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**Behavior studies of the stingless bees, with special
reference to the oviposition process. V. *Melipona
quadrifasciata anthidioides* Lepeletier¹⁾²⁾**

By

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(With 7 Text-figures and 1 Table)

Introduction	579
1. Queen-worker behavior in non-oviposition phase	579
2. Cell construction	581
3. Interval between two successive ovipositions	586
4. Queen fixation	591
5. Provisioning, worker oviposition and queen food intake	596
6. Queen oviposition and operculation	603
Concluding remarks	604
Summary	606
References	606

1) This paper is dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, Japan, in honor of his sixtieth birthday, June 21, 1966.

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As the fifth report of our serial work on the behavior of the stingless bees, especially in the provisioning and oviposition process, the present paper deals with *Melipona quadrifasciata anthidioides* Lepeletier. Some general remarks on this serial work was given in the first report (Sakagami & Oniki, 1963).

Melipona quadrifasciata Lepeletier is one of the commonest *Melipona* species in the southeastern Brazil, distributing along the coastal states from Pernambuco to Rio Grande do Sul. The southern populations form *M.q. quadrifasciata*, while the northern ones are *M.q. anthidioides*. The both subspecies meet approximately at the State of São Paulo, where they form a wide hybridization zone (Kerr, 1951; Moure & Kerr, 1950). The observations presented in this paper were made using a reasonably large colony of *M.q. anthidioides*, which had been captured in Rio Claro, S.P. and maintained for years in the apiary of the Department of General Biology of the Faculdade de Filosofia, Ciências e Letras de Rio Claro. For observations, the colony was transferred to an artificial hive designed by one of us (Cf. Sakagami & Zucchi, 1963). During the observation period, the queen was once replaced on January 23, 1963, for reasons described later.

The behavior patterns of both queen and workers at and between ovipositions by the queen were in general similar to those in the other species already described, so that the description of qualitative aspects will often be given briefly. On the other hand, some quantitative data will be presented in detail. This species is, at least in the Southern Brazil, together with *Nannotrigona (Scaptotrigona) postica* (Latreille) and *Trigona (Tetragonisca) jaty* Smith, one of the stingless bees which are most easily reared under artificial conditions. Therefore, we could observe this species more closely than any other stingless bees except *N. postica*. These data are recorded in detail, because this species, being adaptable to artificial hives, so is likely to be used for future experimental studies. Close observations and descriptions of behavioral aspects are basic to any such work. Henceforth the name of this subspecies and other forms already reported and cited in text for the comparison will be abbreviated as follows:

Melipona compressipes manaosensis Schwarz (Sakagami & Oniki, 1963)=*compressipes*

M. seminigra merrillae Cockerell (Beig & Sakagami, 1964)=*seminigra*

M. quadrifasciata anthidioides Lepeletier=*anthidioides*

M. q. quadrifasciata Lepeletier=*quadrifasciata*

Nannotrigona (Scaptotrigona) postica (Latreille) (Sakagami & Zucchi, 1963)=*postica*

Cephalotrigona femorata (Smith) (Sakagami, Beig, & Kyan, 1964)=*femorata*.

The order of descriptions follows the arrangement adopted in the previous papers. The term, *provisioning and oviposition process* is henceforth abbreviated *POP*. Some related discussions are included in each section.

1. Queen-worker behavior in non-oviposition phase

The behavior of queen in the non-oviposition phase is basically identical to that in *compressipes* and *seminigra*, showing alternation of resting and walking. She often prefers some definite places for resting, usually more or less dark areas such as below combs or between pots. In contrast to all stingless bees so far observed by us, the queen does not perform the regular wing beats. Of course, she moves the wings from time to time, but without regular rhythm neither consisting of simple

strokes as in *compressipes* nor tending of vibrations as in *seminigra*. This lack of wing beats was confirmed in two queens successively observed in the observation hive and in a few others in the outdoor colonies kept in Nogueira-Neto type hives (Nogueira-Neto, 1953).

The responses of workers to the queen are similar to those observed in other congeneric species, including: 1) Gradual formation of royal court when the queen stays quiet for a while. 2) Repetition of rapid advances and retreats by attendants, 3) Escape by workers at a sudden encounter with the approaching queen (This is especially conspicuous when the queen suddenly appears in front of workers, resting in the areas remote from the combs such as pots and outer involucra). 4) Gradual retreat by workers which perceive the approaching queen (even the workers only 3-4 days old with still whitish mesosomal hairs and pale integument escape from the queen.) 5) Rapid dashing of workers to the queen was occasionally observed, but wing beats and exaggerated raising of fore-body by attendants as seen in *postica* did not appear. One important difference exists in the touching by the queen of the workers which crouch in front of her. As previously reported, this is seen both in *compressipes* and *seminigra* but neither in *femorata* nor in *postica*. In *anthidioides* this behavior is seen far more frequently and in a more elaborated manner than in two other *Meliponas*: In front of the approaching queen, a worker often makes a short dashing movement, then she crouches with legs tightly in contact to the sides of the body. In contrast to *compressipes* and *seminigra*, workers of which put their faces rather oblique-dorsally (Cf. Fig. 1 in Sakagami & Oniki), the *anthidioides* workers makes a bow in front of the queen, presenting her vertex to the latter. Then, the queen raises the head and taps the worker's vertex with antennae, often accompanied by forelegs (Fig. 3A). Her mandibles are opened during the manipulation, touching or occasionally mildly chewing the hairs of the vertex. This process continues 1-3 sec., during which the worker maintains her akinetic posture. This vertex tapping is seen everywhere but most frequently on the comb, especially before the queen fixes to the cell ready to be provisioned or after her oviposition.

The queen often begged for food to the workers and buccal contacts between them were occasionally seen. But throughout the observation period, lasting more than three months, we confirmed no definite food delivery of workers to the queens, at least in the form seen in the honeybees.

Being a member of the genus *Melipona*, the virgin queens emerged in high frequency. All these virgin queens are attacked and killed by the workers within one or two days (Cf. Kerr, 1951). The attacks were done as in *compressipes*. Often several workers participated in such attacks, pulling the queen's legs on opposite directions, attempting to cut off her head, or licking her with extended tongues. The lack of any resistance in the attacked queens is also the same to that in *compressipes*.

No systematic observation was carried out on the other aspects of worker behavior. The following two notes are cited here as basic data for the further comparison: 1) Twisting

of heads in the workers receiving regurgitated food was seen as in *compressipes*, but not in such an exaggerated manner as in *postica*. 2) Fanning at the hive entrance is done as in *compressipes*, but the metasoma is not so highly raised and extended as in the latter, rather being slightly bent downward. The last difference is somewhat comparable to that seen between *Apis mellifera* Linné and *A. cerana* Fabricius (Sakagami, 1958).

2. Cell construction

According to Kerr & Santos Neto (1956), cell construction and provisioning of the brood cells are performed in *quadrifasciata* by the workers of respectively 9–21 and 12–21 days old, that is, the same age group. This may be applied to *anthidioides*, too, though no systematic observation with age-marked workers was carried out. All workers participating in cell construction and provisioning were still young. Their mesosoma was, together with hairs, already black, but the metasomal terga, especially parts anterior to yellow bands in each tergum were still brownish. Apparently the same individuals can participate both in cell building and provisioning. Most of them were, as reported elsewhere (Sakagami et al., 1963), provided with well developed ovaries. *Anthidioides* is a typical comb maker like all other species of the genus, the nest structure of which is known. Development of comb is successive, and that of each comb concentric, although spiral combs are rarely found. The manner of cell construction is the same as that in other species already reported. Fig. 3A, B, C and Fig. 1 show the successive stages of cell construction. As in other species, the cell construction reaches the final stage by the formation of the collar at the cell orifice, being 1 mm or more above the comb surface. The time required for the completion of one cell was not accurately measured but was approximately 1 to 2 hours, always less than 3 hours, except for some cells which were begun but abandoned when half completed. The duration is shorter than in *compressipes* (2-4 hours), apparently due to the larger colony size. Correspondingly the number of half-built cells at a given time was larger than in the latter species, usually 3–4, often 6–7 cells. Such cases are due to the simultaneous growth of two combs in the nest. In such instance, either both combs grew in parallel, or one progresses more rapidly than the other. As in the other species already reported, *anthidioides* makes each cell successively, not synchronously, so that there are cells of diverse growing stages at a given time, from those just started to the fully collared. Sometimes because of the upward growth of the combs, cells were constructed too close to the glass lid of the hive, for instance, only 4 mm distant from the glass. In such cases, the cell construction ceased halfway, or half-built cells were destroyed after remaining 1–2 days unused.

As in other species, each cell grows not through the continuous and exclusive activity of a single worker from start to collar formation, but through the successive activities by a number of workers. One worker may leave a cell after a time and may change to other tasks such as building of another cell just left by one worker or elaboration of comb surface. But it is also true that one worker frequently

works on the same cell. Based upon the comparative observations in diverse species, especially in *postica*, it is certain that the stingless bee workers of definite age classes tend to remain more or less in definite areas in the nest, apparently in part due to the architectural peculiarities of the nest in contrast to the homogeneous combs of honeybees, in which house workers very often migrate from one area to the other within the brood area. Therefore the workers on a growing comb tend to stay there, thus having more probability to visit a same cell frequently.

The work duration by the successive builders of the same cell gradually increases parallel to the growth of the cell, four representative phases of which are shown in Fig. 1, each taken from 30 min. continuous record of some particular cells. In Fig. 1, A, which deals with a cell still 1/4 of the height of the comb, the cell was unoccupied most time. A similar record of a cell 1/3 the height of the comb (Fig. 1B, observed synchronously with A) shows the increased building activity.

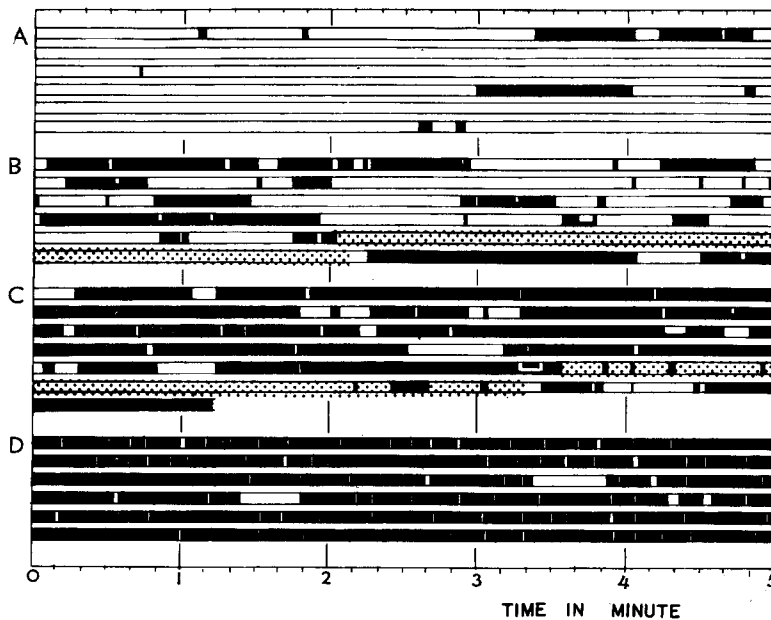


Fig. 1. Four 30 min. continuous records of the successive occupation of cells in different growing stages. A, A 1/4 built cell (Nov. 2, 13: 09-39); B, A 1/3 built cell (Nov. 22, 13: 09-39); C, A 4/4 built cell, that is, the cell margins reached the comb surface (Nov. 29, 10: 00-31); D, A collared cell (Nov. 29, 16: 23-53). White: Unoccupied; Black: Occupied; Half-height white: Reoccupation by the same individual after a brief departure; Dotted: In B, the presence of the queen on the comb. In C, the occurrence of the oviposition process at another cell on the same comb. Intervals less than 1 sec. were rounded to about 1 sec.

The length of such activity distinctly increases except when the queen is on the comb, during which the workers showed either positive (court formation) or negative (escape from or mild avoidance to the queen) responses to the queen, resulting in a decrease of cell construction and occupation. In a 4/4 cell, that is, a cell reaching level of comb surface, the cell is occupied nearly throughout the 30 min. period (Fig. 1, C), except for the 24-29th min., during which a *POP* (=provisioning and oviposition process) occurred at another cell on the same comb, about 3.5 cm part from the cell observed. This process attracted the attentions of nearly all workers on the comb surface, resulting in the decreased building activity.

The final stage (Fig. 1, D) at or after the collar formation, is characterized by the nearly continuous cell occupation, decrease of the time of each occupation (mostly less than 15 sec.) and immediate alternation of occupants. Naturally the number of occupation, duration of each occupation and interval between two successive occupations varied from case to case.

Especially Fig. 1, A, illustrates rather an extreme case. The cells are usually more frequently occupied even at such incipient stages. Nevertheless, the figures sufficiently show the general trend seen during the growth of one cell.

The alternation of individuals occurs, except for the final stage, in the following three ways: 1) The occupant leaves the cell by the probable loss of the motivation to continue the work. This is revealed by her subsequent participation in other tasks. 2) The occupant leaves the cell to collect further building material, usually near the cell but occasionally up to 3-4 cm distant from the cell. If the cell remains unoccupied when she returned, she again occupies the cell (Fig. 1, B and C, half-height white blocks). But often another individual occupies the cell during the absence of the ex-occupant. 3) The occupant leaves the cell probably to collect building material. But after collecting for a while, she changes her work and participates in other tasks, probably caused by the other releasing stimuli. This case is distinct in typical instances, but often difficult to distinguish from the first.

From these three types of alternation, together with other observations partly mentioned above, it is speculated that the cell construction is governed by the following mechanism. This explanation is still assumptive, given here as a mere hypothesis for the future studies:

1) The appearance of each particular response is governed both by external and internal factors. Various releasing stimuli may be provided from cells, other workers, queen, etc. As internal factors, we would like to distinguish general physiological conditions (=general motivation) and specific motivation. The general motivation is defined as the internal conditions, which make possible, if the appropriate external stimuli are given, the release of certain responses. In the present case, the general physiological conditions are regarded partly dependent on age, therefore, exactly said, age-specific general physiological conditions, although the alternation of such conditions from one age class to the next is obviously gradual. For instance, each comb worker is under general physiological conditions, by which they are capable to make some response groups, such as

building response group (cell building, elaboration of comb surface, formation of pillars and involucrum, etc., each further consisting of elementary motor units), *POP* response group (provisioning, worker oviposition, operculation, etc.), etc, but still incapable or difficult to make other response groups such as guarding, foraging, etc.

2) Under such general physiological conditions, each response is released by specific motivation, which is the internal conditions make possible the release of definite response or response sequence, again if the appropriate external stimuli are provided. For instance, a comb worker participating in *POP* is under *POP* motivations, but her building motivation is temporarily blocked at such time. Obviously, the strength of each specific motivation may vary both temporarily and individually. It is known whether or not each particular response (within each response group) is governed by corresponding specific motivation. This may be true in some cases but may be not always in others. On the other hand, it is possible that in some responses, which are superficially simple but really of compound nature, more than one specific motivation participate in the release, or the specific motivation has itself a layer structure, composed of more than one level.

3) In the course of cell occupation, the building (specific) motivation (controlled by the general physiological conditions and external releasers) in each cell occupant may decrease (resulting in the change of the task as in case 1 mentioned above) or may persist resulting in the collection of building material, where it may be directed to other tasks affected by other releasers (case 3) or may still continue until the bee returns to the cell and re-occupies it (case 2). The whole process of cell building may proceed as the integration of such individual contributions.

4) Except for the final building stage, there is no cooperation in the strict sense among builders, that is, there is no communication device which reinforces the process. The presence of one worker at the cell might serve as a releasing stimulus, but the process continues mainly through independent activities of each worker.

5) The cell building is performed under lower excitement than in *POP*: In the latter, many comb workers concentrate on the comb and especially around the queen and the cell to be provisioned. Their movements are rapid and very excited. In cell building, except for the final stage, the number of workers on comb is small and their movements are more sluggish.

6) The relation between cell building process and *POP* is remarkably different from that in the honeybee as follows:

Honeybee	<i>M.g. anthidioides</i> and other stingless bees so far observed
Cell building is made independently from provisioning, mostly by other worker groups	Cell building is always followed by provisioning and both performed by the same worker group
Queen oviposits before provisioning by workers (O→P)	Queen oviposits after provisioning by workers (P→O)

Provisioning is progressive, continuous during six days. Therefore oviposition-provisioning is a slow process like cell building process

Provisioning is in mass, given at one time. Therefore, provisioning-oviposition is a rapid process in contrast to cell building process

At oviposition and provisioning workers do not show particularly excited attitude

At oviposition and provisioning, workers show very excited attitude

The final stage of the cell building process is henceforth called *prefixation phase*, because the behavior pattern of the workers in this stage becomes similar to that in the next phase (fixation phase) described later. This phase is characterized not only by the rapid and uninterrupted alternation of occupants, but also by the formation of a group of workers surrounding the cell and their excited attitude (Fig. 3 D). Such attendants appear already at the 4/5 stage, but their number increases at this stage. In two observations cited in Fig. 1 (C and D), the number of such attendants was counted every 15 sec. The frequency distribution in both cases shows that the attendants are distinctly more numerous at the prefixation phase:

Stage in cell building	Number of attendants counted every 15 sec.							
	0	1	2	3	4	5	6	7
4/4-cell stage (Fig. 1, C)	90	24	5	1				
Final stage (=Pre-fixation phase)	10	0	11	28	32	26	16	6

These attendants have a very excited appearance. Facing the cell, the forebody is pushed forwards, then pulled backwards. During this repetition of for- and backwards movements, the mandibles are half opened and, together with the forelegs, manipulate the outer surface of the cell walls. Except for the trembling of the body, the whole behavior pattern somewhat resembles that of the rocking movements in honeybees. These attendants are responsible to the immediate alternation of occupants cited above. One of these attendants immediately replaces the occupant when the latter leaves the cell. The cell occupation is like that already reported for other species; the body is inserted into the cell up to the anterior 1/4 or 1/3 of the metasoma.

The occurrence of this excitement, the prefixation phase, was confirmed in all species so far observed. Hence it is regarded as characteristic to the stingless bees. Probably the excited movements originated from the cell-building behavior and still might retain partly its original significance by smoothing the cell walls. It is probable, however, that the movements changed their initial significance and took the character of a signal response for the queen conveying information about "cell is ready".

One instance is cited as evidence for this assumption. On Nov. 29, at 15:53, one cell was ready to be provisioned. The queen fixed to it and the provisioning would have

taken place, in all probability, within a few seconds. At that time we were obliged to change the glass lid of the hive for another purpose. The queen and workers fell in a momentary confusion and the process was interrupted. About 10 min. later, the formation of attendants to this cell appeared once again and lasted nearly 30 min., although the cell was structurally ready to be provisioned. This suggests that the attendance has a significance more than a mere final elaboration of the cell. Probably the excited attitude of attendants reciprocally increases the excitation necessary to *POP*.

It is difficult to determine the precise length of the prefixation phase, because the excitation develops gradually. Some measurements of the time between approximate beginning of excitation in attendants and queen arrival were as follows (in min): 4,4,5,8,12,15,18,22,34,45,78. The values show great variability, with mean 22.27 and *s.d.* 22.6. One closely observed instance is cited in Fig. 4, C, in which the duration of each body insertion by encircling workers was precisely recorded starting with the approximate beginning of excitation in attendants. The general trend well corresponds to that in Fig. 1, D. In some prolonged cases such as the three final examples cited above, the strength of excitation, recognized qualitatively by the movements of workers, was not always maintained at the same level throughout, showing irregular fluctuation.

When there were two cells ready to be provisioned, it was occasionally seen that the prefixation excitement appeared simultaneously at both. In such instance, however, as soon as the queen arrived and fixed to one of the cells, the excitement was concentrated to this cell alone, which absorbed all attendants of the other.

3. Interval between two successive ovipositions

Before dealing with each successive phase in *POP*, the interval between two successive *POP* is mentioned. As in *compressipes* and *seminigra*, and in contrast to *postica*, each cell is provisioned one by one, in general with considerable intervals. The best index of such an interval is obviously the time between two successive queen ovipositions. But as we have more accurate data on the arrival of the queen to the cell (=the beginning of the queen fixation), the intervals were obtained from the time between the queen arrivals in two successive *POP*. The frequency of these intervals distributed as follows (in min.):

Interval	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	101-200
Frequency	13	2	5	9	8	8	5	8	1	2	9

It is seen that intervals concentrate in two ranges: one in 0-10 min. (exact records are respectively 2,4, 7,8 min., each one case; 5 min., 4 cases; 10 min., 5 cases) and the other in 31-80 min., although there are some cases in which the intervals are longer than 100 min.

The short oviposition intervals appeared when there were two cells ready to be provisioned. In such cases the prefixation excitement often appeared at both.

In some of these instances, the queen after finishing her first oviposition fixed to the second cell without leaving the comb, or returning to the comb after a brief departure. One record of such rapid succession is shown in Fig. 4, B. These cases obviously indicate the presence of at least two mature eggs in the ovaries of the queen. By the occurrence of such rapid succession of two ovipositions, *anthidioides* approaches to *femorata*, in which each oviposition occurs always successively and often with short intervals. While in *femorata*, however, the succession of three or rarely four ovipositions with short intervals was observed, the rapid succession of more than two ovipositions was never seen in *anthidioides*. Fig. 2 shows the relation between two successive intervals, taken from the records in which the time of the queen arrival in at least three successive ovipositions was accurately noted without interruption of observation. In most cases, namely in 24 out of 32, one interval is distinctly longer than the other. On certain days, four successive ovipositions were observed continuously, the intervals were recorded (in min.): 75-35-36, 7-101-51, 30-80-20-65, 70-25-95, 50-80-40, 60-5-60, 35-110-1, 50-120-10. Although the data are still insufficient, it appears that the length of intervals often tend to alternate in successive ovipositions.

It would naturally be possible that the intervals become shorter under favorable conditions. But it is also certain that the oviposition rate in *anthidioides* does not increase too much beyond the level observed by us. One of us (W.E.K.) once obtained, in another normal colony kept in Nogueira-Neto type hive, the mean oviposition rate of 13/day and the maximum rate 22/day (Kerr, 1948. The mean

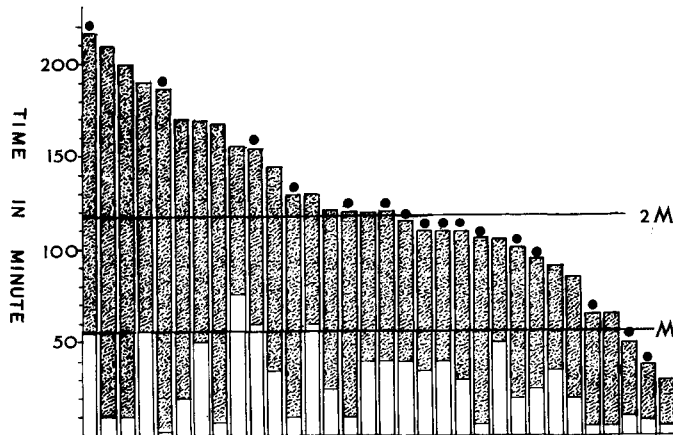


Fig. 2. Relation of intervals in three successive ovipositions. The shorter intervals are given at bottom of each histogram, irrespective of actual chronological sequence. The cases "first interval longer than second" are shown with black circles, the opposite cases not marked. M and 2M are respectively 56 min. (=Mean interval of 72 observations and 112 min. (Doubled mean interval) levels.

rate in another species, *M. rufiventris* Lepeletier as higher, 45/day). The mean interval between two successive ovipositions was 56 min. in our observations. This approximately corresponds to one oviposition per hour, or 24/day, that is, higher than the maximum rate in the colony mentioned above. At any rate, it is obvious that *anthidioides*, or the genus *Melipona*, does not show the high productivity as seen in other prolific stingless bees such as *Trigona* (s. lat.)

The oviposition rate in insect societies is determined by the capacities of both queen and workers. In the honeybee, often the potential oviposition rate of the queen, which exceeds 1,000 or even 2,000 per day under favorable conditions, cannot be realized when the worker population is insufficient. In stingless bees, too, such interrelation between both castes exists. Leaving detailed studies for the future, here is cited an example of the queen productivity without corresponding increase in worker activities, resulting in the multiple ovipositions in one and the same cell.

This phenomenon, which had already been noticed by one of us (W.E.K.) and also by Dr. P. Nogueira-Neto and Mr. R. Zucchi, was especially frequent in two outdoor normal sized colonies kept in Nogueira-Neto type hives. One conspicuous case is shown in Fig. 3, O, left, in which many cells contained 2-3 or even 4 eggs. The fact that such cells are concentrated at the center of the comb suggests the temporary nature of such overproductivity in the queen. This assumption was revealed by inspecting the comb below that cited above. In the lower comb, the central area contained larvae while the periphery had eggs. All 86 egg cells contained only one egg, except for one cell which had three. From the concentric and upward oviposition gradient in *anthidioides*, it is certain that the queen oviposition was made in the order of normal-multiple-normal. Expecting to observe such multiple oviposition, the queen in the observation hive was replaced on Jan. 23, 1964 by the queen mentioned above.¹⁾ But after introduction into the observation hive, the second queen laid always one egg per cell and her behavior seldom differed from that of her predecessor. It is still premature to make a general conclusion on the fate of such eggs laid in the same cell. But in the central area of the lower comb containing larvae of diverse stages cited above, we found only three cells with two larvae, and only one cell with three larvae. All other cells contained a single larva. In three cells with two larvae, two had one well fed living larva and one dead larva, and the other had two medium sized living larvae. In the cell with three larvae, only one larva was well fed and alive. Therefore, it is highly probable that after such multiple oviposition only one larva per cell can survive to the pupation, as is the rule in multiple oviposition in the same cell by the laying workers of the honeybee. In such multiple oviposition, all eggs were located at the center of the cell, each being erect on the larval food and tightly in contact with one another (Fig. 3, O, right).

1) The requeening was tentatively made simply by removing the one and immediately introducing the other on the comb. Throughout this procedure, the second queen showed no excitement and the workers immediately adopted her with no detectable excitement. Thereafter the worker's attitude to the substituted queen did not differ to that to the predecessor. It is still uncertain whether such peaceful adoption at the direct requeening is the rule in *anthidioides* or not.

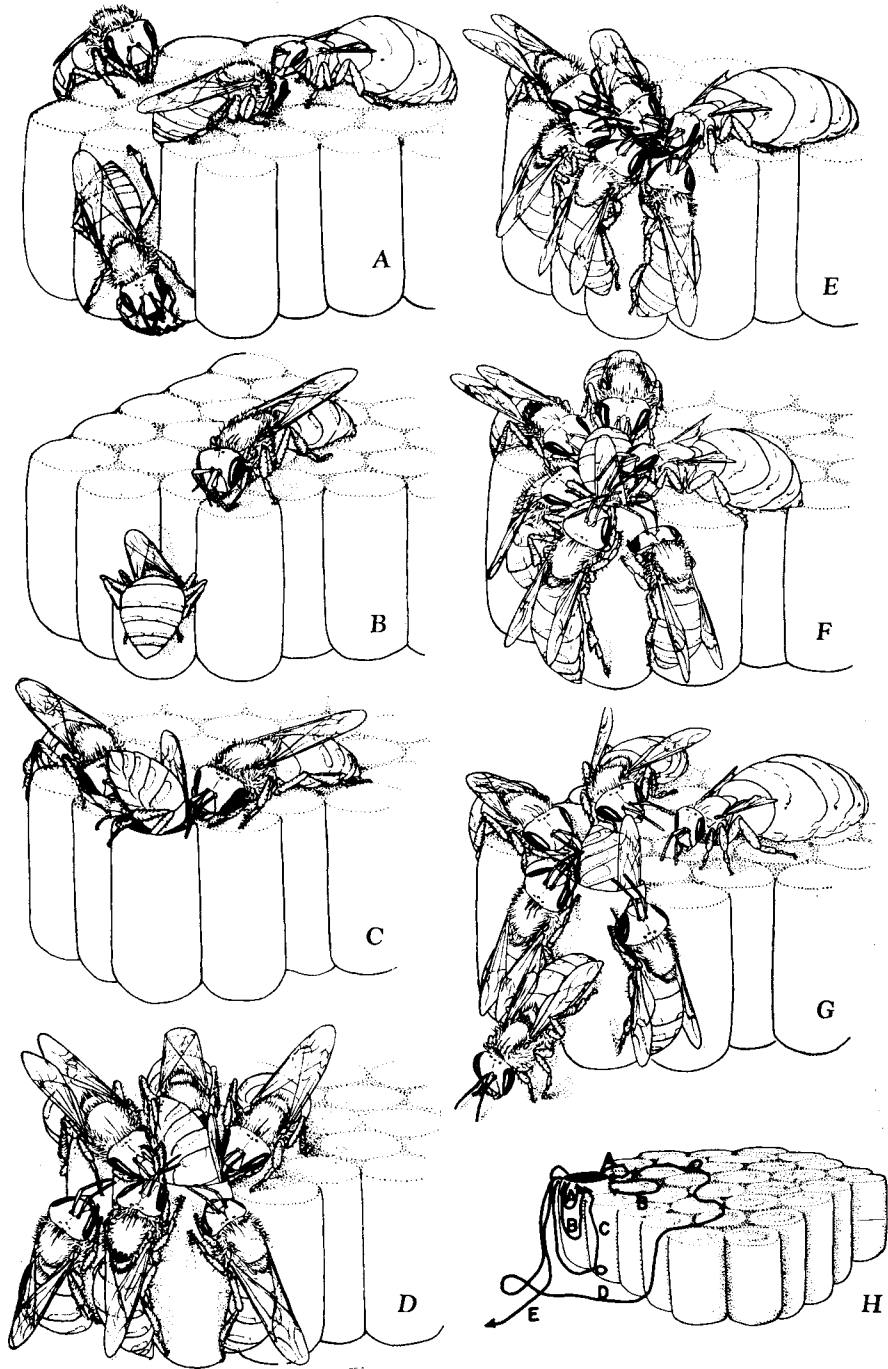


Fig. 3 (Continued)

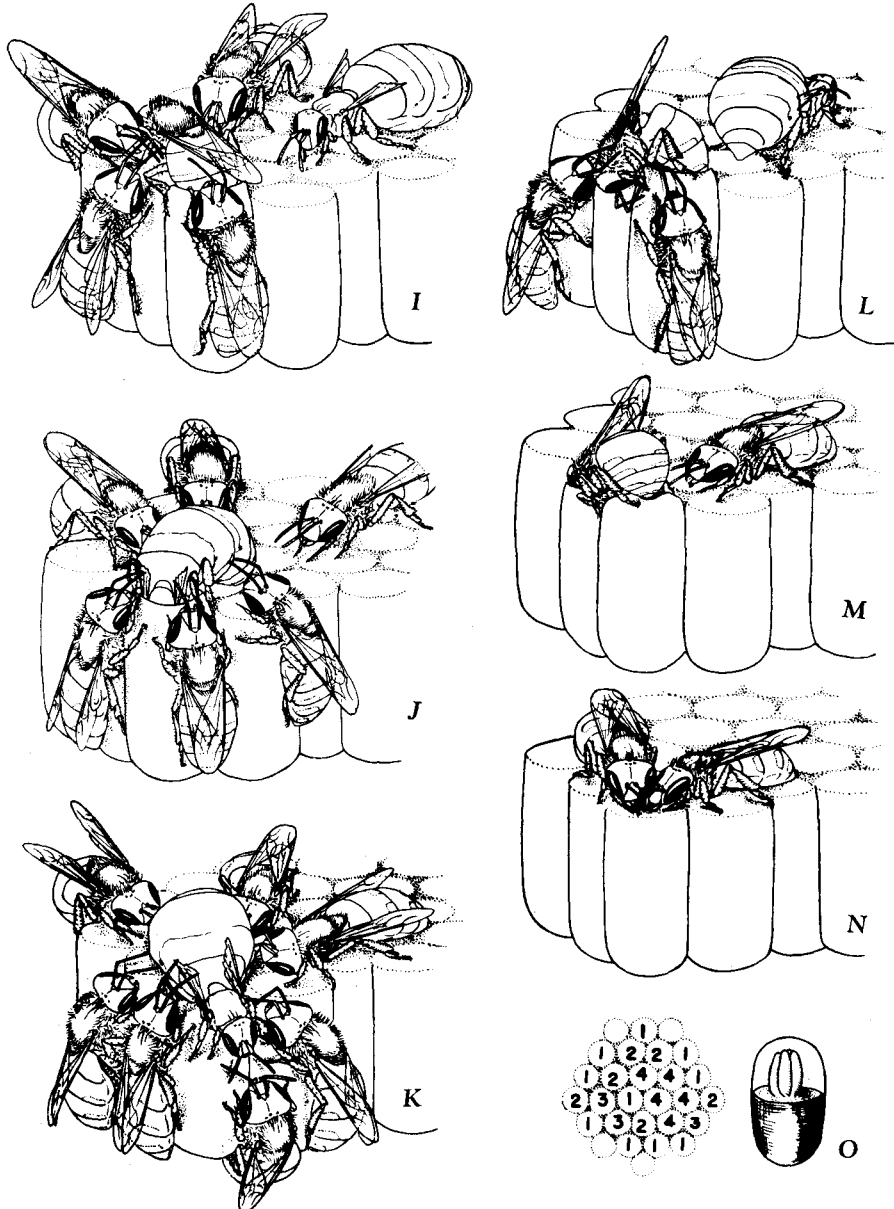


Fig. 3. Behavior sequence from the beginning of cell construction to the completion of operculation; A, Tapping of the vertex of a crouching worker by the queen on comb. One cell is started by one worker (left-below); B, Gradual growth of a cell. The cell reached about 2/5 of the height of comb, occupied by one worker. Another worker manipulating (*continued*)

4. Queen fixation

The *POP* in the strict sense begins by the arrival of the queen at the cell ready to be provisioned and finishes by the operculation of the oviposited cell. Some continuous records of representative cases are shown in Figs. 4 and 5, which deal with the temporal sequence of the process, with observation errors of about ± 0.5 sec. in short term behavior such as each food discharge and ± 1.0 sec. in long term behavior²⁾. The behavior of the queen is indicated above, that of workers below the base line.

When one or more cells are being constructed, the queen visits the comb from time to time, and inspects the growing cells. But she leaves the cell unless it is structurally ready to be provisioned, and unless there is prefixation worker excitement described in Section 2. One example of such repeated visit is cited: Nov. 20 (*V*=visiting, *L*=leaving the cell), 15: 26 *V*, 15: 27 *L*, 15: 28 *V*, 15: 29 *L*, 15: 35 *V*, 15: 36 *L*, 15: 45 *V* and fixed.

In *compressipes* and *seminigra*, especially in the former, the fixation was rather irregular. Often a structurally completed cell remained for a considerable time without prefixation excitement. On the other hand, the queen often fixed to the cell still without prefixation excitement, which developed later and resulted in food discharges, or, still in other cases, did not develop until after the queen abandoned the cell. Further, repetition of fixing, departure and refixing was frequent. All these irregularities suggest the lack of coordination between queen and workers, probably caused by the weak and abnormal colony state in these Amazonian forms observed under unfavorable conditions. In *Anthidioides*, however, such irregularities were relatively rare, both in the queens observed successively. The structurally ready cells always evoked the prefixation excitement, and the queen fixed only to such cells. In other words, the repeated fixing and departures

2) In a previous paper (Sakagami & Oniki, 1963) on *compressipes*, the worker body insertion are shown by white blocks but in captions erroneously explained as vertically striated blocks.

the comb surface; C, The cell reached to the level of comb surface, occupied by one worker. Two other workers attending upon the cell; D, Prefixation phase at a fully collared cell. One worker inserting into the cell, encircled by excited attendants; E, Arrival of queen at the cell; F, Queen tapping the inserting worker with her antennae and fore legs; G, Food discharge (at center), retreat of queen (right) and *escape* from the cell after discharge (left-below); H, Various types of *escape* response after workers withdrew from the cell; I, Worker oviposition; J, Queen oophagy (the same posture is taken at insection and food intake); K, Queen oviposition; L, Beginning of operculation; M, End of rotation subphase; N, Side work subphase; O, Multiple oviposition into the same cell by a queen (Left, Distribution of number of eggs in a comb; Right, Position of eggs laid in the same cell).

were relatively rare. Among 30 instances, in which the prefixation phase was more or less closely observed, the queen abandoned the cell halfway only in one instance (Fig. 4, A).

The duration of queen fixation varied as follows (in sec.) with the mean being 116 sec. and *s.d.* 46.69: 21, 31, 51 (2 cases), 60, 80 (2), 81, 82, 85, 86, 90, 94 (2), 101, 104, 120 (2), 125, 149, 185, 210, 214, 266, 340, 397 (2) 479, 626. Two extreme instances are shown in Fig. 4, B and Fig. 5, D. The prolonged fixation may be related to the lower readiness of the workers to elicit food discharges. Further, such lower readiness might positively correlate to the time spent since the food discharges in the preceding *POP*. As our data on the time of food discharges were less sufficient than that of the queen oviposition, the correlation between duration of fixation phase and time spent since the last *POP* (=interval between two successive ovipositions in the preceding section) was sought as far as the latter is known. But the data show no correlation ($r = -0.18$) within the limit of our sample size.

In general, the fixation did not continue so long as in *compressipes* and *seminigra*. In these species, besides frequent repetition of fixation and departure, the final fixation often lasted for a considerable time. This may result from the unfavorable colony conditions suggested above.

The behavior of queen and workers in the fixation phase is similar to that in *compressipes* and *seminigra*. On arrival on the comb (Fig. 3, E) the queen sits either horizontally on the comb surface, facing the cell to be fixed, or clings vertically to the outer walls of the cell. The cell is inspected by rapid antennal touchings. The workers inserting to the cell receive violent tapping from the queen by means of antennae and forelegs (Fig. 3, F). Further, the queen occasionally inspects the cell by inserting her forebody. The difference of queen behavior from *compressipes* and *seminigra* in the non-oviposition phase is maintained during *POP*, too: The vertex tapping is very frequent after the arrival of the queen on the comb but before fixation, as well as after her oviposition and before departure from the comb. At cell inspections, the queen occasionally shrugs her wings but does not make regular wing beatings as seen in the other two species. The peculiar chasing behavior found in *seminigra* was never seen. On arrival of the queen, the excitement of comb workers increases. A distinct court encircling both queen and cell is formed. The workers show the advance-retreat responses to the queen as in the non-oviposition phase, but in a more vigorous manner. Meanwhile, the rocking movements around the cell increase their excited appearance. Thus, the general excitement on the comb grows greatly as in *compressipes* and *seminigra* but not so exaggeratedly as in *postica*.

The successive body insertions of workers continue and, judging from their movements, their excitement increases. But, probably due to the avoidance component in the response to the queen, the duration of each insertion tends to decrease, whereas the interval between two successive insertions increases. The

durations of accurately determined insertions and intervals between them were gathered from all available records, the frequencies of which are distributed as follows:

Range of interval (in sec.)	Relative frequencies (in %) of durations														Total number observed
	≤1	≤3	≤5	≤7	≤9	≤11	≤13	≤15	≤17	≤19	≤21	≤30	30 <		
Insertion in fix. phase	19.3	31.3	27.8	5.7	5.7	3.7	3.7	2.0	1.2		0.4		0.4		244
prefix. phase	0.5	2.6	10.0	13.8	17.0	12.3	10.6	12.3	4.8	5.3	4.8	3.0	3.0		279
Interval btw. insertion in fix. phase	7.2	32.3	17.9	11.1	10.4	7.9	5.7	0.7	1.2	0.7	1.4	3.5			188
prefix. phase	86.3	12.2	1.0	0.5											189

The decreased duration of insertions and increased intervals are also recognized from the comparison of Fig. 1, D and fixation phase in various cases in Figs. 4 and 5. Probably the violent tappings by the queen are responsible for the rapid insertions. And the immediate occupation of the cell by the next bee may also be inhibited by the avoidance component of the response to the queen, resulting in the prolongation of intervals. Virtually, in numerous instances, the workers attempted to insert but withdrew, showing a *hesitating* attitude in front of the queen. The actual insertion was realized often after repetition of such interrupted attempts. The behavior after body insertion becomes also different. In the prefixation phase, inserting bees usually remain at the sides of the cell after withdrawal. In the fixation phase, the bees, after withdrawal often removed from the cell, though without showing so conspicuous avoidance as in the provisioning phase. Fig. 3, H shows several types of the course of avoidance response after withdrawal. Fifty counts of the responses fluctuated as follows: Type A, 22; B, 8; C, 16; D, 3; E, 1. Therefore about half of the observed bees made avoidance from the cell after withdrawal.

As mentioned above, the queen occasionally inspects the cell, inserting her forebody approximately to the wing bases (Behavior pattern is the same to that in Fig. 3, J). The time spent for inspection was 1-3 sec. (1 sec., 13 cases; 2 sec., 35; 3 sec., 1). The number of inspections in the fixation phase was 1-7, obviously varying according to the length of the phase. The interval between two inspections was 12-130 sec. The record cited in Fig. 4, A, is worth mentioning. In this case, the queen fixed to the cell but abandoned it after 5 min. 32 sec., probably for the worker excitement was still insufficient. In this instance, we can notice rather frequent inspections in the earlier part of the phase, which, though far inconspicuous, resembles the repeated, nearly ceremonial inspections by

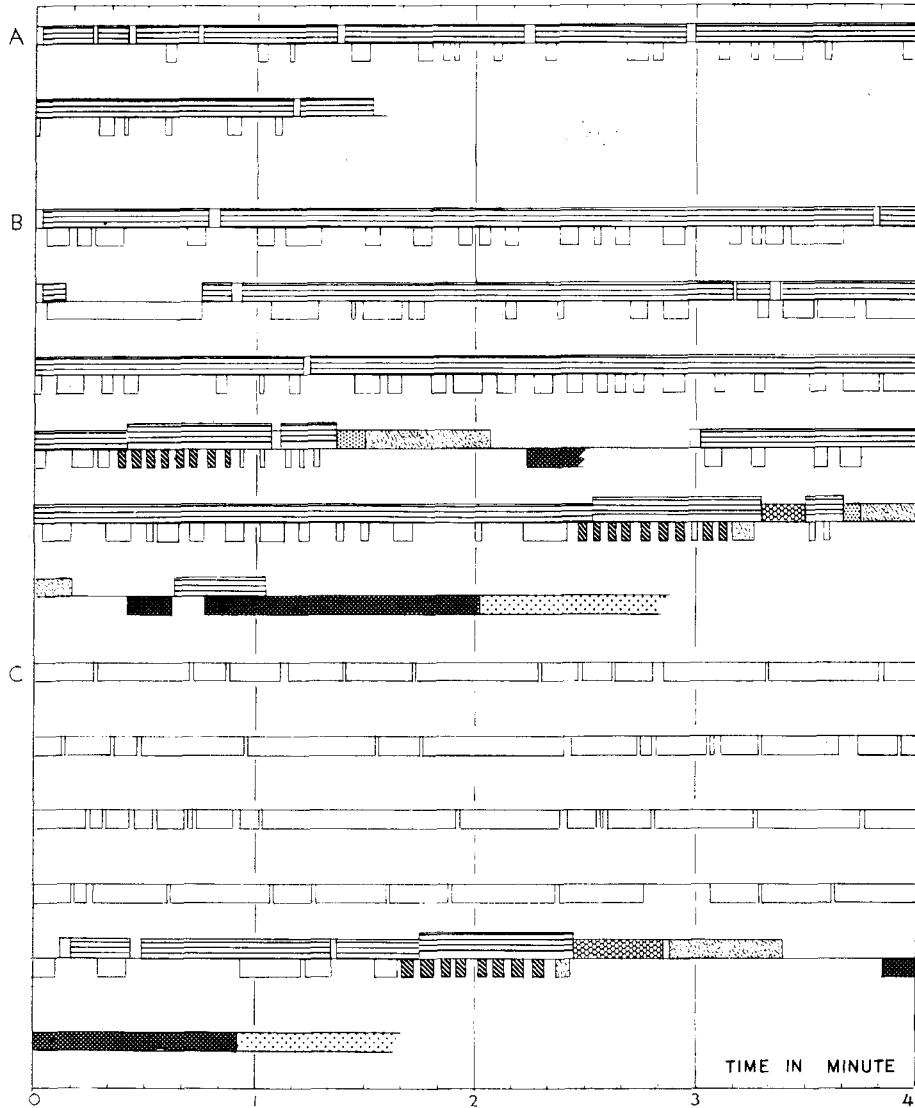


Fig. 4. Temporal sequence in some representative cases of provisioning and oviposition. A, Interrupted queen fixation (Dec. 1, 14: 10); B, Prolonged fixation followed in rapid succession by two ovipositions (Nov. 24, 14: 50); C, Continuous record of prefixation phase, followed by a relatively simple provisioning phase (Nov. 24, 9: 37). For further explanations see caption for Fig. 5.

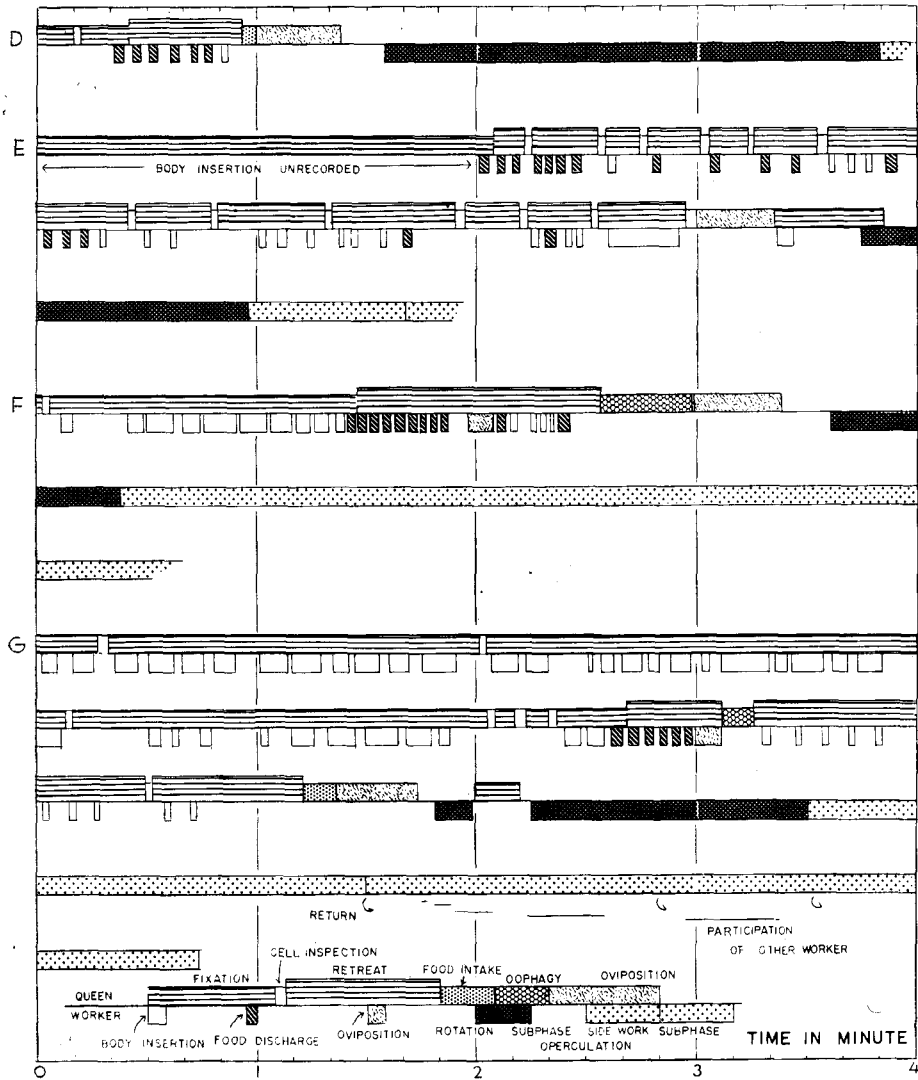


Fig. 5. Temporal sequence in some representative cases of provisioning and oviposition (continued).

D, Simplest case with very short fixation and provisioning phases (Nov. 24, 18:43); E, Irregular distribution of food discharges in provisioning phase (Nov. 23, 11:18); F, Occurrence of additional food discharges after worker oviposition (Nov. 2, 17:48); G, Prolonged postdischarge subphase (Nov. 22, 15:45).

the queen of *femorata*.

5. Provisioning, worker oviposition and Queen food intake

After the queen has been fixed to the cell for about 0.5 to 10 min., one of the workers around the cell discharges the first droplet of larval food. The provisioning phase is defined as the period from the beginning of this first discharge to the beginning of the queen oviposition. During this phase, the workers perform, besides excitement and court formation as mentioned above, *food discharge*, *body insertion* and occasionally *oviposition into the cell*. On the other hand, the queen shows *retreat from the cell*, *occasional inspection*, *Oophagy* and *intake of larval food*.

The behavior pattern of food discharge in *anthidioides* is similar to that in other species. The metasomal contraction is in typical cases clearer than in any other species so far observed, because the bright yellow bands on the terga contact one another at contraction (Fig. 3, G, center). The postdischarge escape or avoidance is usually distinct, though not so exaggerated as in *postica* (Fig. 3, G, lefthand below). In most cases, the escape distance corresponds to Type C in Fig. 3, H. No postdischarge crouching on the cell, as seen in *seminigra*, was observed.

The time for each discharge varied as follows, with the mean 2.4 sec. and *s.d.* 0.4 (54 cases):

Duration in sec.	1.5-2.0	2.1-2.5	2.6-3.0	3.0-
Frequency	17	21	14	2

The first discharge is rapidly followed by the second, third ones and so on. Within a relatively short time the cell is already filled up to 2/3 of its capacity. The interval between such rapidly succeeding discharges fluctuated:

≤ 1.2	≤ 1.5	≤ 1.7	≤ 1.9	≤ 2.1	≤ 2.6	≤ 3.0	≤ 3.2	(in sec.)
4	10	3	4	7	4	3	1	

As in *compressipes* and *seminigra*, the queen retreats at discharges from the cell to which she has been fixing, usually 1.0-1.5 cm, but often to 2.0 cm or more (Fig. 3, G, righthand). This position is maintained until her oviposition. When she occasionally inspects the cell or takes food from the cell (Posture as in Fig. 3J), she advances, performs the act and immediately retreats, again maintaining a definite distance from the cell. This characteristic was observed in three species of the genus *Melipona* but not in *postica* and *femorata*. The retreat began in *compressipes* at a rather indefinite time, either immediately after the first discharge or after several discharges. In *anthidioides*, however, the retreat usually appeared (26/31) soon after the first discharge. It is open to the question whether such is characteristic to the species or depends on the colony state.

The body insertions by workers during the provisioning phase seldom differ from those in the fixation phase in the behavior pattern, but the insertion is usually very short. The duration was measured as follows:

Duration (in sec.)	≤1.0	≤1.5	≤2.0	≤2.5	2.5<
Frequency	3	17	11	1	1

These insertions were mostly accompanied by the distinct escape response as in the case of discharges. Occasionally a response intermediate between typical discharge and mere insertion appeared, showing the subtle metasomal contraction. Such responses may signify the discharges by the individuals whose stomachs do not contain much food. They were tentatively classified in discharges. But the distinction was sometimes very difficult to observe. Workers provide each cell with varying quantities of food, from 87 to 150 mg.

The duration of the provisioning phase varied greatly as shown in Fig. 6, below, by the frequency distributions of actual and net (=subtracting the time for worker oviposition and queen food intake) durations. The most frequent cases were 40–150 sec. in both. In most cases, the food discharges were concentrated to the initial part of this phase (=discharge subphase). This is revealed by the frequency distribution of the discharge subphase, which did not fluctuate so much as the total duration of the provisioning phase, either actual or net, mostly ranging 15–40 sec. (Fig. 6, above righthand). Moreover, there was no clear correlation between the net duration of the provisioning phase and of the duration of the discharge subphase (Fig. 6, above lefthand). On the other hand, the duration of discharge subphase more or less increased parallel to the increase of the number of food discharges (usually 6–8, Fig. 7). Among 30 closely observed cases, additional discharges after the discharge subphase were observed only in two instances, as shown in Fig. 5, E and F. (The separation of discharge subphase intervened only by a simple body insertion was occasionally observed, *cf.* Fig. 4, B). The case E in Fig. 5 is characterized by the prolongation of the provisioning phase,

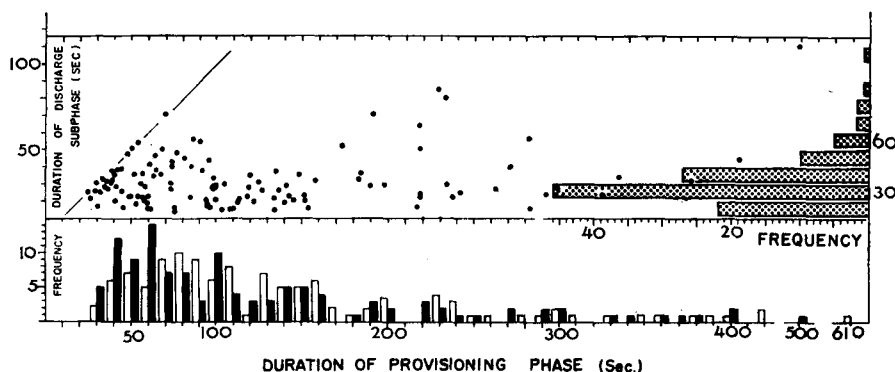


Fig. 6. Above. Correlation between duration of provisioning and of discharge subphase, with (righthand) frequency distribution of duration of discharge subphase; Below. Frequency distribution of actual (black) and net (=excluding time for worker oviposition and queen food intake; white) duration of provisioning phase.

frequent appearance of queen inspection and occurrence of many additional discharges (most of which were the intermediate type mentioned above) after the discharge subphase. The case F is exceptional because two additional discharges appeared after a worker oviposition but before queen oophagy, which was not seen in all other cases, and the queen did not inspect the cell except at the beginning of the fixation.

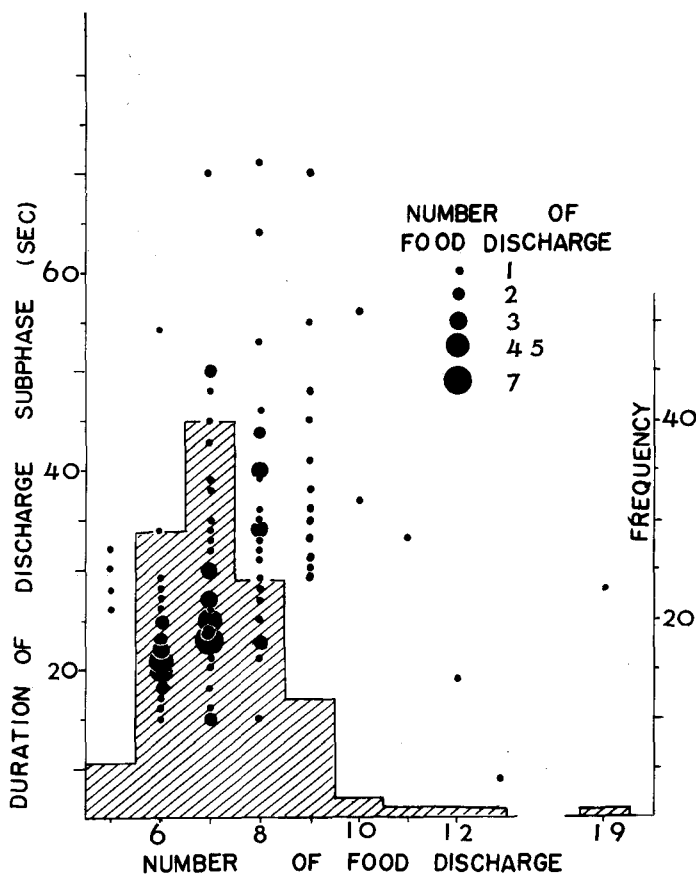


Fig. 7. Frequency distribution of number of food discharges (scale righthand) and its relation to duration of discharge subphase (scale lefthand).

Except for these two instances, all cases showed a concentration of discharges at the initial part of the provisioning phase. Next, all available records except case E mentioned above, which was too irregular, were classified according to the relative lengths of discharge and postdischarge subphases into six types (O, A-E) as defined in Table 1. From the table the segregation of two groups is

Table 1. Types of provisioning phase, in relation to oviposition interval and fixation phase

Type of provisioning phase		Relation to oviposition interval					Relation to fixation phase						
Symbol	Definition and example in Figs. 4 and 5 (in parentheses)	Total number	Time since last oviposition to queen fixation (in sec.)					Total number	Duration of fixation phase (in sec.)				
			1-20	21-40	41-60	61-100	101-		30-60	61-120	121-150	151-	
O	$y=0$, or $y < 0.5x$ (B ₂ , C, D)	20	3	6	5	5	1	9	3	1	1	1	3
A	$0.5x \leq y < x$ (B ₁)	6	1	2	1	1	1	4		1	3		
B	$x \leq y < 1.5x$	2	1				1	2				1	1
C	$1.5x \leq y < 2x$	3	2		1			2			1		1
D	$2x \leq y < 4x$ (F)	19	2	3	1	1	12	3			1		2
E	$y \leq 4x$ (G)	24	4	6	5	7	2	10	1	1	5		3

x: duration of discharge subphase

y: duration of post-discharge subphase

obvious, that is, the postdischarge subphase is mostly either very short, sometimes practically absent, or quite prolonged, often longer than the discharge subphase. Further the relation of various types to the time between the last oviposition and queen fixation and to the duration of the fixation phase were sought, but within the limit of the present sample size, no clear correlation was obtained as seen in Table 1. Before considering this postdischarge subphase further, however, the observations concerning worker oviposition and queen food intake are referred to.

The behavior pattern of worker oviposition is similar to that in *compressipes* and *seminigra*. After a brief inspection of the cell, the worker advances one step and inserts the apical half of her metasoma. The duration of oviposition was measured as follows (in sec., number of observations in parentheses): 3 (1), 4(2), 5(4), 6(7), 7(11), 8(5), 9(1), 10(2), 13(1), 14(1). The mean duration was 7.0 sec. and *s.d.* 2.0, statistically without significant difference from *compressipes* (4.8 sec.) and *seminigra* (5.3 sec.). During this period the worker remains quiet, closing her wings, without moving any appendages (Fig. G, I). As in food discharge, the distinct escape response appears after the oviposition. The egg is distinctly smaller than the queen's one, approximately 2/3 in length, standing erect on the center of the surface of larval food as does the queen's egg.

The behavior seen from the outside at oophagy and food intake by the queen is the same as that in cell inspection. The queen advances to the cell, slowly inserts the forebody approximately to the wing bases and maintains this posture during the intake (Fig. 3, J). No peculiar wing and appendage movements are seen at intake. The duration of these two acts were measured as follows:

Duration (in sec.)	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	24	25	29	30
Oophagy						1	3	1	3	2	2	8	2	1	1	2	2	1	1	2	2
Food intake	14	23	23	13	8	7	6	2	3												

The mean duration of oophagy (16.9 sec., *s.d.* 6.2) is similar to that in *seminigra* (16.2 sec.) while shorter than in *compressipes* (27.0 sec.), although probably not significantly different. A large variability from 10 to 30 sec. is noteworthy. In all three species of *Melipona*, the worker eggs are laid at the center on the cell, not at the cell margin as in *postica* and *femorata*, so that the act of egg devouring is externally invisible. Hence, in so prolonged oophagy as that lasting 20–30 sec., it is possible that the queen ingested some quantity of larval food after devouring the egg.

The duration of intake of larval food was measured only in a few instances in *compressipes* (four; 3,4,4,7 sec.) and *seminigra* (two, 5,3 sec.), probably there is no difference between these species and *anthidioides* on this point. There is a doubt as to the lower limit of the duration of food intake. As shown previously, the cell inspection by the queen continues from 1 to 3 sec. Therefore, it is possible that some of the short food intakes, for instances, those lasting only 4 sec., merely represent prolonged inspections. At any rate, it is reasonable to suppose that the prolonged duration means the ingestion of larger quantity. We divided the oophagy and food intake arbitrarily into the following classes: Oophagy, I (10–18 sec.), II (19–30 sec.); Food intake, a(4–5 sec.), b(6–7 sec.), c(8 sec. or more). The distribution of these classes and their combinations were sought among 129 cases as follows (0 means the absence of oophagy or food intake and I+I, two worker ovipositions, consequently two queen oophagies):

Food intake	0	a	b	c	Total
Oophagy 0	6	35	23	20	84
I	4	10	5	5	24
II	7	1	0	1	9
I+I	1	0	1	0	2
Total	18	46	29	26	129

We know: 1) The intake of larval food occurred more frequently than oophagy and 2) the duration of food intake decreased when both oophagy (especially prolonged oophagy) and larval food intake occurred in the same *POP*. Therefore, the above mentioned assumption, the possibility of additional food intake at the prolonged oophagy, seems justified. At the same time, it is plausible that the amount of nutrition, either egg or larval food or both combined, taken by the queen in each *POP* does not vary too much. The occurrence or not of the correlations between food intake (*s. lat.*), oviposition interval (*cf.* Section 3), duration of fixation phase and type of provisioning phase (O, A-E *cf.* Table 1) was examined but no apparent positive correlations were obtained.

The temporal position of worker oviposition, queen oophagy and queen food intake in the post-discharge subphase was in some accurately observed cases as follows:

1) In nine cases, in which only intake of larval food was observed, the latter appeared always at the end of the postdischarge subphase, irrespective of the type of provisioning phase (Type A,B,C,D each one case; E, five cases). In Type O, all three phenomena logically appeared immediately after the discharge subphase. 2) Among five cases, in which only worker oviposition and queen oophagy were observed, in one instance (Type D) both appeared at the end of the postdischarge subphase and in four instances (Type A, 1; E, 3) the worker oviposition appeared immediately after the discharge subphase while the oophagy at the end of the postdischarge subphase. 3) Among three cases, in which three phenomena were observed in one and the same *POP* (Type A, 2; D, 1), the first two appeared immediately after the discharge subphase, while the food intake at the end of the postdischarge subphase.

From these results and those in other, not so accurately observed cases, the general trend seems to be as follows: 1) Worker oviposition tends to occur soon after the discharge subphase, 2) Intake of larval food tends to occur immediately before the queen oviposition, and 3) oophagy either immediately follows the worker oviposition or appears immediately before the queen oviposition, in the latter case, probably occasionally followed by the food intake. In other words, the intake of either egg or larval food tends to be followed by the queen oviposition. But if the intake of larval food did not immediately followed by the queen oviposition, it often appears again later, immediately before the delayed queen oviposition.

When the oophagy does not immediately follow the oviposition, the surrounding workers mostly show a distinct avoidance reaction to the worker egg, repeatedly advancing, touching the egg with antennae and retreating with a shock reaction. The oophagy by workers seen in *postica* and *femorata* was not seen in *anthidioides* as in *compressipes* and *seminigra*. The deposition of two worker eggs in the same *POP* was observed only in two instances among 129, and of more than two eggs, as seen in *compressipes*, never happened. We tried, for several times, to increase the amount of food, by pipetting small quantity of food in the brood cell. In all instances, the workers were very upset and took out all the food, and the egg in cases where the queen had already laid.

Returning to the temporal pattern of the provisioning phase, the concentration of successive discharges was seen in all species so far observed except *compressipes*. As to the more or less prolonged postdischarge subphase, this appeared in *postica* at the first cell to be oviposited in each *POP* (which involves, in this species, numerous cells) often with a marked delay. The prolonged postdischarge subphase was practically absent in *femorata* and appeared in *seminigra* in a manner similar to that in *anthidioides*. In *compressipes*, the segregation of two subphases was relatively rare and the whole provisioning phase often showed an irregular sequence, as in case E (Fig. 5). As repeatedly referred to, this state seems to be caused by the abnormal conditions of this Amazonian form observed under unfavorable conditions. In general, the concentration of successive discharges within a relatively short time appears to be the rule in stingless bees. The factors

which govern the appearance or omission of a prolonged postdischarge subphase is still unknown. We failed to find a correlation between this and oviposition interval or duration of fixation phase. But it is still premature to conclude the absence of any relations among these variables. Probably several factors may contribute to the facultative appearance of the postdischarge subphase. It is certain, however, that the prolonged postdischarge subphase and the delayed queen oviposition do not always correlate with insufficient amount of larval food *judged by the queen*, or with insufficient amount of food ingested by herself. In most cases of the prolonged postdischarge subphase, the queen inspected the cell, often more than once. Yet additional food discharge occurred only exceptionally (In *compressipes*, on the contrary, additional food discharge after the first unsuccessful attempt of the oviposition once observed. Cf. Sakagami & Oniki, 1963). Moreover, the frequent occurrence of delayed queen oviposition after oophagy suggests that the queen did not wait for her own food supply. Some stimuli, probably relating to the degree of worker excitement, may be necessary to elicit the queen oviposition. Delayed queen oviposition may be an outcome of low intensity of such stimuli, or of the lower sensitivity of the queen to them, or of a combination of both. The closer relationship must be analysed experimentally in the future. All accurately observed ovipositions since Nov. 20, 1962 to Jan. 31, 1963 were chronologically arranged. Each oviposition was classified into several types according to the length of postdischarge subphase. The sequence of diverse types showed sometimes a tendency for succession of the same or similar types but was in general irregular.

Finally, two comments are added in connection to the provisioning. In two instances, we disturbed the colony at the middle or the end of the discharge subphase. The queen left the comb. The worker excitement disappeared. In both instances, discharged food was ingested by the workers and these cells were never used later. Similarly, opening of cells and devouring of the contents were also occasionally seen but not so frequently as in *compressipes*, probably due to the favorable colony conditions.

The second comment relates to the nature of larval food. When the cells containing eggs or young larvae of stingless bees are opened, we confirm the observation by Kerr (1948), that is, there is a separation of larval food into two layers, upper thin, semitransparent layer and the rest, consisting of opaque yellowish liquid or mass which including abundant pollen. At feeding, the larvae may ingest at first the upper layer, later the lower, more viscous food. According to Landim (1963) the upper layer contains secretion for hypopharyngeal glands. If such would be the case, body insertions by workers in the postdischarge subphase, at least some of them, could be responsible to such secretion. Keeping this in mind, one of us (S.F.S.) repeated closer observations soon after discharge subphase but found in no case the formation of this semitransparent layer, nevertheless such layer was always found when the cells were opened one or two days later. Therefore, we consider the upper layer as an outcome of a simple separation of the pollen by gravity. This is easily demonstrated by centrifuging the food immediately after discharge. One of us (W.E.K.) determined that the upper part contained sugars only 7%, while honey of this bee contained 72% and showed a pH of 4.2 (Kerr, 1949).

6. Queen oviposition and operculation

The queen oviposition, the climax of *POP*, is in itself a simple act. After brief cell inspection she advances and inserts the apex of her metasoma. During oviposition her metasoma is slightly contracted but neither wings nor appendages move specially (Fig. 3, K). The duration of oviposition was measured as follows (in sec., number of corresponding cases in parentheses; only one case if unmentioned): 18(3), 19(5), 20(11), 21(5), 22(11), 23(13), 24(6), 25(9), 26(6), 27(6), 28(9), 29(4), 30,33(2), 34,35,48,52, with the mean 24.57 and *s.d.* 5.24, that is, shorter than in *compressipes* (36.3 sec.) and statistically not significantly different from *seminigra* (25.7 sec.). All three *Melipona* species observed by us can be characterized by long queen oviposition, distinctly longer than in *postica* and *femorata*. The duration is not related to the occurrence of a prolonged postdischarge subphase. The oviposition act seemingly corresponds to the fixed pattern behavior (Tinbergen, 1951). In two instances, the queen failed to oviposit normally due to the unfavorable position of cells, too close to the glass lid of the hive. In both cases, she did not adjust her metasoma to the position but repeated the same effort a new.

The operculation of a cell after laying is performed in the manner same to that seen in other species already reported upon. At first, a single worker closes the orifice, resting on the cell, inserting her metasomal tip and rotating the body on the apex of the cell (rotation subphase, Fig. 3, L, M). After the orifice is considerably reduced, she works from the side of the cell (Fig. 3, N, side work subphase). The temporal sequence of rotation subphase is seen from Figs. 4-5, B-G. The side work subphase was accurately observed only in case G.

The operculation always begins in *postica* and *femorata* soon after the queen has finished oviposition, and the rotating bee is indifferent to the excitement on the comb surface, which gradually decreases, and to the occasional interference by the queen still nearby. In *compressipes* the operculation is often delayed but not always, mostly due to the interference by the queen. The data in *seminigra* are insufficient but suggest intermediacy between these two types. In *anthidioides* such delay is rather a rule. The time between the removal of the queen metasoma from the cell and the beginning of the operculation was measured as follows (in sec., number of cases in parentheses, each single case if unmentioned): 0(3), 5(2), 7, 10, 12(2), 13, 14, 15(3), 17, 18, 20, 22(2), 25*, 26, 27*, 27(2), 28, 36, 38*, 41, 55*, 58, 90*, 135. Most of the delays were caused by the interference by the queen, but in cases marked with asterisks, the delay appeared without such interference. In these cases, many shock reactions by workers to the cell were noted, these were similar to the reactions to worker eggs described in the preceding section. After the repetition of such responses, one of them finally climbs over the cell and begins to close it. Even after the operculation begins, the performer frequently abandons the work when the queen approaches and taps her with the antennae, as seen from the following records (Duration of the first attempt to operculate — duration from the abandonment to the resumption by the same or different (with*) individual):

5-5*, 27-5, 10-15*, 1-82*, 2-21, 8-2, 11-7 (all in sec.). Such interruption of the operculum is shown in Figs. 4-5, B and G. Subtracting this interruption, the duration of rotation subphase was measured (in sec., number of cases in parentheses, if unmentioned, only one case): 45(2), 47, 50, 54(2), 55, 60 (2), 64(2), 74, 75(2), 84, 89, 90(2), 97, 100, 125, 126, 147, 225. The last case was delayed by the position of the cell, which was so close to the glass lid, that the complete rotation could not be executed. Excluding this case, the mean duration was 77.0 sec. After the rotation subphase was finished, the cell orifice was usually still open. No detailed observation was made until the end of the side work subphase but the orifice was in most cases completely closed about 2-3 min. after the beginning of the rotation subphase, a period a little shorter than in *compressipes*.

After the orifice was more or less completely closed, the work still continued for a while. At this stage often one or two other workers participate in the task. But they were usually less persistent in the work and often abandoned the cell sooner or later (Figs. 4-5, B, G). It was also often observed that the original performer started to leave the cell, then, returned and again began the work, as shown in the same figure (shown as *return*),

Concluding remarks

As mentioned in the first report (Sakagami & Oniki, 1963), closer comparisons of the behavior of various stingless bee species observed by us will be given at the end of the present serial work. Therefore, here is given only some general and comparative remarks concerning the observations described above.

First of all, the temporal pattern of *POP* in *anthidioides* well coincides with that of the other species already reported. The sequence of cell construction, queen fixation, provisioning of the cell, queen oviposition and operculum is thus regarded the basic pattern common to the stingless bees. Furthermore, the following elementary behavior patterns were also more or less common among the species observed: 1) Advance-retreat responses of workers to the queen, 2) Appearance of excited and alternate body insertions of workers in the structurally ready cells (defined as the pre-fixation phase in the present paper), 3) Successive food discharge (discharge subphase), followed or not by the postdischarge subphase, 4) Escape response of workers after food discharge, 5) Frequent worker oviposition and queen oophagy as well as the intake of larval food by the queen, 6) Operculum, first by rotation, then by manipulation from the side of the cell (distinguished in the present paper as rotation and side work subphases).

Within this general framework, there were found several features, which were identical in the three species of *Melipona* observed but different in the other two species: *Singular* and *successive* queen oviposition, *retreat* of the queen from the cell after worker food discharge, worker oviposition shorter than that of the queen, worker eggs smaller than the queen's eggs and deposited on the center of the larval food, and oviposition taking a considerable time.

Finally *anthidioides* behaved in some aspects differently from the other two species of *Melipona*. Most of such differences could be reduced to the different colony conditions, but the *vertex tapping* performed by the queen on crouching workers, absence of rhythmic wing beats of the queen, and absence of crouching of workers after food discharge (observed in *merrillae*, cf. Beig & Sakagami, 1964) seem to be specific to *anthidioides*.

Wheeler (1923) and subsequent authors considered mass provisioning by the stingless bees as an archaic heritage in this socially highly developed group. However, it is obvious that the stingless bees developed a highly complicated behavior pattern as to *POP* (cf. also Sakagami & Zucchi, 1963), accompanied by the marked differentiation among species or supraspecific taxa, which cannot simply be equalized to the undifferentiated mass provisioning in solitary bees. This is also seen from the contrast between cell construction and provisioning. The former lasts for a considerable time, whereas the latter is characterized by rapid sequence of acts under high excitement. It is indubitable that the stingless bees took a direction which is evolutionarily different from, not always inferior to, the path followed by the honeybee.

As to the functional aspects of *POP*, the influence of colony conditions was, though not directly studied, suggested from the comparison between *anthidioides* and *compressipes*. The latter was observed under very unfavorable conditions resulting from damage received when the nest was taken in equatorial Amazonas and during transport by air to southern Brazil and then reared under unfavorable weather conditions there. On the other hand, the colony of *anthidioides* was taken in Rio Claro and has been kept there for years in an artificial hive. In addition to the probable specific differences cited above, *anthidioides* differed from *compressipes* in the higher cell construction and oviposition rates, closely successive queen ovipositions, constant appearance of pre-fixation excitement, regular sequence in fixation phase, appearance of food discharge in rapid succession and consequently clear segregation of discharge and postdischarge subphases, and finally, absence of more than two, and rarity of more than one worker oviposition within the same *POP*. Most of these differences can tentatively be explained by the difference in colony observed of each species, some features, especially quantitative ones, can vary according to the environmental conditions. It also indicates a promising field on the functional aspects of colony organization and dynamics to be studied in the future.

Finally a few words are added as to the food intake of the queen. Among 129 accurately observed cases, only in 6 instances, that is only in 4.6%, neither oophagy nor intake of larval food by the queen was observed (cf. table in page 600). On the other hand, direct food delivery from workers to the queen was rare. Short buccal contacts between queen and worker were occasionally seen. But at least we did not observe the prolonged cases comparable to those seen daily or hourly in the honeybee hives. Even considering the difference of egg productivity between

honeybee and *anthidioides*, it is obvious that the queen could not survive and function with such rare and small food intake from the workers. We are inclining to the opinion that the gravid queen takes most, if not all, of her necessary nutriment from the worker eggs and larval food, because, at least in the colony observed by us, it was certain that the food taken by the queen from the cell during *POP* formed a considerable part of her nutriment. This again suggests, as already mentioned (Sakagami & Zucchi, 1961; Sakagami et al., 1963), the way taken by some stingless bee species in the social evolution, which is different in various aspects from that in the honeybee.

Summary

In the present paper the observations upon *Melipona quadrifasciata anthidioides* Lepeletier were described as the fifth report on the behavior studies of the provisioning and oviposition process (*POP*) in the stingless bees.

The temporal sequences within *POP* corresponds well, in general, to that found in other species already reported, with the regular succession of cell construction, queen fixation, provisioning of the brood cell, queen oviposition and operculation. Many elementary behavior, such as excitement in prefixation phase, escape reaction after food discharge into a cell, worker oviposition and subsequent queen oophagy, food intake by the queen from the brood cell, etc., also showed the pattern common to other stingless bees. Further, the behavior patterns were in many aspects the same or similar to those found in two other species of the same genus, *M. compressipes manaosensis* Schwarz and *M. seminigra merrillae* Cockerell. Most features, in which *anthidioides* differed from the two other species seem to depend on the different conditions in the colony observed. Only tapping by the queen of the vertex of workers and absence of rhythmic wing beats by the queen were regarded as clearly specific to *anthidioides*.

The aspects more fully analysed than in other species are: Cell construction, prefixation phase, and food intake by the queen. Cell construction was explained as an integration of independent contributions by various workers, whose work intensity might vary according to the motivation and releasing stimuli. The interval between oviposition processes occasionally becomes very short, especially compared to that in two other *Meliponas*, but not so frequently as in *Cephalotrigona femorata* (Smith). The provisioning phase was subdivided into discharge and postdischarge subphases; the latter often did not appear. Finally, it was concluded that the food intake by the queen during the oviposition process played an important role in her food economy.

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