stingless bees. For the time being, our results seem to support Kerr than Wille, but the establishment of the most plausible phylogeny of stingless bees requires a synthesis of various characters, where oviposition behavior and social control types must be regarded, at least as important as many other characters.

Summary

The oviposition behavior of *Trigona* (*Duckeola*) *ghilianii* Spinola, 1853, an aberrant species within *Trigona-Tetragona* Complex of the stingless bees, was observed by using a colony taken near Manaus, Amazonas, and reared in Rio Claro, São Paulo, in an artificial hive. In many behavior characters, the species resembles *T.* (*Tetragona*) *clavipes* (Fabricius) and *Cephalotrigona femorata* (Smith), especially in the lack of exaggerated interactions between queen and worker, frequent, nearly ritualized cell inspection by the queen before food discharges by workers, and poor differentiation of rotation and sidework subphases in cell operculation. The characteristic features of this species are exclusive batch formation in oviposition, prolonged wing vibration by the queen with geopositive orientation, absence of worker oviposition and leg twitching by the queen at oviposition. By the absence of ovarial development in and oviposition by workers under queenright condition, this species is assumed to have the honeybee type social control by the queen, representing a primitive stage, in contrast to many other stingless bees with the stingless bee type social control.

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