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Oviposition Behavior of Two Dwarf Stingless Bees, *Hypotrigona* (*Leurotrigona*) *muelleri* and *H.* (*Trigonisca*) *duckei*, with Notes on the Temporal Articulation of Oviposition Process in Stingless Bees[1)]

By

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(*With 27 Text-figures and 8 Tables*)

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The aims of the present paper are twofold. As a ninth report of our serial work on the behavior of stingless bees (Sakagami, Sakagami *et al.* 1963～'73), it deals with the oviposition behavior and related accounts of two dwarf Brazilian species, *Hypotrigona* (*Leurotrigona*) *muelleri* (Friese 1900) and *H.* (*Trigonisca*) *duckei* (Friese 1900). As in other species so far described, the oviposition behavior of these two species is definitely articulated in temporal sequence. But our previous explanations on this aspect were not always free from ambiguities, and the adopted terms were often inadequate. Therefore, some general accounts are preceded before the descriptions of the behavior of two species.

In subsequent pages the species observed by us will be cited, unless necessary, with generic or subgeneric names alone. The specific names, authors and years of publication on behavioral accounts are given in references at the end of the paper. The term "group" will frequently be used for these higher taxa.

1. Some remarks on the temporal structure of oviposition process in stingless bees

Through our previous papers it has been revealed that the oviposition process of stingless bees has a definite temporal structure, involving characteristics common to all groups observed as well as those varying from one group to another. A closer comparison of various groups must be postponed until the results of other groups would be analysed. But it has been noticed that our previous explanations contained some ambiguities, and the adopted terms were not always appropriate. In order to remove these defects, some interim comments are given below, which complement and correct previous explanations and terminology.

In this section, some assumptions on the stimuli which seem to release and direct the oviposition process are introduced, all of which are not crucially proved but are fairly likely from the context of the process. But the main aim of the following comments is to visualize the structure of the process accurately, leaving the interpretation of underlying mechanisms for the future, though a tentative hypothesis will be presented in Concluding remarks **3.** For the convenience's sake, the term "comb" is frequently used, but the explanations could also be applied to the cluster-making species without essential modifications.

1.1. *Cell construction*: The presence of one or more completed cells, i.e. those provided with collars slightly higher than the comb surface (more difficult to determine in cluster-makers) is a prerequisite to the oviposition process. Up to the present, three types of cell construction have been recognized (Fig. 1):

1. *Successive* (S_c): Cells are started at any time so that various stages of cells

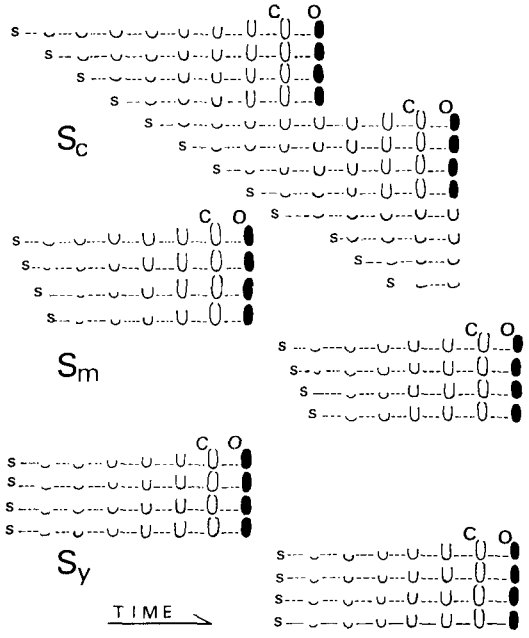


Fig. 1. Three types of cell construction in stingless bees. Two successive oviposition processes (IOP), each involving four cells, are schematically presented by the gradual growth of cells. S_c. Successive type; S_m. Semisynchronous type; S_y. Synchronous type. s. Start of cell construction; c. Completed, i. e. collared cell; O. oviposited cell.

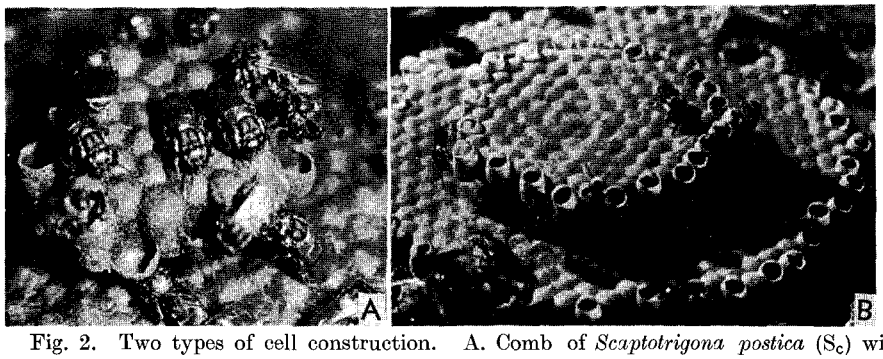


Fig. 2. Two types of cell construction. A. Comb of *Scaptotrigona postica* (S_c) with various growing stages of cells. B. Comb of *Plebeia emerina* (S_y) with same staged cells (all completed).

are found at a given time (Fig. 2, A). 2. *Synchronous* (S_y): Cells are nearly synchronously started usually a little after the prior oviposition, and only cells of the same stage are found at a given time (Fig. 2, B). 3. *Semisynchronous* (S_m): Cells are rather successively started but the stage difference becomes gradually smaller.

In S_y and S_m, therefore, no half-built cells are found soon before or after an oviposition process (Fig. 2, B). The start of new cells is seemingly inhibited once the cells under construction attained an advanced stage.

1.2. *Formation of batches*: In most groups so far observed several ovipositions take place in rapid succession, separated from subsequent ones by a considerably long interval. Three types of batch formation are distinguished (Fig. 3, I):

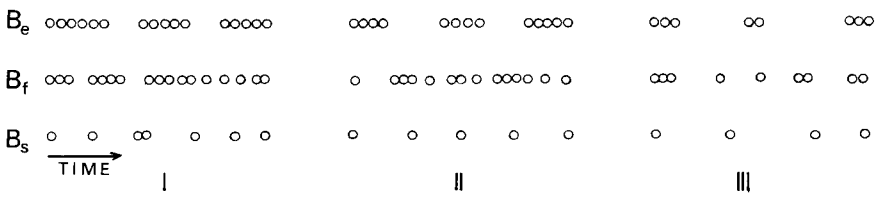


Fig. 3. Three types of batch formation in stingless bees under three hypothetical colony conditions, I~III. B_e. Exclusively batched; B_f. Facultatively batched; B_s. Predominantly singular. I. Favorable; II. Intermediate; III. Unfavorable conditions.

1. *Exclusively batched* (B_e): Ovipositions always batched. Number of cells per batch as well as interval between batches relatively large and stable. 2. *Facultatively batched* (B_f): Ovipositions loosely batched. Number of cells per batch and interval between batches variable, occasionally resulting even in singular ovipositions (Fig. 3, II). Delimitation of batches more or less arbitrary. 3. *Predominantly singular* (B_s): Each oviposition usually separated by sufficiently long interval, though occasionally two or more (up to four) ovipositions forming a batch.

Distinction of these types is not always clear, sometimes becoming subtle, because the temporal sequence of ovipositions is affected by colony conditions, and the criterium distinguishing B_e and B_f, the relative stability of batch size and interval, is literally "relative". Under less favorable conditions, the interval between "batches" prolongs in B_s (Fig. 3, II). The same is true to B_e but here the average batch size also decreases. On the other hand, B_f type is more easily determined under slighly unfavorable conditions (Fig. 3, II), because under favorable conditions B_f-tendency can be masked by increased batch size as seen in *Tetragona*. In extreme cases it is possible that all types converge to a similar pattern (Fig. 3, III), as suggested by the appearence of singular oviposition in *Friesella* (B_e). Moreover, in the groups with poor capacity of thermal regulation, the oviposition pattern is affected by external factors, even if internal colony conditions, for instance, population size and amount of food storage, are favorable. Thus the batch type must ultimately be identified through observations under diverse colony conditions (cf. Conclud. remarks **5**), which is not easy to practice in some groups being rare and difficult to rear. In such groups the identification of batch types is obliged to be provisional. Combining cell construction and batch formation types, the groups so far observed are classified as follows (In this and subsequent pages the asterisked groups are those the detailed results on which are still not published):

S_cB_s: *Melipona* (Exception, *M. rufiventris* Lepeletier, Camilo 1972), *Meliponula**, (*Trigonisca*).
S_cB_f: *Tetragona, Cephalotrigona.*
S_cB_e: *Scaptotrigona.*
S_mB_e: *Duckeola, Friesella, Leurotrigona.*
S_yB_e: *Plebeia,** *Nannotrigona.**

1.3. *Patrolling phase* ($\bar{P}$): Our previous explanations on the temporal structure of oviposition process are summarized as follows:

> The oviposition process is divided in six phases, prefixation, fixation, provisioning, oviposition and cell operculation. Excluding B_s group, each batch or IOP (Integrated oviposition process) involves several ovipositions or UOP (Unit oviposition process), i.e. IOP $=n$ (UOP).

Three last mentioned phases, provisioning, oviposition and operculation, are obligatorily repeated in each UOP. But the explanations have been ambiguous as to the first two phases and their relation to IOP and UOP.

A certain time after the previous IOP, either batched or not, the queen visits the comb where new cells are under construction (=the comb with *advancing edge*, or the cell cluster with *advancing front* in case of cluster-makers, cf. Michener 1961), apparently driven from the revived laying motivation. She cruises on the comb, occasionally stays by growing cells or inspects them and leaves the comb away. Repetition of this arrival — cruising and stay — removal accelerates cell construction, congestion of young workers on the comb, especially at cells under construction and their gradual excitation. Certainly these worker responses reciprocally stimulate the queen, increasing the frequency of her visits and lengthening her stay on the comb. The later half of this period with increases of both queen visits and worker responses mentioned above is defined as the *patrolling phase* (=prefixation phase in previous papers), excluding the final part, which will be discussed in **1.4.** and especially in **1.5.**

The beginning of patrolling phase could formally be delimited by, A. Increased visits of the queen with, and prolonged stay by her on the comb. B. Appearance of completed cells with collars. C. Congestion of workers at cells with frenetic body movements, usually accompanied with alternate insertions of fore-bodies into the cells. (cf. Fig. 8, *I*).

But the clear delimitation of this phase is difficult because all above mentioned responses increase rather gradually. Moreover, 1. In some groups (*Nannotrigona**, *Geotrigona**), the queen spends her most daily life on the comb even out of the oviposition process, which makes the application of item A difficult. 2. Adoption of item B is relatively easy in S_y and S_m types (**1.1.**) by taking the time of the completion of all cells. This is less obvious in S_c, though even here workers tend to concentrate their effort upon the completion of several well grown cells near the beginning of an oviposition process. Further, in some groups with poor thermal regulation, ovipositions in completed cells often take place the next day (cf. Section **3**). 3. Appearance of item C is relatively abrupt in *Melipona*, *Scaptotrigona* and

*Plebeia**, making its use to delimit the phase easy, but rather gradual in *Tetragona, Duckeola, Friesella* and *Leurotrigona.*

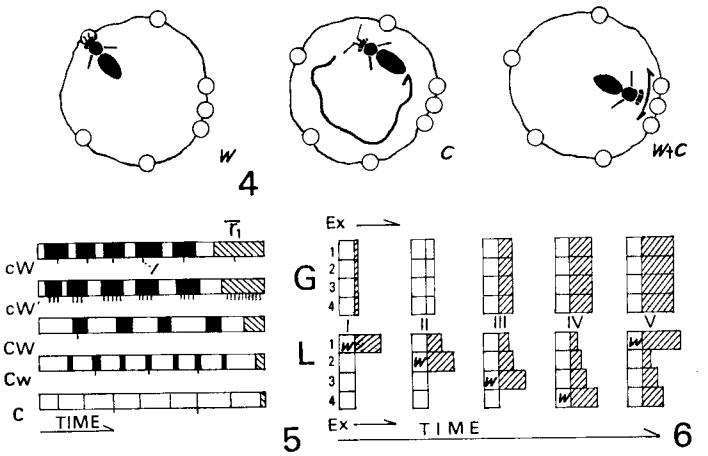
Nevertheless the recording of the time of items B and C is, if possible, useful for the comparison of approximate lengths of the phase $\bar{P}$ among different groups. The end of $\bar{P}$ is more easily delimited as given in **1.4.** The patrolling phase is meaningful only as a phase of IOP, because each UOP is still not crystallized at this period.

1.4. *Arousal phase* ($\bar{A}$): The period since the final arrival at the comb of the queen to the beginning of the first UOP (or $\bar{u}_1$ as closely discussed in **1.5.**) is defined as the *arousal phase*, $\bar{A}$, (approximately corresponding to the first part of fixation phase in the previous usage). Virtually $\bar{A}$ is the mere final part of $\bar{P}$ without qualitative behavioral differentiation. Its separation from $\bar{P}$ is made rather for a technical reason: To trace the behavior sequence throughout $\bar{P}+\bar{A}$ is tedious. Because of the absence of qualitative difference in behavior sequence between $\bar{P}$ and $\bar{A}$, a closer observation made at $\bar{A}$ is often sufficient to know the behavior pattern specific to the species concerned. If an IOP covers more than one comb widely separated for each other, the final arrival at the comb with the cell first receiving food could be used to delimit the beginning of the arousal phase. While the delimitation of this phase is easier than of $\bar{P}$ (except *Trigonisca*, cf. **3.3.1.**), its temporal structure has previously not adequately been explained.

The queen behavior in $\bar{P}$ and $\bar{A}$ basically consists of the alternaton of two components, *cruising* on the comb (*C*) and *waiting* by particular cells (*W*) with or without cell inspection (*E*) (Fig. 4, cf also Fig. 8). In previous papers this waiting was called *fixation.* Besides the expression is somewhat inappropriate, its application to the phase name has given an impression as if the queen has continued *W* throughout the phase. To avoid a misunderstanding it is stressed here that *W* involves even a momentary stop at the cell. The main function of $\bar{A}$ certainly lies in lowering the threshold to release food discharge into the cell, by raising an excited mood on the comb which already has been evoked since $\bar{P}$ through reciprocal stimulations between queen and workers. The term "arousal phase" was adopted to stress this function. Repetition of frenetic body insertions in cells by workers in $\bar{A}$ (Fig. 8, *I*) represents, in our opinion, the subliminal or *intentional* food discharges.

The queen behavior in $\bar{P}$ and $\bar{A}$ is thus expressed as $m.(CW)$ (m: replication mark). The relative length of two components enables the distinction of several behavioral types (Fig. 5):

$C<W$	cW :	*W* is relatively long. *Friesella, Melipona* and probably *Trigonisca.*
	cW′ :	Ditto, but *W* involves ritualized repetition of cell inspections, *Cephalotrigona, Tetragona, Duckeola* (Fig. 8, *E*).
$C>W$	CW :	*W* is relatively short. *Leurotrigona.*
	Cw :	The queen continuously cruises on comb with brief stays at cells. *Nannotrigona**.
	C :	The queen nearly runs about the comb with only occasional and momentary stops at cells. *Plebeia.**



Figs. 4~6. Excitement arousal by the queen. 4. Queen behavior patterns in patrolling and arousal phases. *W*. Waiting; *C*. Cruising; *W*+*C*. Swinging in *Scaptotrigona*. 5. Various types of queen behavior in arousal phase (cf. in text). Black. Waiting; White. Cruising; *I*. Queen cell inspection; $\bar{r}_1$. First predischarge phase (cf. **1. 5.**). 6. Excitement arousal in generalized (G) and localized (L) types. Each IOP is assumed as involving four (1~4) cells. Gradual increase of excitement (Ex) through successive periods (I~V) is shown by hatching. *W*. Queen waiting (In G type, period II, hatching is erroneously omitted).

Independent of, but intimately linked with these types, some modifications appear in other groups. For instance, the queen of *Scaptotrigona* often swings between two or more cells placed nearby (Fig. 4, *W*+*C*), i.e. *W* is short as far as each cell is concerned but long when a group of neighboring cells are regarded as forming a section.

By such localization of queen behavior, another important aspect comes into question: Two behavior components, *W* and *C*, correspond respectively to two different aspects of excitement arousal, *W* more to the increase of excitement at particular cells and *C* to the spread of excitement over the comb. Therefore different behavioral types mentioned above relate to the relative prevalence of these two aspects of excitement arousal (Fig. 6). Suppose that the advancing edge of a given comb is provided with four completed cells. In $C>W$ type the excitement grows rather homogenously at all cells (Fig. 6, G), while in $C<W$ type the general excitement increases through repetition of localized ebbs and flows (Fig. 6, L). This difference is maintained till the later sequence of the process as explained below.

1.5. *First predischarge waiting* (R_1, $\bar{r}_1$) *and relation of* IOP *to* UOP: In previous papers the fixation phase was defined as the period from the final arrival of the queen at the comb to the first food discharge in the cell first receiving food. In **1.4.** the term fixation phase was replaced by arousal phase, Ā, but the delimita-

tion of its end was merely stated as "beginning of the first UOP." First the end of $\bar{A}$ is clarified here. At the end of $\bar{A}$, the queen makes her final waiting at a cell until the first droplet of larval food is discharged in it by a worker. This final or *predischarge waiting*, or, to be clearer, the *first predischarge waiting*, R_1, is distinguished by its particular position in the temporal sequence of IOP from subsequent predischarge waitings at a second, third cell, etc., R_2, R_3, ... R_n, in general, *secondary predischarge* waitings, R_i ($i=2\sim n$) (=secondary fixation in previous papers). Previously R was called the final fixation and was included within the fixation phase as its final part. Here we remove it from $\bar{A}$ and regard it as the predischarge phase of the first UOP ($\bar{u}_1$) or $\bar{r}_1$ (R indicates the behavior while $\bar{r}$ the period) (In some groups this expression becomes invalid as discussed in **1.7.**). In other words, $\bar{A}$ corresponds to the fixation phase in the previous usage excluding the final fixation at the cell first receiving food (cf. Fig. 7). This distinction is necessary not to meticulately delimit the temporal sequence but to apprehend its basic structure, especially the relation of IOP to UOP. Further explanations are given with hypothetical cases.

Suppose that a whole oviposition process involving five cells was accurately filmed in two species, each representing $C<W$ and $C>W$ type and also in *Scaptotrigona*. The behavior sequence at these cells would be such as given in Fig. 7. In any types there obviously exists the behavior sequence during $\bar{P}$ and $\bar{A}$ but it is not well articulated. In $C<W$ type the queen would stay at each cell only intermittently and workers would repeat ebbs and flows of excitement without showing a definite phase sequence as realized later at each cell, arranged, *predischarge waiting* ($\bar{r}$), *food discharge* ($\bar{d}$), *oviposition* ($\bar{o}$) and *operculation* ($\bar{s}$). In other words the primordium of each UOP is obscured within an unlocalized general excitement of IOP. This tendency is more conspicuous in $C>W$ type, in which each UOP is virtually concealed in IOP, sharply contrasting to the sequence after the food discharge to the first cell, where IOP gives way to UOP, serving as a mere background for the definite succession of the latter. Only in *Scaptotrigona* the primordium of the first UOP can appear before the beginning of $\bar{r}_1$ due to its trait to localize W. Thus we regard $\bar{r}_1$ as the first phase of 1. UOP or $\bar{u}_1$, serving as a prelude to its definite succession. This means the delimitation of the end of $\bar{A}$ by the start of R_1 or $\bar{r}_1$. The rest of IOP, continuing until the end of the operculation of the cell finally receiving an egg, is simply called the *oviposition phase*, $\bar{O}$ (cf. Fig. 7). Thus we change our previous formula, IOP=n (UOP) as follows: $\mathrm{IOP}=\bar{P}\cdot\bar{A}\cdot n\bar{u}_i=\bar{P}\cdot\bar{A}\cdot\bar{O}=\bar{P}\cdot\bar{A}\cdot n(\bar{r}_i\cdot\bar{d}_i\cdot\bar{o}_i\cdot\bar{s}_i)$. We know well that the delimitation of each phase is itself of minor importance and always involves some artificial or arbitrary judgements. For instance, it will be a matter of opinion whether $\bar{r}_1$ is included in 1. UOP or not. The important matter is that such delimitation facilitates a clearer apprehension of the complicated temporal structure and a closer comparison of the oviposition process among different groups.

Retruning to $\bar{r}_1$, it is quite long in *Melipona*. But in this genus each oviposition is principally discrete (B_s, cf. **1.2.**) so that, if the queen continues W at one

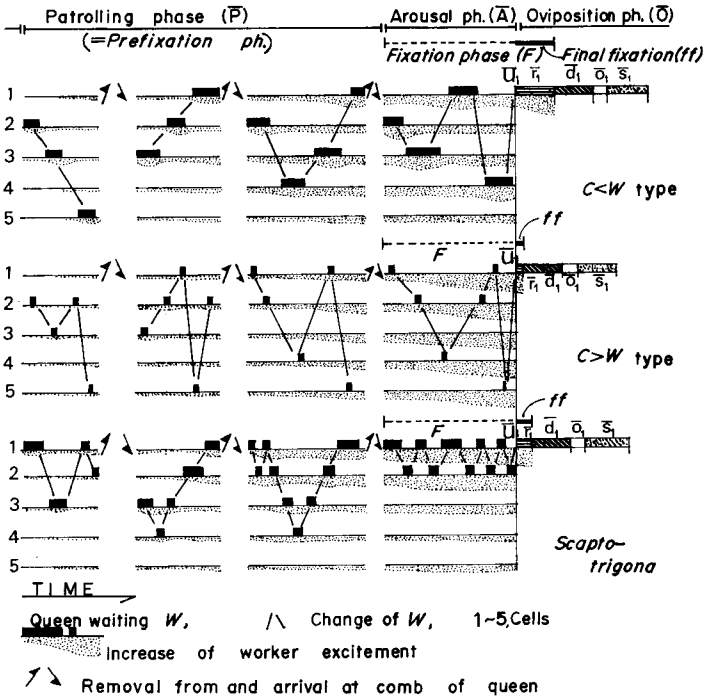


Fig. 7. Behavior sequence in each cell during patrolling phase, arousal phase and the first part of oviposition phase (=first UOP, $\bar{u}_1$), in C<W type, C>W type and *Scaptotrigona*. Each process is assumed as involving five cells (1~5) and in *Scaptotrigona*, as cells 1~2 and 3~4 making each one section. Correspondence between previous and revised terminologies are given at top.

cell since her final arrival at the comb, Ā virtually becomes synonymous to $\bar{r}_1$. If such prolonged $\bar{r}_1$ would appear in other B_f or B_e groups, it means the replacement of a fairly long part of Ā by $\bar{r}_1$, implying the promotion of the process more by localized than general excitement. Hitherto no such group has been confirmed. But the relative length of $\bar{r}_1$ is variable among groups, being fairly long in *Duckeola*, variable but often long in *Tetragona* and *Friesella*, relatively short in *Leurotrigona* and very short in *Nannotrigona** and *Plebeia**. In *Scaptotrigona* $\bar{r}_1$ is short. But if the localization to a definite section involving two or more cells (cf. **1.4.**) is regarded as a whole, the type is similar to that with a prolonged $\bar{r}_1$. Although the number of observed groups is still insufficient, the prolongation of $\bar{r}_1$ seems more conspicuous in *C<W* type than in *C>W* one. Consequently, two different modes of excitement arousal mentioned at the end of **1.4.**, *generalized* (G) and *localized* (L), seem to have a correlation with the relative length of $\bar{r}_1$, too:

L ($C<W$, longer $\bar{r}_1$)			G ($C>W$, shorter $\bar{r}_1$)
Duckeola	*Tetragona*	*Leurotrigona*	*Nannotrigona**
	Friesella		*Plebeia**
(*Scaptotrigona*)			

In L type the role of $\bar{r}_1$ is important to release the food discharge in $\bar{u}_1$ whereas in G type $\bar{r}_1$ is less important because of a higher general excitement.

1.6. *Types of food discharges*: The first UOP begins with $\bar{r}_1$, followed by subsequent phases. Revised terms for phases of Ō given in **1.5.** are cited again together with previous ones in parentheses: predischarge ($\bar{r}$=final fixation), food discharge ($\bar{d}$=provisioning), oviposition ($\bar{o}$) and operculation ($\bar{s}$). Symbols $\bar{r}$, $\bar{d}$, $\bar{o}$, $\bar{s}$ could be replaced by *R* (*W*), *D*, *O*, *S* to designate the behavior prevailing in respective phases (Fig. 8).

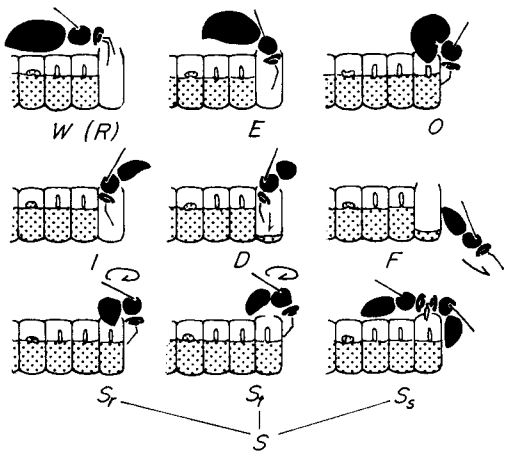


Fig. 8. Prevailing queen (top) and worker (middle and bottom) behavior patterns. *W*(*R*). Waiting; *E*. cell inspection; *O*. Oviposition; *I*. Body insertion; *D*. Food discharge; *F*. Post-discharge escape; *S*. Operculation; S_r. Rotation with metasomal insertion; S_f. Rotation without metasomal insertion; S_s. Sidework.

The first UOP is soon followed by a second, third and subsequent ones. In this serial performance, the queen oviposition is obligatorily successive, but food discharge phase $\bar{d}_i$ takes one of the following four types: 1. *Successive* (D_c). Discharge phase of a given cell, $\bar{d}_i$, appears always after the oviposition in the prior cell, $\bar{o}_{i-1}$, usually preceded by $\bar{r}_i$, i.e. $\bar{r}_i \cdot \bar{d}_i$ always after $\bar{r}_{i-1} \cdot \bar{d}_{i-1} \cdot \bar{o}_{i-1}$. *Duckeola, Cephalotrigona, Tetragona, Friesella, Leurotrigona*, and, if two or more ovipositions form a batch, *Melipona* and *Trigonisca*. 2. *Synchronous* (D_y). Discharges in each cell appear in rapid succession, not always after the oviposition to the prior cell and not necessarily preceded by $\bar{r}_i$. *Nannotrigona**, *Plebeia**. 3. *Semisynchronous* (D_m). First as in D_c later D_y. *Scaptotrigona*. 4. *Singular* (D_0). *Melipona* and *Trigonisca* at singular ovipositions.

This grouping corresponds to that on the relative length of *C* and *W* as well as of $\bar{r}_1$ given in **1.4.** and **1.5.**, suggesting a further extension of the two modes of

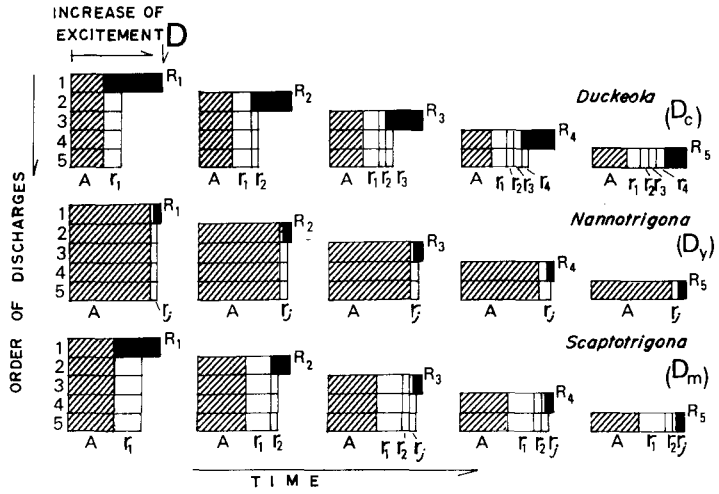


Fig. 9. Models showing excitement arousal in three discharge types, D_c. Successive discharges, D_y. Synchronous discharges, D_m. Semisynchronous discharges. Each IOP is assumed as involving five cells (1∼5). Level of excitement to release food discharge (**D**) is same-scaled in all cells of all groups. A. Excitement attained during $\bar{A}$, R_i. Excitement attained by r_i at cell i (=1∼5), $r_{1,2\cdots i}$. Fractions of excitement by prior discharges at each cell, r_j. Ditto, fractions at several cells combined.

excitement arousal, L and G to this stage. Fig. 9 schematizes, though still incompletely, this difference in three representative groups, *Duckeola* (D_c), *Nannotrigona** (D_y) and *Scaptotrigona* (D_m), assuming that each batch consists of five cells. In *Duckeola* $\bar{r}_1+\bar{d}_1$ raise G only a little so that subsequent $\bar{d}_i$ must be preceded by $\bar{r}_i$. Further in *Duckeola*, *Tetragona* and *Cephalotrigona* *R* must be reinforced by ritualized cell inspections by the queen. In *Nannotrigona* a higher G already attained before $\bar{r}_1$ results in a nearly synchronous appearance of $\bar{d}_i$. In *Scaptotrigona* the sequence is first as in D_c type but later as in D_y. However, even in D_c, especially in *Tetragona* and *Duckeola*, the influence of increasing G upon the subsequent UOP is suggested by the shortening of $\bar{r}_i$ ($i=2\sim n$) compared with $\bar{r}_1$. Therefore, the possible roles of various stimuli participating in excitement arousal could be expressed by two models given in Fig. 10.

1.7. *Phase differentiation in UOP*: In **1.6.** the basic temporal structure of each UOP ($\bar{u}$) was given as $\bar{r}\cdot\bar{d}\cdot\bar{o}\cdot\bar{s}$. Some additional comments are given concerning the phase differentiation within each UOP, first on those common to all UOP.

In previous papers two subphases of $\bar{d}$ were distinguished. These are adopted in the present revised system, too: 1. *Discharge subphase* ($\bar{d}_d$), which covers the time from the start of the first discharging act to the end of the last one. It must be mentioned that each $\bar{d}_i$ involves several discharging acts (Fig. 8, D_j, $j=1\sim m$) appearing successively ($\bar{d}_{di}=m\cdot D_j$). This subphase is essential to $\bar{d}$, occuring

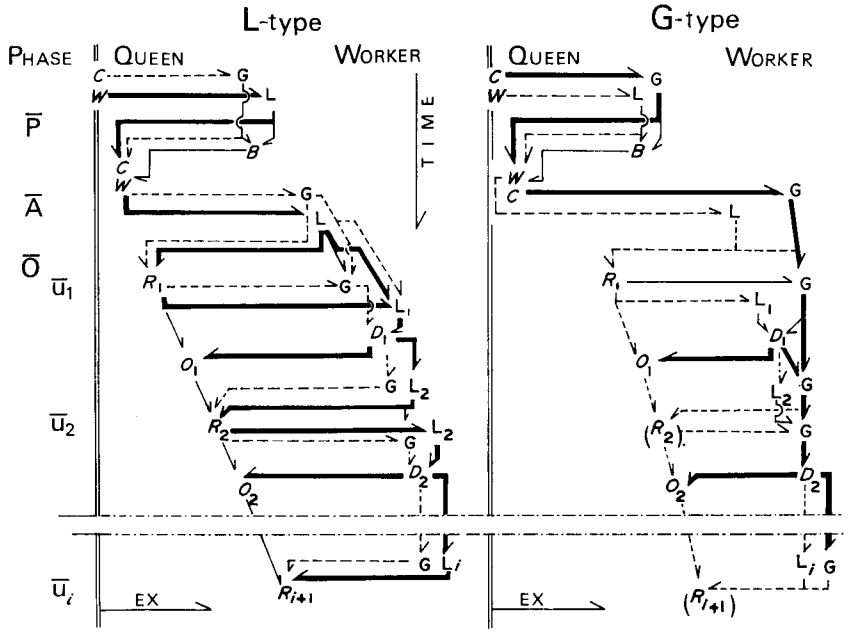


Fig. 10. Models showing relative importance of various releasing stimuli during oviposition process in L and G- types. EX. Increase of excitement, *C*. Cruising; *W*. Waiting; *B*. Cell construction; R_i. Predischarge waiting, O_i, Oviposition; D_i. Food discharge; G, L_i Generalized and licalized excitement. Thick and broken arrows. Strong and weak influence; Thin arrows. Influence not specified.

obligatorily in each $\bar{d}_i$. 2. Postdischarge subphase ($\bar{d}_p$), from the end of $\bar{d}_d$ to the beginning of the queen cell inspection soon before her oviposition. This subphase appears facultatively in the same species or even on the same day in the same colony (*Melipona quadrifasciata*, *Tetragona*, etc.). Further discussion on this interesting subphase is not given here but it must be mentioned that this subphase appears, using our revised terminology, only in D_c type as explained below.

Previously only the duration of queen oviposition (*O*, Fig. 8) was regarded as forming $\bar{o}$, but here the cell inspection immediately before *O* by the queen (Fig. 8, *E*) is included in $\bar{o}$, because this behavior is intimately linked with *O*. Concerning $\bar{s}$, previously two subphases were recognized, rotation and sidework subphases. In some groups, however, the consistent operculation occasionally starts with a considerable delay after the end of $\bar{o}$ (*Melipona quadrifasciata*, *Tetragona*). Further in *Tetragona*, *Duckeola*, *Trigonisca*, etc., rotation often continues after the removal of metasomal tip. Therefore four subphases may be recognized in $\bar{s}$: 1. *Preoperculation subphase* ($\bar{s}_p$): Since the end of $\bar{o}$ to the beginning of consistent rotation, involving delay as well as interrupted attempts of rotation. This subphase is

facultative and could be omitted from the consideration in many cases. 2. *Main rotation subphase* ($\bar{s}_r$): From the start of rotation until the first removal of the metasomal tip (cf. Fig. 8, S_r). This is the most essential part of $\bar{s}$, being obligatory except for *Friesella*. 3. *Transient subphase* ($\bar{s}_t$): From the first removal of the metasomal tip to the disappearance of rotation (Fig. 8, S_t). This subphase is conspicuous in *Tetragona*, *Duckeola*, *Cephalotrigona* and *Trigonisca* but virtually absent in *Melipona*, *Scaptotrigona*, etc. This subphase is often difficult to delimit accurately because rotation and sidework can appear alternately in due course. 4. *Sidework subphase* ($\bar{s}_s$): From the final disappearance of rotation to the end of operculation. In this subphase, the work is made by the bee sitting aside the cell, usually accompanied by one or more helpers (Fig. 8, S_s). Rotation is absent in *Friesella* so that its operculation phase consists of only $\bar{s}_p$ (facultatively) and $\bar{s}_s$. The end of $\bar{s}$ is occasionally difficult to delimit clearly. Three events are useful, closure of cell orifice, departure of the principal operculator (=the worker participating in rotation) and final departure of the bee working on the cell (either principal operculator or helpers, usually the latter). The first event is easy to confirm but the work often continues long after the cell closure. The last event frequently appears with a considerable delay. The confirmation of both first and second events is recommended to give the approximate length of $\bar{s}$. Summarizing, each UOP basically takes the following sequence (facultative parts in parentheses): $\bar{r}\cdot\bar{d}_d\cdot(\bar{d}_p)\cdot\bar{o}\cdot(\bar{s}_p)\cdot\bar{s}_r\cdot(\bar{s}_t)\cdot\bar{s}_s$.

Nextly let us examine the difference between successive UOP, which appears intimately linked with the types of food discharges given in **1.6.** In D_c type the temporal structure of each UOP does not show any essential difference (Fig. 11, D_c). Often $\bar{r}_{2\sim n}$ are shorter than $\bar{r}_1$ under the influence of general excitement, G. The interval between the end of $\bar{o}_i$ and the start of $\bar{r}_{i+1}$ is usually short, though occasionally prolonged by the insertion of *C* or *C* and *W* by other cells.

In D_y type (Fig. 11, D_y), besides $\bar{r}$ is often very short or even virtually obsolete (**1.5.**), the temporal correspondence between $\bar{d}_i$ and $\bar{o}_i$ does not necessarily exists. When food discharges in each cell, $\bar{d}_i$, appear very synchronously and $\bar{r}_i$ is very brief, a cell receiving food earlier is not necessarily that oviposited earlier, in extreme case, resulting in a complete discrepancy of two temporal orders (Fig. 11, X). Therefore, the order of successive IOP or $\bar{u}_i$, in D_y type is determined by two ways, either by $\bar{d}_i$ or by $\bar{o}_i$. The determination by $\bar{d}_i$ is often difficult because of its synchronous appearance. The determination by $\bar{o}_i$ is easier for its successive appearance but can be made only later. Because of this possible discrepancy between two orders, the ways adopted to determine the temporal order of $\bar{u}_i$, either by $\bar{d}_i$ on by $\bar{o}_i$, must clearly be mentioned when the oviposition process of D_y type is described.

In connection with this discrepancy, there appears another peculiarity in D_y. As the end of $\bar{d}_i$ appears earlier than the beginning of $\bar{o}_{i-1}$, a peculiar phase is inserted between the end of $\bar{d}_i$ and the beginning of $\bar{o}_i$. This phase consists of two parts (Fig. 11, D_y): 1. Time since the end of $\bar{d}$ to the arrival of the queen

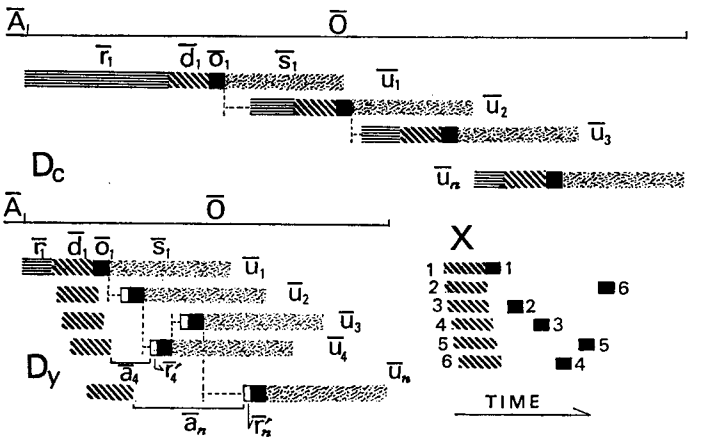


Fig. 11. Temporal articulation between and within each UOP (ū) in D_c and D_y types. X. Possible discrepancy of food discharge and oviposition sequence in D_y type (Further explanations in text).

(=$\bar{a}$) and 2. Time since the arrival of the queen to the start of $\bar{o}$ (=$\bar{r}'$). The latter phase, postdischarge waiting ($\bar{r}'$) is functionally similar to $\bar{r}_i$ in D_c type but the chronological position is inverted. The first part, $\bar{a}_i$, corresponds to $\bar{d}_p$ in D_c type, but differs by the absence of queen waiting (*W*), and its duration gradually lengthens in later $\bar{u}_i$ due to the increasing interval between $\bar{d}_i$ (synchronous) and $\bar{o}_i$ (successive). This phase, ā, is called here the *attending phase* by a peculiar worker behavior appearing there. Usually one worker continuously stays by the cell as if guarding it until the arrival of the queen (*Plebeia**, *Nannotrigona**, *Lestrimelitta** and later $\bar{u}_i$ in *Scaptotrigona*). Consequently the phase differentiation of the oviposition phase ($\bar{O}$) in D_c and D_y types are formulated as follows (Subphases not mentioned. Facultative ones in parentheses):

$D_c = \bar{r} \cdot \bar{d} \cdot \bar{o} \cdot \bar{s}$

$D_y = (\bar{r}) \cdot \bar{d} \cdot \bar{a} \cdot (\bar{r}') \cdot \bar{o} \cdot \bar{s}$

In *Scaptotrigona* the phase sequence is first as in D_c, later as in D_y.

1.8. *Systematization of temporal articulation of oviposition process*: Thus we came to the provisional systematization of the temporal articulation of oviposition process in various stingless bee groups as schematized in Fig. 12. The results are formulated (subphases excluded):

$$\mathrm{IOP} = \bar{P} \cdot \bar{A} \cdot \bar{O}, \quad \bar{O} = n\,(\mathrm{IOP}) = \overset{n}{\underset{i=1}{n}}\, \bar{u}_i = \overset{n}{\underset{i=1}{n}}\, \bar{r}_i \cdot \bar{d}_i \cdot \bar{o}_i \cdot \bar{s}_i \quad \text{in } D_c \text{ type}$$

$$= \overset{n}{\underset{i=1}{n}}\, (\bar{r}_i) \cdot \bar{d}_i \cdot \bar{a}_i \cdot (\bar{r}_i') \cdot \bar{o}_i \cdot \bar{s}_i \quad \text{in } D_y \text{ type.}$$

From the previous results of observations, several behavioral types are distinguished as follows:

Type	Excitement arousal	Cell construction	Batch formation	Queen behavior	Food discharge	Groups
I	G<L	S_c or S_m	B_f or B_e	cW or cW′	D_c	*Duckeola, Cephalotrigona, Friesella*
II	G≒L	S_y	B_e	CW	D_c	*Leurotrigona*
III	(G≳L)	S_c	B_e	(Cw)	D_m	*Scaptotrigona*
IV	G>L	S_y	B_s	Cw, C	Dy	*Plebeia*, Nannotrigona*
V	(G=L)	S_c	B_s	cW	D_o (D_c)	*Melipona*

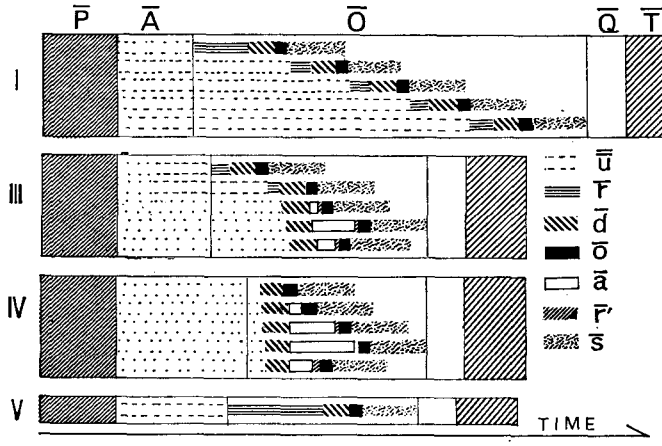


Fig. 12. Temporal articulation of oviposition process in relation to other phases in circadian colony cycle in four behavioral types, each represented by *Duckeola* (I), *Scaptotrigona* (II), *Plebeia* (IV) and *Melipona* (V). Except *Melipona* each Ō is assumed as involving five ū, ūi. Primordium of each ū. Other explanations in text. Duration of each phase does not represent real relative length.

Although further studies may reveal the occurrence of other types or may necessitate some modifications of the system presented, the synopsis given above suggests, together with Fig. 12, two opposite tendencies among various groups concerning the integration of UOP into IOP, which is stronger in type IV than in II and I (III and V are difficult to incorporate in this order. Cf. **1.5.**). Considering the stingless bees as a whole against other bees, a higher integration must indicate a specialized condition. As to the relation among groups exhibiting different types, however, it is not easy to conclude their relative antiquity without comparing various other characteristics, both morphological and non-morphological ones. Our basic working hypothesis is to regard less integrated types as more primitive (Sakagami, Camilo and Zucchi 1973), simply because we need a provisional starting point for further explorations. But the distintegration of IOP accompanied with a relative independence of UOP is possible and must always be

kept in mind. For instance, ritualized cell inspections by the queen appearing in Type I could be regarded as a specialization.

Finally we would like to incorporate the extra-oviposition period in our phase delimitation, in order to complete our picture on the circadian colony cycle. A tentative system is given as follows (cf. Figs. 12 and 13):

Ā. *Extraoviposition period.*
  Q̄. *Quiescent phase.* Comb surface is completely free from oviposition excitement. Time since the end of the prior Ō to the revival of some primordia of oviposition excitement, such as the first visit of the queen with the comb in S_c type or the beginning of cell construction in S_y type.
  T̄. *Transient phase.* From the end of Q̄ to the beginning of P̄.
B̄. *Oviposition period* (IOP), consisting of P̄, Ā, Ō as explained above.

Here again, the exact phase delimitation is impossible and meaningless, just as in case of dividing historic or prehistoric eras. What important is to apprehend rhythmic ebbs and flows of oviposition excitement, by which the stingless bees are

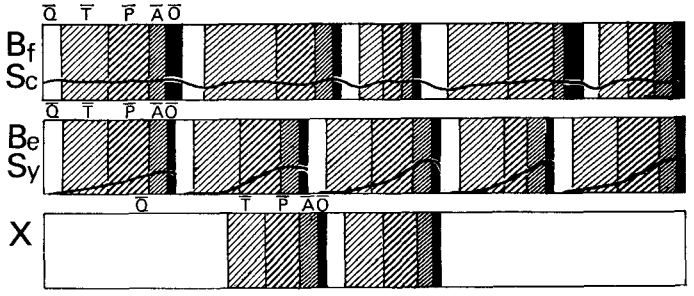


Fig. 13. Circadian colony cycle in B_f-S_c and B_e–S_y types. X. Limitation of oviposition process during favorable hours in some groups with poor capacity of thermoregulation.

unique among all eusocial insects. The relative length of these five phases could be in general T̄>P̄>Q̄>Ā>Ō as shown in Fig. 13 but varies from one group to another. As pointed out in **1.5.** the phase distinction may be difficult in *Nannotrigona** and *Geotrigona** except for Ā and Ō, for the queens spend their most daily lives on the combs. The rhythmicity produced by such phase differentiation must also be variable among groups, being more regular in S_y or B_e types than in S_c or B_f types as schematized in Fig. 13. The bottom schema in Fig. 13 (X) shows the restriction of ovipositions to favorable hours during adverse season in some groups with poor capacity of thermal regulation (cf. **2** and **3**). Such prolonged quiescent phase can extend to an entire season in some species, e.g. *Plebeia droryana* (Friese), (Terada unpub.), resulting in the cessation of cell construction and oviposition as well as the prolongation of worker longevity, which are comparable to the winter dormancy of honeybee colonies in the temperate and frigid regions.

2. *Hypotrigona* (*Leurotrigona*) *muelleri* (Friese)

This and the next species are each one of the smallest bees in the World, with the body length of dried worker specimens seldom exceeding 2 mm. They are, together with some other Brazilian *Hypotrigona* species, familar by the popular name, "lambe-olhos" (=eye lapper) for their habit to frequent the exposed human skin, especially the eyes, to lap perspiration and tear, which gives under blazing heat a nuisance often much more insufferable than attacks by mosquitoes and gnats (e.g. cf. Lévi-Strauss 1955). *Leurotrigona* is monobasic (Moure 1950). *L. muelleri* is distributed in southern Brazil (States of Saõ Paulo, Paraná, Santa Catarina) and Paraguay.

Observations were first made in Rio Claro, the State of São Paulo, in 1963, with a colony taken in Martinópolis, S.P. Later, additional studies wre made in Ribeirão Prêto, S.P., especially in 1971~'72. These studies have clarified some interesting aspects of colony cycle with reference to the production of sexuals, etc. (Terada unpub.). In the present paper only observations on the oviposition behavior are described, accompanied with some general accounts as a basis for further studies.

2.1. *General traits out of oviposition process*: As in other groups so far described, some observations on nest structure and queen-worker coactions are briefly given in this subsection.

2.1.1. *Nest structure and related features*: Nests are mostly made within narrow cavities of hollow trees, either branches or thin trunks. The species is a typical cluster maker. Cells are often connected with pillars (Fig. 14, B, C, Fig. 16, J) and form an irregular cluster or clusters which develop upwards as in *Frieseomelitta* (Kerr *et al.* 1969). But contrary to the latter, cells are shorter and honey and pollen pots are homomorphous (Fig. 14, A, B, Fig. 16, A, C). The involucrum is absent as in most cluster makers. Both cells and pots are built with no or little admixture of resin, giving a pale and semitransparent appearance, being quite different from nests of other stingless bees and rather resembling newly built honeybee combs. The nest entrance is narrowed but allows the passage of more than one bee or the presence of up to four guards at a time. Previous observations on nest architecture are referred to Schwarz (1948).

As in most other stingless bees, the excrement and other waste materials are once deposited at definite places within the nest cavity, each forming a small heap. However, rubbish is not carried away on the wing, simply thrown down from the entrance, resulting in a small heap below the entrance. In this trait, *Leurotrigona* resembles *Hypotrigona* s. str.* and, interestingly, two cleptobiotic groups, *Lestrimelitta* (Sakagami and Laroca 1963) and *Cleptotrigona* (Araujo 1958).

The first colony observed in Rio Claro was introduced into a double-walled heat controlled observation hive (Sakagami 1966). But reflecting the nest site preference, the colony refused to use the inner box with an ample space, occupying the narrow corridor connecting the inner box to the nest entrance. Subsequent

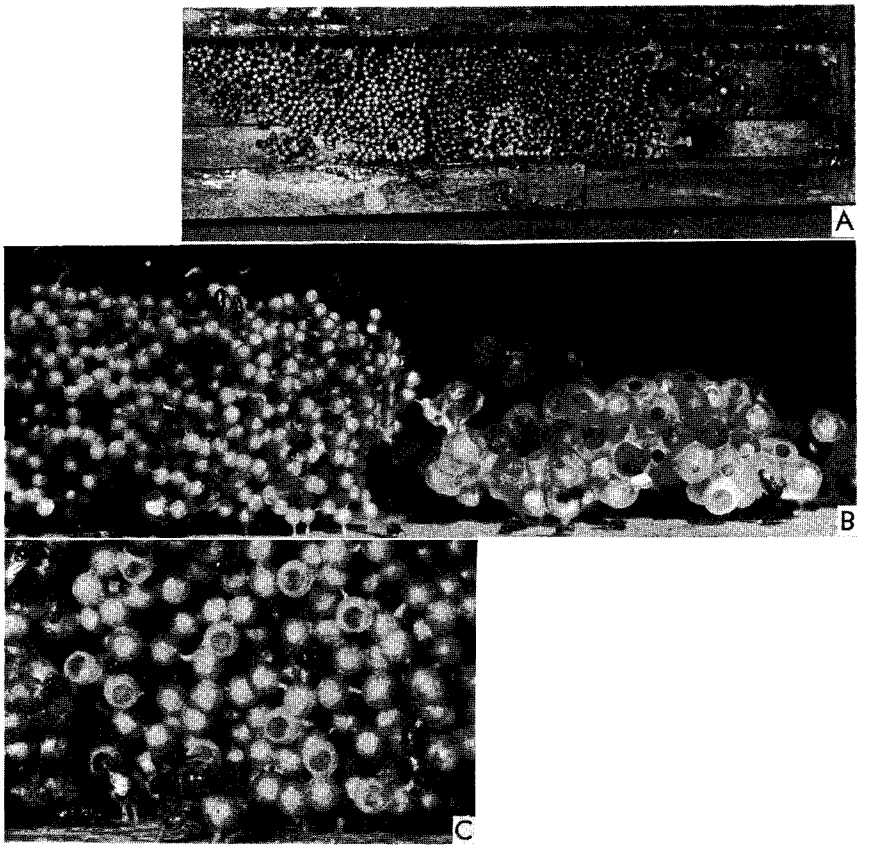


Fig. 14. Nest structure of *Leurotrigona*. A. Colony in a simple elongate wooden box. B. Ditto, enlarged. Left. Brood cell area, Right. Storage pot area. C. Several cells ready to receive larval food. Queen at bottom.

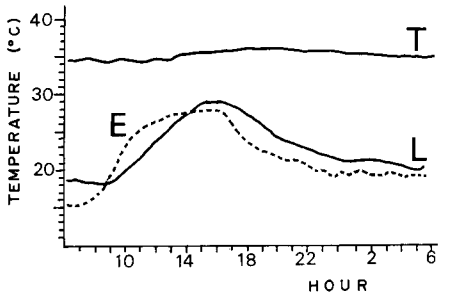


Fig. 15. Fluctuation of nest temperature in *Trigona* (*Trigona*) *spinipes* (T) and *Leurotrigona muelleri* (L), measured synchronously with that of environment (E).

trials showed that *Leurotrigona* colonies could easily be reared in such narrow cavities (Fig. 14, A). Moreover, two interesting findings were obtained: 1) This species has virtually no capacity of heat regulation. On cold days, especially at night, colony activities cease by cold stupor. Fig. 15 (from Zucchi and Sakagami 1972) shows the temperature change at brood rearing area from a 24 hours periodical recording. The nest temperature fluctuates just as that in the environment, sharply contrasting to the excellent regulatory capacity in *Trigona* (*Trigona*) *spinipes* (Fabricius) measured on the same day in the same room. 2) In spite of such susceptibility, the colony is quite resistant to temperature fluctuation. Immatures hardly perish by a cyclic exposure to the cold and colony activities readily recover by a rise of temperature in the daytime. Moreover, the queen and workers are relatively indifferent to exogenous disturbances, for instance, not ceasing their activities by translocation of nests, etc. This facilitates the studies of behavior, though for closer observations occasionally the use of a hand-lens or a binocular microscope is required for their tiny size.

2.1.2. *Queen behavior and queen-worker coactions*: In contrast to other groups so far studied, the queen tends to stay at a definite place out of the oviposition period. In the colony X observed in Rio Claro, she spent most of her extra-laying time at a spot about 3 cm apart from the nest entrance. From there she made excursions from time to time to visit the cell cluster. A similar tendency was observed in other colonies kept in Ribeirão Prêto. Her walk is more rapid than *Melipona* and especially *Scaptotrigona* but not so rapid and abrupt as in *Plebeia.** Wings are virtually not beaten at rest but make regular or intermittent vibrations at walk. Either at rest or at walk, antennae are constantly but not violently shaken.

Behavior coactions between queen and workers are simple. Four patterns basic to all other groups are found: 1) Gradual formation of royal court around the resting queen, 2) Repetition of rapid pushing followed by retreat by attendants in front of the queen, 3) Escape by workers at sudden encounter with the queen and 4) Gradual retreat by workers perceiving the approaching queen. But all are rather sluggish. No ritualized responses are performed by workers to the queen, contrasting to *Scaptotrigona*, *Nannotrigona** and *Plebeia*,* though a brief and weak akinetic response and a mild darting in front of the queen are rarely observed. Food begging by the queen to workers and resulting buccal contact between them with hand shaking (Fig. 16, L) are frequent, sometimes lasting even 3 min. But real food delivery by workers was difficult to confirm even if occurs. We observed only a few plausible instances.

No systematic observation on worker behavior out of oviposition process was undertaken. Fig. 16 illustrates some behavior patterns found in the colony. At food regurgitation workers tend to twist heads (Fig. 16, B) and at out-door fanning fore bodies are raised and heads direct against the nest entrance, both common to other stingless bees so far observed. Interferences in nests are responded virtually

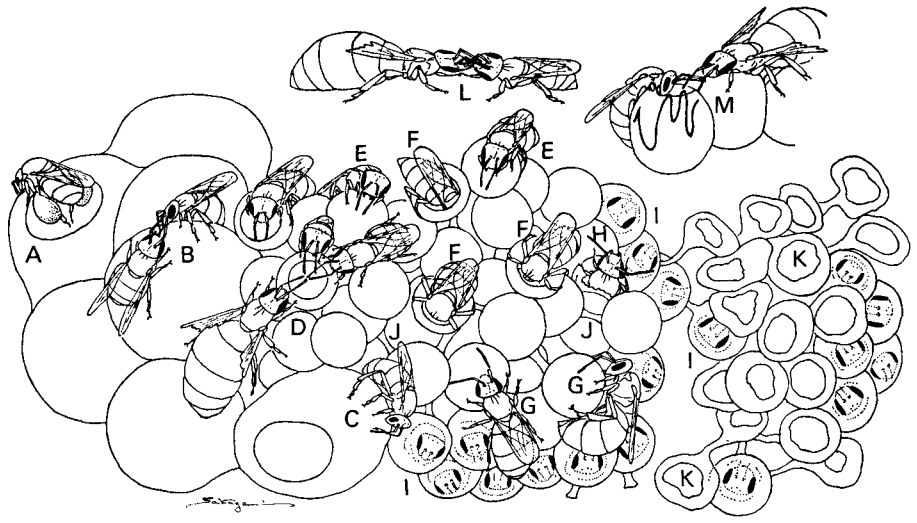


Fig. 16. Nest structure and colony life of *Leurotrigona* drawn as five brood cells are ready to receive larval food. A. Pollen pot where a pollen forager is disloading; B. Foor regurgitation, the donor twisting the head;. C. Honey pot with one worker manipulating the surface; D. Arrival of queen at a completed cell, resulting in repetition of pushing-retreat by three workers; E. Workers manipulating brood cells; F. Workers inserting fore-bodies in completed cells; G. Workers with swollen metasomas; H. Worker at emergence; I. Old cells. Semitransparent cocoons exposed by removal of waxen cell walls, making pupae visible; J. Pillars connecting cells; K. Brood cells destroyed after consumption of contents; L. Buccal contact between queen and worker; M. Appearance of intentional body insertions in worker by the arrival of queen. The course of worker movement shown by thick line.

by no defensive response.

In one feature *Leurotrigona* differs from other groups. As is usual workers participating in cell construction and food discharges have paler coloration and enlarged metasomas compared with aged foragers. The latter feature is, however, very conspicuous. There are frequently seen the workers whose metasomas are swollen to the extent intermediate between foragers and a gravid queen (Fig. 16, G). Probably this peculiarity relates to the low number of food discharges per UOP described in **2.3.2.**

2.2. *Oviposition pattern and cell construction*: Before describing the behavior sequence in oviposition process, the pattern of oviposition in *Leurotrigona* is referred to, followed by observations on cell construction.

2.2.1. *Oviposition pattern*: The oviposition process is exclusively batched (B_e, **1.2.**). The number of cells involved in each IOP is given in Table 1, together with some related measurements taken in Colony X. The number of cells ovipo-

Table 1. Some numerical data on the oviposition rate in *Leurotrigona* (Colony X, 1963)

No. Case	Date	No. cells oviposited (No. not oviposited)	No. cells built for the next IOP	Duration since the prior IOP (h:min.)	Approximate oviposition rate per hour
1	May 13	3			
2	14	7	8		
3	〃	8	7	3:33	2.3
4	15	10(1)	10		
5	〃	10	9	6:32	1.5
6	〃	9		4:05	2.2
7	16	13(2)	13		
8	〃	9	7	4:59	2.6
9	18	5			
10	〃	6	5	4:59	1.2
11	20	6			
12	21	6	7		
13	〃	7	5	1:53	3.7
14	〃	5		5:12	1.0
15	22	6	5		
16	24	6	5		
17	〃	5	6	4:22	1.2
18	25	?	7		
19	〃	7(1)		2:24	2.9
20	26	5			
21	〃	3(1)	5	5:06	0.6
22	〃	5	5	2:11	2.3
23	June 8	5			
24	10	5			

sited in each IOP (mean 6.6) was relatively high during May 14 ~16, but decreased later down to about one half, probably related with some unfavorable conditions in the colony. The interval between two successive IOP is variable (Table 1, 5th column, mean 3 h. 56 min.) and not particularly correlated with batch size of the prior IOP, though further observations would prove such relation confirmed in *Friesella.*

The mean oviposition rate in Colony X was 1.95 per hour or 36.8 per day in May and June. But this rate varies in *Leurotrigona* much more than in other groups due to its limited capacity of thermoregulation. In colder season colonies not artificially heated often stop the oviposition for days or even much longer. Moreover, under adverse conditions the destruction of once ovipositied cells and intake of their contents frequently appear. This event is occasionally seen in other groups but seemingly frequent in weak colonies of *Leurotrigona.* For instance, the destruction started in Colony X on May 18 and about one half of cells before pupation were consumed until June 10 (Fig. 16, K).

When one of the workers opens the cell, many workers gather around the cell, which is similar to that found in orphan colony (Fig. 19, F). A marked excitement

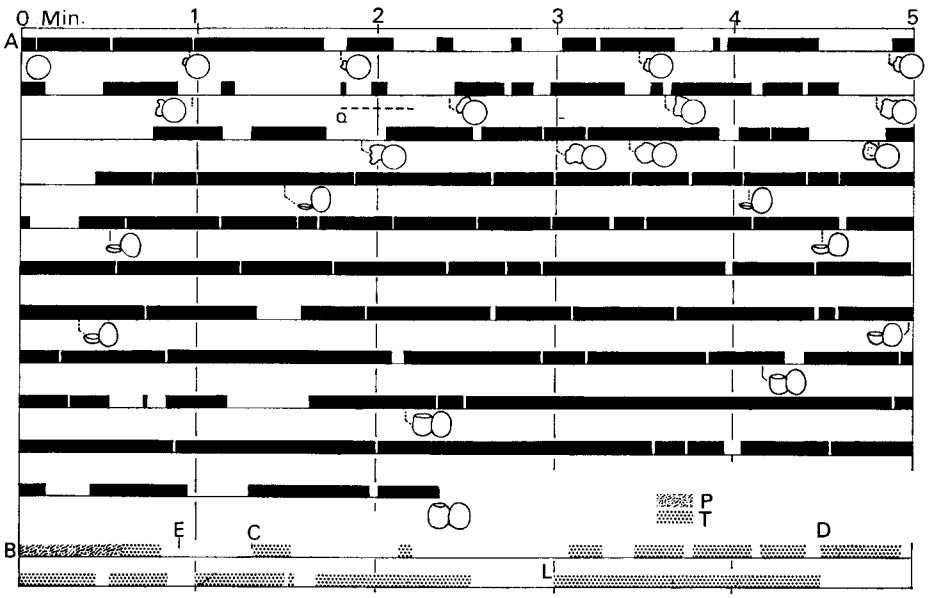


Fig. 17. Chronological sequence of cell construction (A) and consumption of cell contents (B) in *Leurotrigona*, each shown by a representative case. A. Each horizontal block shows participation of a worker in construction. Cell growth is given for first 15 min. by dorsal view, later by profile view. B. P. Perforation; T. Occupation of cell by a worker; E. Beginning of excitement; C. Beginning of worker congestion; D. Decrease of congestion; L. Cell content nearly empty.

develops among workers. The intake is fairly rapid so that within 15 min. cell content is absorbed as shown in Fig. 17, B. Several workers successively ingest the content but the replacement among them is often disturbed because more than one worker simultaneously seek for the content.

This ingestion of cell contents is noteworthy, because it can coexist with the oviposition process. Although we have no crucial evidence, the workers ingesting cell contents and those discharging larval food in the cell to be oviposited are of the same age class. It is likely that one and the same individual makes the two acts on the same day. Apparently the result is of less survival value than the cessation of oviposition. But it suggests two possibilities: 1) The queen is more important than workers in realizing a regular rhythmicity in oviposition process. 2) Some basic structures of oviposition process can persist, to some degree, without changing behavioral context, even under unfavorable colony conditions.

Under favorable conditions the batch size increases and the interval between two IOP decreases. But the maximum oviposition rate have seldom exceeded 100 per day though no systematic recording has been taken.

2.2.2. *Cell construction*: Cell construction is synchronous (S_y, **1.1.**). After

Table 2. Speed of cell construction in *Leurotrigona* (in min.)

No. case	A	B	C	D	E	F
1	9	1>	58	48	106	
2	20	20	25	40	85	443
3	22	12	65	5	82	133
4	4	19	32	25	76	214
5				10		
6			35	20		205
7				25		
8	15					
9	9	15	55	1>	70	69

A. From last oviposition to start of construction of first cell
B. From start of first cell to that of last cell
C. From start of last cell to completion of first cell
D. From completion of first cell to that of last cell
E. Durations B~D combined
F. From completion of last cell to first oviposition

the end of an IOP, there is no half-built cell except occasional permanence of one completed cell or rarely two, which have remained unoviposited in the prior IOP (Table 1, 3rd column). New cells are soon started after the IOP nearly synchronously (Table 2). As in other groups, cells are built by the summation of successive activities by a number of workers (cf. Sakagami, Montenegro and Kerr 1965). Fig. 17, A shows the temporal sequence of the construction of one cell from start to completion. The first 15 min. were mainly spent for accumulation of materials at the side of one cell, later the cell gradually increased its height until the orifice was constricted about 52 min. after the start of construction.

The approximate time spent for construction was measured in several cells as follows (in min., each one case unless parenthetically mentioned): 32 (5 cases), 33, 40 (2), 42 (4), 45 (3), 50 (2), 51 (7), 55 (5), 57 (3), 58 (2), 65 (2), 75 (2), in average 49.3 min. The construction is relatively rapid and made more laboriously than in other groups, especially at incipient stage. In this species not only cells but storage pots are also built rapidly. The motor pattern exhibited at cell construction appears as if more excited than in oviposition process.

2.3. *Oviposition behavior.*: The oviposition process in *Leurotrigona* is characterized by the simplicity of behavior patterns exhibited as described subsequently.

2.3.1. *Behavior before food discharges in brood cells*: A considerable time after the prior IOP the queen leaves the resting place, visits the cell cluster, cruises around there and returns to the resting place. This patrolling becomes gradually more frequent in parallel with the growth of cells. As given in Table 2, the duration between completion of the last cell and the next IOP, roughly comparable to patrolling and arousal phases combined in Section **1**, $(\bar{P}+\bar{A})$, is occasionally quite

long. No continuous observation was made on the queen behavior during $\bar{P}$, but some instances are cited below (**X**. All cells completed; ⌊⌈ Arrival at and removal from the cluster by queen; *Y*. Final arrival at cell cluster=beginning of $\bar{A}$; D_1. first food discharge; ···· Not observed):

May 14, A: **X** 9:40 ···· 11:00, 11:12 ⌊⌈, 11:13 ⌊⌈, 11:14⌊ –11:15 ⌈, 11:16⌊ –11:17 ⌈, **Y** 11:19⌊ –11:23 D_1 ($\bar{P}$=99 min.).

May 14, B: **X** 13:50 ···· 14:00, 14:15⌊ –14:16 ⌈, 14:25⌊ –14:26 ⌈, 14:19⌊ –14:50 ⌈, Y14:54 ⌊–14:56 D_1 ($\bar{P}$=64 min.).

May 15: **X** 17:05, 17:20⌊ –17:21 ⌈, 17:24⌊ –17:28 ⌈, 17:31⌊ –17:34 ⌈, 17:34⌊ –17:35⌈, 17:36⌊ –17:37 ⌈, 17:43 ⌊, ···· **Y** 19:18⌊ –19:19 D_1 ($\bar{P}$=133 min.).

May 16: **X** 10:40, 12:32 ⌊, 12:43 ⌈, 12:50 ⌊, 13:10 ⌈–13:14 ⌊, 14 45 ⌈, ···· **Y** 14:13 ⌊–14:14 D_1 ($\bar{P}$=213 min.).

May 18: **X** 15:25, 16:25 ⌊–16:28 ⌈, ···· 18:01 ⌊⌈, 18:07 ⌊⌈–18:08 ⌊⌈, 18:10 ⌊⌈–18:13 ⌊, 18:14 ⌊⌈, 18:48 ⌊⌈, *Y* 18:50 ⌊–D_1 ($\bar{P}$=205 min.).

Regarding **X** as the start of $\bar{P}$, the length of $\bar{P}$ parenthetically given in each case ranges from one hour to more than three. During this phase the queen repeats visits with the cell cluster, each visit lasting one to several minutes. Both queen and workers behave rather quiet in $\bar{P}$ and the most part of $\bar{A}$. At visiting cells, the queen sits on a neighbouring cell already oviposited and touches the upper margin of the cell with her antennae, but only rarely inspects it by inserting the head. The worker attendants by each cell are very few, usually only one or two (Fig. 16, D). The insertions of fore bodies into the cell are repeated as in other groups but not so excitedly. By the arrival of the queen, worker attendants often rather slowly leave the cell after repeated but unrealized attempts of body insertions (=intentional insertions, cf. **1.4.**, Fig. 16, M). Workers touched by the queen at body insertions also mostly escape after rapid withdrawals from cells. Neither overt darting by workers to the queen nor ritualized behavior appear during $\bar{P}$ and $\bar{A}$. Throughout $\bar{P}$ the queen stays only for a brief time by each cell, spending her most time by cruising on the cell cluster (Type CW in **1.4.**). Workers are rather quiet than in other groups. The frenetic body movements at body insertions, which delimit the start of $\bar{P}$ in some groups, e.g. *Melipona* and *Scaptotrigona*, appear only in $\bar{A}$.

Some numerical data on arousal phase ($\bar{A}$) and the incipient part of oviposition phase ($\bar{O}$), i.e. predischarge phase of 1. UOP (=$\bar{u}_1$), $\bar{r}_1$, measured in several IOP are given in Table 3, and the chronological sequence of five accurately observed IOP are presented in Fig. 18. The comparison of column $\bar{A}$ and W in the table reveals that most part of $\bar{A}$ is spent by cruising in many cases. This is also recognized by the rarity of prolonged waitings (cf. column *W* and *w*) as well as the low number of cells visited (column C). The result is sharply contrasting to that in *Tetragona* (Sakagami and Zucchi 1967, Table 1. Values for $\bar{A}$ and W are obtained by subtracting values in column D from the corresponding values in columns A and B), in which $\bar{A}$ is much longer and the queen spents her most time by waiting. The difference is also recognized by comparing W and $\bar{r}_1$ in both species. The

Table 3. Some numerical data on behavior in arousal phase ($\bar{A}$) and first predischarge phase ($\bar{r}_1$) in *Leurotrigona*

No. Case	Duration (sec.)			Frequency of behavior							Correspondence to Fig. 18
	$\bar{A}$	W	$\bar{r}_1$	*W*	*w*	C	*E*	*e*	*I*	*i*	
1	212	18	4	0	4	2(7)	1	0	0	2	
2	113	30	11	3	4	4(8)	0	0	4	4	A
3	104	16	9	1	4	2(9)	2	1	3	0	B
4	128	53	13	4	2	5(10)	2	1	2	3	
5	49	4	31	0	4	4(9)	4	0	0	11	C
6	48	1	41	0	1	1(9)	1	0	0	13	D
7	72	2	38	0	2	2(13)	2	0	0	12	
8	95	3	20	1	1	2(6)	4	2	0	5	
9	15	3	33	1	3	2(5)	0	1	1	5	
10	28	26	2	0	2	2(6)	0	0	5	0	
11	210	8	2	1	2	2(7)	1	0	0	1	E

$\bar{A}$: Duration of arousal phase: From final queen arrival at cell cluster to beginning of $\bar{r}_1$.
W : Ditto, subtracting time spent for cruising.
$\bar{r}_1$: Duration of first predischarge phase.
W : Number of prolonged queen waitings (longer than 5 sec.) at cells during $\bar{A}$.
w : Ditto, of shorter waitings (less than 5 sec.).
C : Number of cells receiving visits or waitings (in parentheses, number of completed cells present).
E, *e*: Number of queen inspection during $\bar{A}$ and $\bar{r}_1$.
I, *i*: Number of worker body insertions during $\bar{A}$ (during queen waiting alone) and $\bar{r}_1$.

first predischarge phase or $\bar{r}_1$ is in average longer in *Tetragona*, *Duckeola* and *Friesella*. Another peculiarity in *Leurotrigona* is the scarcity of both queen inspections and worker body insertions in $\bar{A}$ and $\bar{r}_1$ compared with *Tetragona*, which is characterized by ritualized repetition of queen inspections in $\bar{A}$ and $\bar{r}_1$. On this point *Duckeola* resembles *Tetragona* (Type cW' in **1.4.**) and *Friesella* is similar to *Leurotrigona*, though only $\bar{r}_1$ was closely observed in these two groups. The comparison of columns *E* and *e* indicates that queen inspections are very scarce, and seemingly more frequent in $\bar{A}$ than in $\bar{r}_1$, both being similar to *Friesella*. Further the scarcity of worker body insertions in *Leurotrigona* also resembles *Friesella* and depends on the same cause, the replacement of actual insertions by intentional ones in the presence of the queen. In the absence of the queen, body insertions are often frequent, which are not illustrated in Fig. 18, but in C, cell No. 7 frequent insertions made alternately by two workers are partly mentioned (Y). Thus, *Friesella* and *Leurotrigona* are similar for each other by the simplicity of queen and worker behavior during $\bar{A}$ and $\bar{r}_1$. However, *Leurotrigona* differs from *Friesella* by, 1) Shorter waiting in $\bar{A}$ and $\bar{r}_1$, i.e., belonging to type CW, not cW (**1.4.**). 2) The queen does not beat workers attending the cell violently. 3) Workers do not show an overt avoidance of the queen arriving at the cell. 4) Workers do not form a "frozen rosette" around the cell.

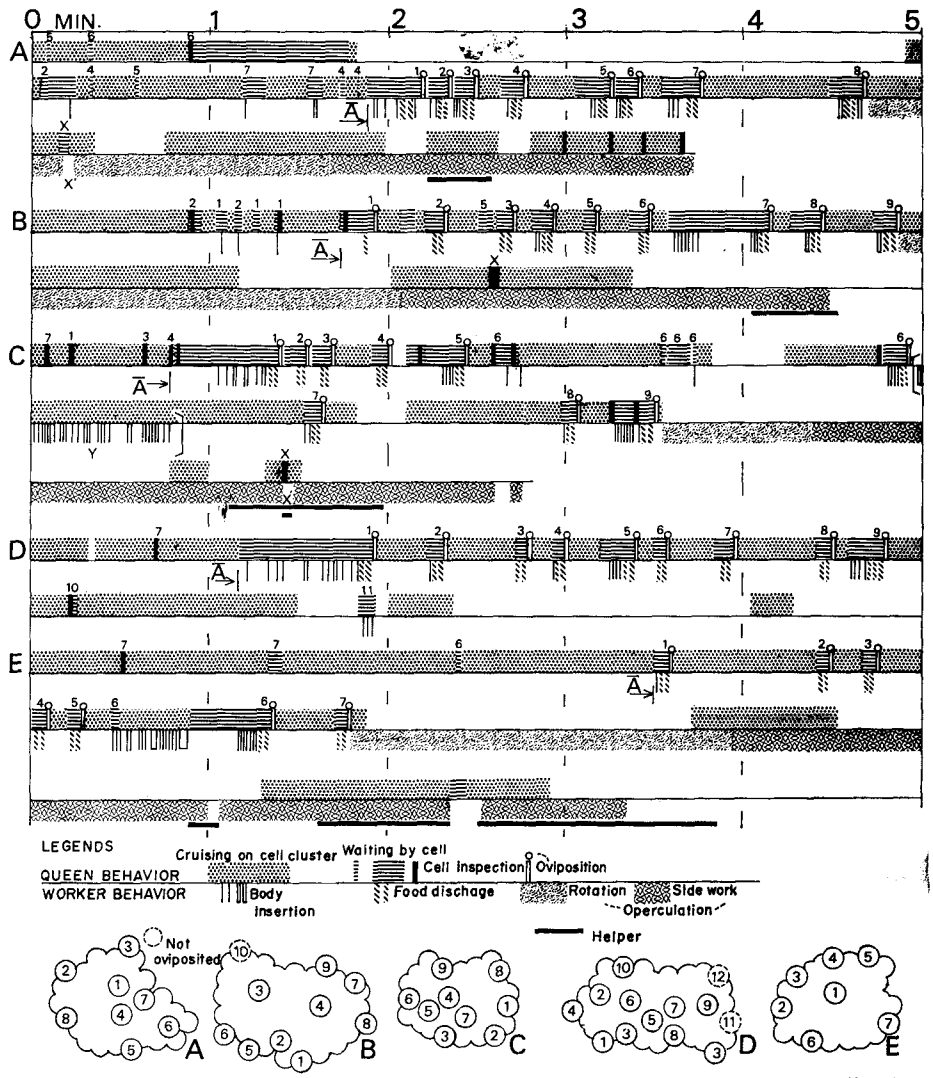


Fig. 18. Chronological sequence of five IOP in *Leurotrigona*. Numerals are cells given in the order of oviposition (Spatial arrangement at bottom). Visits (*W*) of queen to particular cells in arousal phase (Ā) are shown by the same numerals. X and X′. Interference by queen at operculation and resulting escape of operculator. Y. Body insertions by workers in cell 7 in the absence of queen waiting. Cell inspection by queen before her oviposition is not mentioned. Other symbols are given in legends.

Leurotrigona shows the type intermediate between L and G in excitement arousal (**1.5.**) and belongs to type D_c in food discharges (**1.6.**). The excitement aroused at each cell, which is weaker than in other groups, does not culminate in a high excitement over the cell cluster. The first food discharge by a worker in each cell is released always after the arrival of the queen at the cell, i.e. each UOP possesses the predischarge phase, $\bar{r}_i$, even if short. Hence, the food discharge phase in each cell, $\bar{d}_i$, proceeds always successively. Nevertheless, *Leurotrigona* differs from typical L groups by the relative prevalence of *C* against *W*.

In the groups exhibiting a similar temporal pattern, the shortening of $\bar{r}$ in a second and subsequent UOP or $\bar{u}_i$ is known in *Tetragona*, *Duckeola* and, to a certain degree, in *Friesella*, in the first two groups accompanied with the decrease of both queen inspections and worker body insertions compared with their frequencies in $\bar{r}_1$. These simplification indicates the influence of general excitement upon each UOP even in these L type groups (**1.6.**). In *Leurotrigona*, however, secondary predischarges $\bar{r}_i$ ($i=2\sim n$), do not much differ from $\bar{r}_1$ as to the behavioral aspect, merely because the behavior of both queen and workers are simple even in $\bar{r}_1$. Among 47 precisely observed $\bar{r}_i$, queen inspections were noted only in five cases, all in the same IOP (Fig. 18, C). The frequency distribution of worker body insertions during $\bar{r}$ was as follows: No insertion (21 cases), 1 (8), 2 (9), 3 (4), 4 (2), 7 (2), 17 (1). The average frequency seems lower than in $\bar{r}_1$ (Table 3, *i*) but not so conspicuously as in *Tetragona* and *Duckeola*. The duration (in sec.) of $\bar{r}_i$ is distributed as follows (Number of cases in parentheses): $0\sim5$ (5), $6\sim10$, (10), $11\sim15$ (10), $16\sim20$ (12), $21\sim25$ (8), $26\sim30$ (4), $31\sim40$ (3), $41\sim60$ (5), $61\sim100$ (4), $100\sim$ (3). The length is in average slightly but not remarkably shorter than in $\bar{r}_1$ (Table 3, $\bar{r}$). Therefore *Leurotrigona* resembles *Friesella* in the relation of $\bar{r}_i$ ($i=2\sim n$) to $\bar{r}_1$, exhibiting no conspicuous simplification of the former.

Further a characteristic recognized in Ā, longer cruising compared with shorter waiting by the queen, is also observed during the interval between two successive UOP, or exactly, the time since the end of $\bar{o}_i$ to the beginning of $\bar{d}_{i+1}$. As shown in Table 4, the queen spent most of her time by *C* than *W* and the prolonged interval $\bar{o}_i\sim\bar{d}_{i+1}$ results in an increase of time spent by *C* without that by *W*,

Table 4. Relative length of cruising (*C*) and waiting (*W*) by the queen during oviposition phase ($\bar{O}$, excluding 1. UOP or $\bar{u}_1$) in *Leurotrigona*. Duration =Time since the end of $\bar{o}_i$ to the start of $\bar{d}_{i+1}$ in sec.)

Duration	0∼5	6∼10	11∼15	16∼20	21∼25	26∼40	41∼60	61∼
With *W* before $\bar{r}_{i+1}$				2		1	1	2
Without *W* before $\bar{r}_{i+1}$	5	9	6	8	6	4	2	3
C=0%	3							
≤25	2	1						
≤50		2	3					
≤75			1	2	3			
>75		6	2	6	3	4	2	3

which is distributed: 0~5 sec. (36 cases), 6~10 (11), 16~30 (2). Thus, throughout IOP involving phases $\bar{P}\cdot\bar{A}\cdot\bar{O}$, *Leurotrigona* is characterized by longer cruising and few and brief waitings, contrasting to *Tetragona* and *Duckeola* and representing a type intermediate between L and G. The similarity to *Friesella* was already cited above, together with some differences. As illustrated in Fig. 18, bottom, there is no definite relation between temporal sequence of IOP and spatial arrangement of cells.

2.3.2. *Food discharge, oviposition and operculation*: Exhibiting type D_c, each UOP in *Leurotrigona* is articulated as $\bar{r}\cdot\bar{d}\cdot\bar{o}\cdot\bar{s}$ and each $\bar{d}_i$ is always successive (**1.7.**). Behavior sequence in discharge phase, d, is very simple. As mentioned in **2.3.1.**, body insertions by workers (Fig. 18, C, Y) are mostly replaced by intentional ones by the arrival of the queen, so that the first food discharge can arise not preceded by body insertions (cf. Fig. 18, B. Nos. 1~6; C, Nos. 2~4; D, nos. 3, 6~8; E, Nos. 2~5, 7). The food discharge behavior does not much differ from that in other groups, made with a rapid metasomal contraction. The duration of each discharge is short, mostly one second or only slightly longer. After discharge, the worker "escapes" from the cell as in other groups. But the escape is the least exaggerated among all groups so far studied, often being no more than a slight withdrawal towards the lower part of the cell, at most removing the cell only less than 1 cm, from which she again approaches the cell. The first discharge is rapidly followed by a second and subsequent ones, but the number of discharges per $\bar{d}$ is very small and quite regular, distributed in Colony X as follows: One discharge (1 case), 2 (98), 3 (4, cf. Fig. 18, D, No. 9). In additional observations with two other colonies, all confirmed cases, about 30, were always two per $\bar{d}$, the lowest figure so far obtained in various groups. Probably this correlates to a large amount of larval food deposited in crops of nurse age workers as suggested in **2.1.** Corresponding to the lower number of food discharges, the discharge subphase, $\bar{d}_d$ is very short. Cases accurately measured are: 1 sec. (1), 2 (2), 3 (72) 4 and 5 sec each one case (both with three discharges). No queen inspection was observed during $\bar{d}_d$ and worker body insertion only once. Postdischarge subphase $\bar{d}_p$, is also very short and simple, virtually absent in most cases as shown from the following records: 0 (71 cases), 1 (6), 2 (1), 3 (1). Worker body insertions in $\bar{d}_p$ were observed twice, two times in one case (Fig. 18, A, No. 8) and once in the other (Fig. 18, A, No. 1). No queen inspection and worker oviposition during $\bar{d}_p$ were confirmed throughout our observations. The queen oviposition, always preceded by a brief cell inspection, is very short, less than 1 sec., accompanied with no particular behavior pattern.

The cell operculation starts after oviposition without delay, i.e. preoperculation subphase, $\bar{s}_p$ is virtually absent (**1.7.**). The operculation is first made by a worker, who sits on the cell, inserts her metasomal tip into the cell and works by rotating herself on the cell as in other groups except *Friesella*. The rotation subphase, $\bar{s}_r$, lasts: 50 sec. (1 case), 99 (2), 129 (1), 132 (2), 137 (1), 140 (1) and 144 (1).

The segregation of rotation and sidework subphases ($\bar{s}_r$ and $\bar{s}_s$) is distinct because rotation does not continue after withdrawal of metasomal tip, contrasting to the case in *Tetragona* and *Duckeola*, that is, transient subphase ($\bar{s}_t$ in **1.7.**) is virtually absent. The duration of $\bar{s}_s$, defined by the removal of the principal operculator, who worked since $\bar{s}_r$, was measured in some cases as follows (in sec.): 98, 118, 124, 189, 190, 204, 222. The total duration of $\bar{s}$, operculation phase, is 230, 239, 250, 268, 289, 344, and 351 sec., or, delimited by the disappearance of the orifice, 113, 144, 184, and 191 sec. In these figures, the time spent by the temporal absence of the operculator due to the interference by the queen is subtracted. The total duration is more or less comparable to that in other groups except *Tetragona*, but $\bar{s}_r$ is relatively long. Another peculiarity during $\bar{s}$ is the interference by the queen, which is occasionally seen in other groups, e.g., *Melipona quadrifasciata anthidioides*, but rather frequent in *Leurotrigona*. After the final oviposition, O_n, the queen often stays on the cell cluster and visits the cells operculated by workers. Workers are either indifferent, in particular at the earlier half of $\bar{s}_r$, or escape therefrom (Fig. 18, A, B, C, shown with X and X′). In the latter case, the queen often briefly sits on a neighbouring cells, touches the cell under operculation with antennae and rarely manipulates the cell with mandibles, behaving as if a worker in $\bar{s}_s$. By this interference the change of operculators can appear even in $\bar{s}_r$.

Table 5. Length of IOP in some accurately measured cases in *Leurotrigona* (min.-sec.) (*n*. Final cell oviposited; b and e. Beginning and end of each phase)

No. Case	No. cells oviposited	$\bar{A}$	$\bar{r}_1{}^b \sim \bar{s}_n{}^e$	$\bar{r}_1{}^b \sim \bar{o}_n{}^e$	$\bar{o}_1{}^b \sim \bar{o}_n{}^e$	$\bar{d}_1{}^b \sim \bar{d}_n{}^e$
1	8	0-49	8-32	7-41	7-00	7-08
2	13	1-12	8-32	7-20	6-59	7-00
3	7	3-30	5-27	3-15	3-12	3-14
4	9	1-44	5-21	3-10	2-58	2-56
5	8	1-53	4-47	2-48	2-28	2-30
6	7	3-32	4-35	3-40	3-10	3-09
7	10	2-08	4-17	3-40	3-30	3-28
8	7	0-48	4-00	3-20	2-09	2-13
9	6	0 15	2-59	2-01	1-40	1-32
10	9	0-48	-	3-48	2-56	3-01
11	4	-	-	-	0-39	0-39

The duration of oviposition phase of IOP, $\bar{O}$, was measured in several cases as given in Table 5. The duration is in average distinctly longer than in *Nannotrigona** and *Plebeia** showing L-D_y type, but shorter than in *Duckeola* and *Friesella*, in part due to the small batch size in the cases observed but also due to the simpler behavior sequence with shorter queen waiting.

2.4. *Behavior of laying workers*: Workers of *Leurotrigona* do not develop their ovaries under queenright conditions. The worker oviposition characteristic

to many stingless bees do not take place in the presence of the queen. On the other hand, ovarian development and resulting worker oviposition appear in orphan colonies. In this aspect *Leurotrigona* behaves like as the honeybee and ovarian development is probably inhibited by the queen as in the latter (cf. Sakagami, Camilo and Zucchi 1973). Leaving the detailed accounts of orphan colonies elsewhere (Terada unpub.), here the behavior of laying workers is briefly described, based upon observations made in August, 1971 with two orphan colonies. Because of its irregular temporal sequence and the absence of the queen, who can serve as a reference point of observations, quantitative studies are so difficult that descriptions must be confined to qualitative aspects.

2.4.1. *Worker oviposition*: The probable laying workers in orphan colonies are recognized by their peculiar behavior pattern. Sitting by a structurally completed cell, she shakes the body erratically, accompanied with brief but violent wing vibration (Fig. 19, C). Thereafter she inserts her fore body into the cell with an excited manner (Fig. 19, A), which is followed by an immediate withdrawal and a second and subsequent insertions. When she is lonely attending at a cell, an excited tour around the cell with wing vibration is interwoven between insertions (Fig. 19, B). But in the absence of other workers, this behavior complex does not further culminate in food discharge and oviposition. When other workers (abbreviated XX) attend at the cell together with the excited worker mentioned above (abbreviated V), they also make body insertions, which are usually longer, often lasting more than one minute. In one instance, ten successive insertions, excluding those shorter than five sec. were measured as follows (min.-sec.): 6–50, 1–45, 1–50, 2–12, 1–25, 1–15, 1–05, 1–30, 2–40, 3–20. During these insertions V touches the inserting X rapidly with antennae and mouthparts, and makes instantaneous and excited insertions often successively between prolonged insertions by XX. These alternate body insertions do not necessarily lead to the release of food discharge. Often the excitement of V ceases or she shifts to another cell. However, all food discharges observed were invariably preceded by this "differentiated" alternation of body insertions. (Fig. 19, D).

The first larval food is always discharged by V. Occasionally alternate body insertions are made by two such individuals, both with frenetic body movements and wing vibration, called V and V′ respectively. Although not precisely measured, the food discharge seems to be released more quickly in the presence of V and V′ than in V alone. The posture and duration of food discharge do not differ from those in queenright conditions (Fig. 19, E) with an important exception, the absence of postdischarge escape from the cell. After the first food discharge, the subsequent course of the process is variable but divided into two major types: I. Those culminating in oviposition and, II. Those not, followed by the intake of once discharged food. Seemingly II is more frequent than I. Among 67 cases observed 26 belonged to I and 41 to II.

Type I is further divided into several subtypes according to behavior sequence

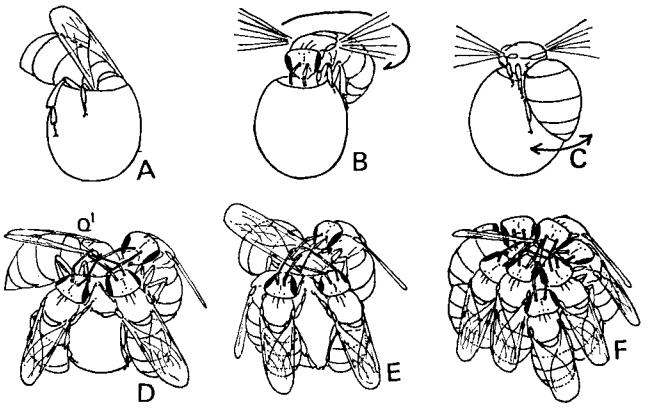


Fig. 19. Behavior patterns at worker oviposition in *Leurotrigona*. A. Body insertion in cell; B. Tour around cell with wing vibration; C. Body shaking with wing vibration; D. Body insertion by laying worker (Q′) in cell surrounded by other attendants; E. Ditto, food discharge; F. Worker congestion around cell.

as summarized in Table 6. During *D*, *B* and *b* by XX (or V′) V continuously touches them with antennae, accompanied with frenetic body shaking and wing vibration. Naturally V′ behaves similarly toward XX and V. The appearance of worker congestion is gradual. The arrows given in the table indicate only approximate time.

Some tentative conclusions from the result are enumerated: 1) In all cases two *D* are noted, that is, at least two discharges seem necessary to release *O*, which is the amount comparable in queenright colonies (**2.3.2.**). 2) Only one excited worker, V, is sufficient for the release of *D*. The attendance by two, V and V′, was observed only in 6 out of 17 cases. 3) When V and V′ discharge the food, *O* seems to be made more frequently by the worker who discharged first (13/17). Occasionally both V and V′ attempt to lay synchronously, but one of them gives the way without any struggle or submissive posture. 4) Intervention of *b*, *B*, ⌊ and ⊦ are relatively rare in Type I.

Summarizing, the behavioral sequence is relatively simple in Type I. The posture and duration of oviposition do not differ from those in normal queen oviposition. After oviposition the worker does not leave the cell and immediately start operculation by body rotation. The operculation phase ($\bar{s}$) is divided into $\bar{s}_r$ and $\bar{s}_s$ as in queenright colonies (**2.3.2.**), without difference in behavior sequence and posture. The duration of $\bar{s}_r$ was measured (in sec, each one case unless mentioned): 155 (2 cases), 165, 167, 175, 180, 190, 215, 235 (2), 245, 250, in average lasting longer than in queenright colonies (**2.3.2.**). This is in part but not exclusively caused by worker congestion around the cell. In an extreme case not included above, the operculator could not rotate till 195 sec. The rotation finished at 337 sec. and she left the cell 485 sec. after the oviposition.

Table 6. Temporal sequence in various types of food discharge followed by oviposition (Type I) in orphan colonies of *Leurotrigona*

B or *b* afterfirst *D*		Worker congestion around the cell after *D*		
		Absent	Mild (⌊)	Strong (⌊)
Absent		V: *D* *O* (5) X: *D*	V: *D* ⌊ *O* *D*	V: *D* ⌊ *O* X: *D*
		V: *D* *O* V′: *D*		
		V: *D* V′: *D* *O*		
Present		V: *D* *b* *b* *O* X: *D* *b*		
		V: *D* *O* X: *b* *b* *b* *b* *b* *D*		
		V: *D* *b* *O* X: *b* *b* *B* *b* *D* *b*		
		V: *D* *B* *O* X: *B* *D* *b* *B*		
		V: *D* *b* *O* V′: *D*	V: *D* *b* ⌊ *O* V′: *D*	V: *D* *b* ⌊ *b* *O* V′: *D* *b*

Abbreviations: V. Excited worker with wing vibration, always one individual. V′. The other worker with same behavior, always one. X. Other workers, occasionally only one but usually more. *D*. Single food discharge. *B* and *b*. Prolonged (≥5 sec.) and short body insertions. *O*. Oviposition. Temporal sequence follows in each case from left to right. Each type represents a single case unless parenthetically mentioned.

2.4.2. *Food ingestion after discharge*: Type II mentioned in **2.4.1.**, the ingestion of discharged larval food was precisely observed in 26 cases. Among them a mild worker congestion appeared once and a stronger one in all other cases, which often made further observation difficult (Fig. 19, F). But as the congestion appears, in some cases distinctly while in others plausibly, after the beginning of food ingestion just as in case of the consumption of cell content described in **2.2.1.**, it cannot be the main cause to disturb the realization of oviposition. The behavior sequence in II is so variable that several subtypes are separately presented (Abbreviations. *I*. Ingestion directly confirmed by a gradual distention of the metasoma. n. Replication mark. Other abbreviations see in Table 6. It is possible that many *B* are actually *I* with a small amount ingested. Appearance of congestion omitted).

		General pattern	Examples
$II_{1.1}$	V:	*D*	*D* ; *D*
	X:	^{n}b ^{n}B ^{n}I	*b b b R B* ; *B b B*
$II_{1.2}$	V:	*D* ^{n}b	*D b b* ; *D b b* ; *B D b b b*
	X:	^{n}b ^{n}B ^{n}I	*B B B*; *B B B*; *I b B B*
$II_{1.3}$	V:	*D* ^{n}b *I*	*D b I*
	X:	(^{n}b ^{n}B)	*b B*
II_{2}	V:	*D* ^{n}b	*D b* ; *D b B* ; *D*
	X:	(^{n}I ^{n}B ^{n}b *D*)	*D B*; *B D B B*; *D I D B*
II_{3}	V:	*D* ^{n}b *D*	*D b b b I*
	X:	^{n}b ^{n}B	*I B B b b D b b*
II_{4}	V:	*D I*	a) *D I* b) *D b I*
	X:	^{n}b	*b b B E I*
	V:		c) *D b b b b I* d) *D I*
	X:		*b b B b D I b*
	V:		e) *D I*
	X:		*D I*

Subtype II_1, with only one discharge, was most frequent, involving 14 out of 26 cases. Subtype II_2, with two or more discharges intervened by one food ingestion or more, either confirmed (*I*) or inferred (*B*), was observed five times. II_3, food ingested probably by XX, then definitely by V, the discharger herself, was observed twice and II_4, food ingested by V herself before probable intake by XX, six times.

Subtypes $II_{1\sim3}$ could be interpreted as such that discharged food was ingested before reaching a quantity enough to elicit the oviposition. But subtype II_4 is difficult to explain by the same assumption. In this subtype, examples c), e) and probably b) could be explained by a possible change of motivation in V when waiting further discharge by X. But this cannot be applied to a) and especially d), in which the food was soon re-ingested by the discharger herself.

These cases suggest a marked instability of motivation system in orphan colonies. The excited posture and food discharge by one worker do not automatically lead to her oviposition, though these two items are indubitably linked for each other and oviposition is always preceded by food discharge. The examples mentioned also reveal that the worker congestion must not be regarded as the main factor inhibiting oviposition. It is probably released by a chemical stimulus emitted from the discharged food, but its facultative appearance remains for the time being unsolved.

2.4.3. *Temporal sequence of cell construction and oviposition*: Two features characteristic of queenright colonies of *Leurotrigona*, batched ovipositions and synchronous cell construction, are remarkably disintegrated in orphan colonies. However, the vestiges of these features seem to persist. In the early morning, there were usually an accumulation of many cells ready to receive food and eggs. Potential dischargers and layers, V, were relatively rare before 10:00 and ovipositions were observed mostly in afternoon:

Date (August 1971)	14	17	20	23	24	25	27	28
Approximate time of first oviposition	13:00	15:10	13:05	12:45	13:00	14:00	10:55	11:20

Certainly diurnal change of nest microclimate must affect the sequence, for the observed colonies were not kept in heat conditioned hives and diurnal change of temperature was conspicuous at the end of southern winter (cf. also **2.1.1.**). Nevertheless, daily observations, two successive records of which are reproduced in Fig. 20, show that new cells were usually constructed after many cells were oviposited and operculated, and the construction proceeded more or less synchronously. It is interesting that cell growth was rapid and construction was made under high excitement as in queenright colonies (**2.2.2.**). Moreover, food discharges and ovipositions in several cells often appeared in rapid succesion after a marked latent time. Probably the food discharge in one cell affects the workers staying nearby to release further discharges in other cells.

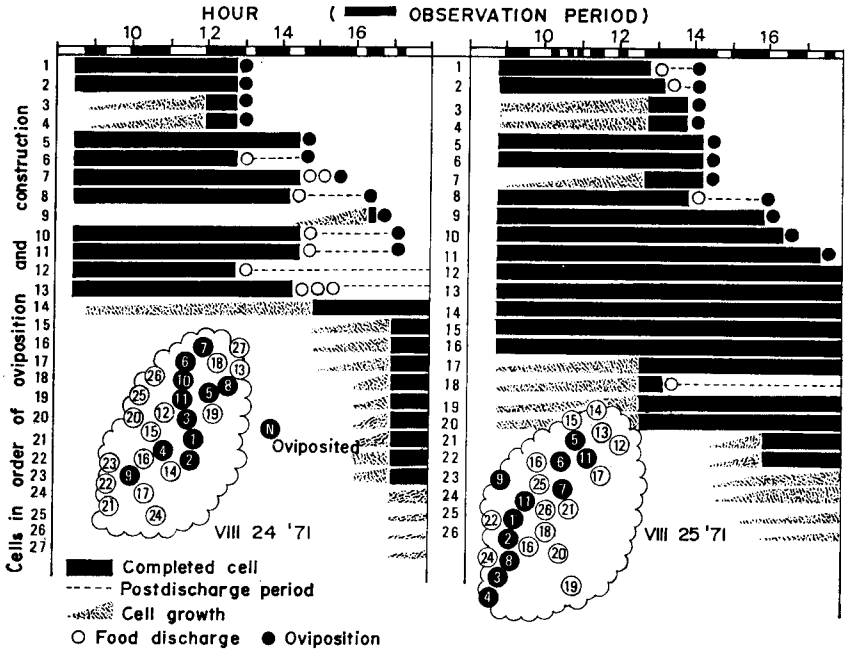


Fig. 20. Chronological sequence of cell growth, food discharge and oviposition in an orphan colony of *Leurotrigona* on two successive days. Spatial distribution of cells shown at bottom with corresponding numerals. Ovipositions were either confirmed directly or indirectly by completion of operculation. Food discharges were not always confirmed in all cases.

2.5. *Ethological characteristics of* Leurotrigona: The oviposition process of *Leurotrigona* proceeds with a distinct phase sequence as in other stingless bees so far studied. Further there are several ethological characteristics common to the other groups: 1) Increased body insertions in patrolling and arousal phases. 2) Rapid succession of food discharges by workers. 3) Escapes of workers from the cell after food discharge. The relation of workers to the queen also shows some patterns common to other groups: 1) Gradual formation of royal court around the resting queen. 2) Repetition of a rapid pushing followed by a retreat by attendants in front of the queen. 3) Avoidance by workers of the approaching queen.

Excluding these items, a provisional ethological description of *Leurotrigona* is given below, with special reference to queen-worker coactions and oviposition process. The groups compared are abbreviated: *Scaptotrigona* (*S*), *Melipona* (*M*), *Cephalotrigona* (*C*), *Tetragona* (*Tt*), *Duckeola* (*D*), *Friesella* (*F*), and *Trigonisca* (*Ts*). The following symbols are used for comparison: Similar to ($\fallingdotseq$), different from ($\neq$), different from all other groups ($\not\fallingdotseq$), similar but more conspicuous than others (others$\leq$), similar but less conspicuous than others ($\leq$ others), more, and less conspicuous than others (others$<$, $<$others), partly (t), probably (p).

1) Cells arranged in cluster ($\fallingdotseq Ts$), neither incompletely ($\neq D$, *F*) nor completely combed ($\not\fallingdotseq S$, *M*, *C*, *Tt*). 2) Involucrum absent ($\fallingdotseq D$, *F*, *Ts*, $\neq S$, *M*, *C*, *Tt*). 3) Differentiation of honey and pollen pots absent ($\fallingdotseq S$, *M*, *C*, *Tt*, *D*, *F*, *Ts*). 4) Royal cells larger than workers cells ($\neq M$, $\fallingdotseq S$, *Tt*, F^b, D^p, C^p, Ts^p), though some interesting traits exist as to the formation of royal cell (Terada unpub.). 5) Cells and pots prepared with little admixture of resin ($\fallingdotseq Ts$, $\neq S$, *M*, *C*, *Tt*, *D*, *F*). 6) Waste materials thrown down from nest entrance ($\fallingdotseq Ts$), not carried away on the wing ($\fallingdotseq S$, *M*, *C*, *Tt*, *D*, *F*). 7) Capacity of thermal regulation poor ($\fallingdotseq Ts$, *F*, $\neq S$, *M*, *Tt*, *C*). 8) Queen tending to rest at definite place ($\not\fallingdotseq$). 9) Queen walking with moderate speed ($=M$, *C*, *Tt*, *F*, *Ts*), not slowly ($=S$, *D*). 10) Rhythmic wing movements of queen present ($\neq M^t$, $Ts<$, $<S$, M^t, *C*, *Tt*, *D*, *F*), being vibration ($\fallingdotseq D$, *Tt*, *F*), not of single strokes ($\neq S$, *M*, *C*), being intermittent ($\fallingdotseq F$), not continuous ($\neq Tt$, *D*). 11) Queen not taking prolonged geonegative resting ($\neq S$, $\fallingdotseq S$, *M*, *C*, *Tt*, *F*, *Ts*). 12) Exaggerated queen worker coactions out of oviposition process absent ($\neq S$, *M*, $\fallingdotseq C$, *Tt*, *D*, *F*, *Ts*). 13) Cell construction synchronous, S_y, not semisynchronous, S_m ($\neq D$, *F*, *Ts*), or successive, S_c ($\neq S$, *M*, *C*, *Tt*), made with excitement ($\not\fallingdotseq$). 14) Cell construction not started by accumulating a large amount of cerumen ($\neq D$, $\fallingdotseq S$, *M*, *D*, *Tt*, *F*, *Ts*). 15) Oviposition exclusively batched ($\fallingdotseq S$, *D*, *F*), not singular ($\neq M$. Ts^p) nor loosely batched ($\neq C$, *Tt*). 16) Food discharges in cells successive, D_c ($\neq S$, $=M$, *C*, *Tt*, *D*, *F*, *Ts*). 17) Queen more cruising than waiting by cell in patrolling and arousal phases ($\not\fallingdotseq$). 18) Excitement arousal localized ($\neq S$, $\leq M$, *C*, *Tt*, *D*, *F*, *Ts*). 19) Shortening of secondary predischarge waiting inconspicuous ($\fallingdotseq F$, $M<$, $<Tt$, *D*, Ts^p, $\ll S$). 20) Worker excitement in arousal phase inconspicuous ($\neq$S, M, C, Tt, Ts^p, $\fallingdotseq$F, D). 21) Queen cell inspection in arousal and discharge phases rare ($\fallingdotseq F$, *Ts*, $<S$, *M*),

never rhythmically repeated (≒*C*, *Tt*, *D*). 22) Queen not violently touching workkers attending cell (≒*S*, *C*, *Tt*, *D*, ≒ *M*, *F*, *Ts*). 23) Worker body insertions before food discharge rare (≒*M*, *C*, *Tt*, *S*, *D*), mostly replaced by intentional insertions (≒*F*). 24) During arousal phase worker behavior simple (≒*M*, *C*, *Tt*, *D*), without exaggerated darting (≒*S*), ritualized submission (≒*Ts*) and formation of frozen rosette (≒*F*). 25) During discharge phase queen normally not wandering to other cells (≒S), nor making peculiar retreat from cell (≒*M*, ≒*C*, *Tt*, *D*, *F*, *Ts*). 26) Number of food discharges per cell small (≠). 27) Postdischarge escape inconspicuous (≠). 28) Postdischarge subphase virtually absent (≒*F*, *D*, *Ts*, ≒*S*, *M*, *C*, *Tt*). 29) Worker oviposition during oviposition process absent (≒*D*, *F*, *Ts*, ≒*S*, *M*, *C*, *Tt*). 30) Queen oviposition very short (≒*C*, <*S*, *Tt*, *Ts*, ≪*D*, *M*), made without peculiar leg twitching (≒*S*, *M*, *C*, *Tt*, *F*, *Ts*, ≒*D*). 31) Cell operculation starting without much delay (≒*S*, M^t, *C*, *D*, *F*, *Ts*, ≒M^t, *Tt*). 32) Rotation and sidework subphases well differentiated (≒*C*, *Tt*, *D*, *Ts*, *F*, ≒*S*, *M*). 33) Operculation not very long-lasting (≒*Tt*, *Ts*, ≒*S*, *M*, *C*, *D*, *F*.).

Obviously some of above mentioned features may vary according to colony conditions. But many if not all could be characteristic of *Leurotrigona*. In general, this group is noteworthy by the simplicity of behavior and the absence of a high excitement during oviposition process. There is found no exaggerated or ritualized behavior patterns. The oviposition process is released only by a brief waiting at the cell by the queen, without complicated queen-worker coactions. The features exclusive to *Leurotrigona* among all groups compared are 8) 13) 17) 26) and 27), all except for the first two are also related to the simplicity in behavior.

3. *Hypotrigona* (*Trigonisca*) *duckei* (Friese)

According to Moure (1950) this species is widely distributed in the Equatorial South America, from the Peruvian Amazonic basins eastward to the State of Ceará and from Guianas southward to Central Mato Grosso. The colony observed was sent from Mossoró, the State of Rio Grande do Norte, Brazil, possibly representing the easternmost record of the distribution.

Consulting the key by Moure (op. cit.), the specimens run straight to *H. duckei*. Yet the identification is still not conclusive, because Moure reports the variability in coloration and relative body size among different populations, assuming the presence of several allied forms within *duckei*-complex. Some body parts of the specimens from the observed colony were measured. The result is given below, together with those by Moure (converted to relative values). Some marked deviations from other populations are shown in Gothic.

Population	Airão Amazonas	Belém Pará	Carolina Maranhão	Mossoró RN	Ponce Mato Grosso
Interocellar distance/ ocellorbital distance	**1.12**	1.47	1.47	**1.29**	1.45
Head length/head width	0,82	0.81	0.83	**0.94**	0.82
Head width (mm)	1.09	0.96	0.97	1.07	**1.23**

The specimens from Mossoró are characterized by the long head and higher value of IOD/OOD. As to the coloration some intranidal variations were observed. Some specimens possessed well-defined facial mark, darker metanotum and propodeum and relatively paler metasomal dorsum. In others, head and mesosomal darkening were less developed but metasoma was nearly blackish brown. One specimen showed an interrupted pale band at the base of mesoscutellum, pointed out by Moure in the specimens from Airão.

3.1. *General traits out of oviposition process*:

Unfortunately the colony was observed under the pessimal condition. Arriving at Ribeirão Prêto it contained only 100 odds workers. The storage pots were nearly empty and the contents of more than a half of brood cells had been consumed (cf. **2.2.1.**). The colony had been kept in an elongate wooden box with a nailed glass window, which was very narrow to make closer observations (Fig. 21). The transference into an observation hive was abandoned for fear of the collapse by manipulation, which was probable by poor colony conditions and tiny body size. For lack of space the box was placed at a very narrow interspace between an window and an instrument at a corner of the room, being so inconvenient that even recording of observations was often difficult. Observations were made during July and August, 1972. Therefore, the results are inevitably incomplete, requiring supplementary studies for the future. Nevertheless, we believe, some ethological characteristics of the species were clarified as described below.

3.1.1. *Nest structure and related features*: Nest structure is virtually identical with that of *Leurotrigona*, including those features such as clustered brood cells (Fig. 21, C, D), each being relatively short and spherical at top and bottom, absence of involucrum, cells and storage pots being pale and semitransparent, built with no admixture of resin. Brood cells were 1.6 mm long and 1.1 mm wide. Storage pots found in the nest were more elongate than those of *Leurotrigona* (cf. Figs. 14 and 16 and Fig. 21, E, F), in average 7 mm long and 3 mm wide, joining for one another without differentiation of honey and pollen pots. The entrance tube was prepared with a slight admixture of resin, about 5 mm in diameter (Fig. 21, A). The entrance was of the diameter allowing the passage of more than one bee, but, probably for the small colony size and severe climate, was usually closed with porous resinous network, leaving a small hole which was closed at night (Fig. 21, B). When interfered the guards made a mild darting with mandibles opened, but soon retreated without

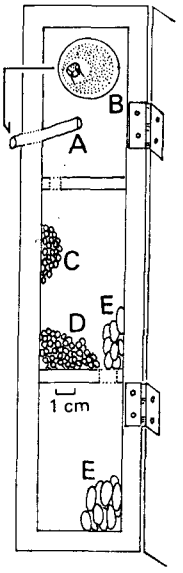


Fig. 21. Nest box containing the observed colony of *Trigonisca*. A. Entrance tube; B. Entrance, enlarged; C. New cell cluster; D. Old cell cluster; E. Storage pots.

further expression of defense. The rubbish was thrown down from the entrance. No systematic observation was made on workers, but their behavior showed no particularity and conspicuous differences from *Leurotrigona*. As in the latter, *Trigonisca* has little capacity of thermoregulation. Complete paralysis of activities appeared on cold days and at night.

3.1.2. *Queen behavior and queen-worker coactions*: The queen was about one third longer than a worker, rather homogenously brownish without dark markings. At rest she preferred for a spot on the wall of the box near the newly made cell cluster, occasionally staying there more than one hour. But this cannot be regarded as having a definite resting place as in *Leurotrigona*. Resting at other places including cell clusters was not rare. At walk, which is slightly slower than in *Leurotrigona*, the antennae are moderately shaken. The most conspicuous feature in queen behavior out of oviposition process is the rarity of rhythmic wing movements characteristic of all other groups except *Melipona quadrifasciata*. Once she made three beats successively with intervals of ten and five sec. but this was exceptional. Often she does not beat wings through more than one hour, either at rest or at walk, or under oviposition excitement. When performed the wing beats are either a single stroke or a rapid succession of 2~3 strokes, forming a brief and incomplete vibration.

Queen-worker coactions are simple. At encounter with the passing queen, workers mildly, never exaggeratedly, avoid her, either by a total withdrawal or, in case of bees working at cell clusters, by inclining the body. The permanence of the queen at a definite spot leads, though not always, to the formation of royal court. Reflecting the poor colony conditions, the number of attendants was low, usually 2~3, rarely 4~6. Workers shake the antennae against the queen but seldom touch her body except for the individual standing in front of the queen, who exchanges the antennal contact with her, the queen touching rather slowly while the worker more vividly. Workers repeat a mild pushing toward the queen, which is soon followed by a retreat. When the queen begins to proceed slowly, the worker in her front gently retreats, or occasionally escapes without exaggerated manner. Or, the worker crouches against the approaching queen, lowering the head. Occasionally a mild darting with mandibles opened appears after crouching but no severe or ritualized aggressiveness is demonstrated. Buccal contacts between queen and workers were occasionally observed but food delivery by the worker to the queen was confirmed only once, lasting about five sec., nevertheless food delivery among workers were frequent.

3.2. *Oviposition pattern and cell construction*: Nest structure identical with that of *Leurotrigona* suggests a poor capacity of thermal regulation (cf. **2.1.1.**). Moreover, the colony was very weak and observed during southern winter, the thermal conditions of which could be recognized from Curve E in Fig. 15. Indubitably, the conditions must have been severe for this equatorial species reared without heat conditioning. On cold days and at night, colony activities completely ceased,

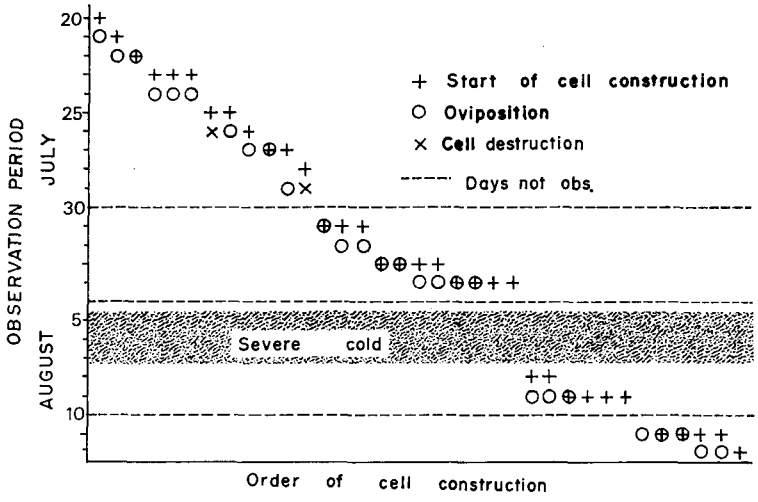


Fig. 22. Temporal order of cell construction and oviposition in *Trigonisca* during July 20~August 12, 1972.

all individuals entering into cold stupor. Nevertheless it is interesting that ovipositions took place in the warm daytime, which suggests that *Trigonisca* is fairly resistant to temperature fluctuation, even if not so as *Leurotrigona*.

Caused by such circumstancial influence, the oviposition rhythm obtained must be different from that under favorable conditions. The result of continuous observations from July 20 to August 12, 1972 (Fig. 22) shows that both cell construction and oviposition rates were very low. Excluding August 4~8, on which both activities ceased for severe cold, the average construction rate was 2.1 (0~4) and the average oviposition rate 1.6 (0~4) per day. Among 34 cells confirmed as to the start of construction, only 10 were oviposited within the same day, 15 on the next day, one on the third day, while two were destroyed on the next day and six not confirmed as to its destination.

Correspondingly the interval between the start of cell construction and oviposition markedly differs between cells oviposited on the same day and those on the next day. In the former case, cells are completed within 2~4 hours and oviposited 1~1.5 hrs later (Fig. 23, Nos. A, E, F, G, H). On the other hand, in cells started at late afternoon, the work stops at night until the next morning, to 9~10:00 (Fig. 23, other cases) on cold days, though continues with a slow tempo on warmer days (Fig. 23, G′, H′).

Cells are built by the summation of successive activities by a number of workers as in other groups. Construction is not so laborious as in *Leurotrigona*, requiring much time for completion (cf. **2.2.2.**), but it is uncertain whether this is specific or due to poor colony conditions. Because only a few ovipositions were made per day, it is difficult to determine the type of batch formation. The interval

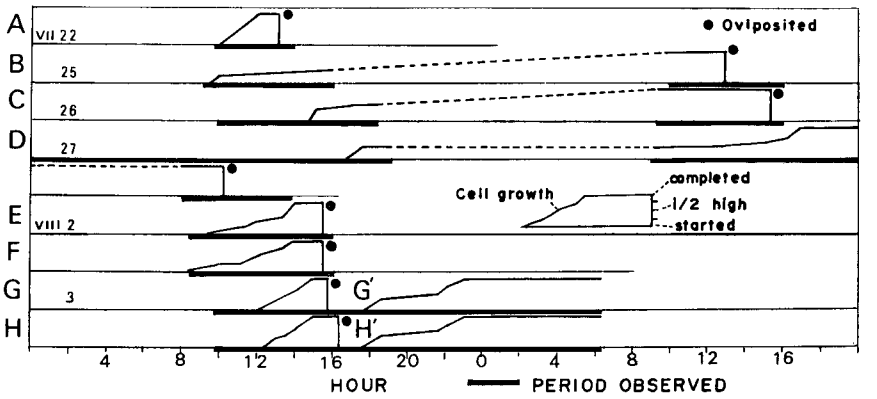


Fig. 23. Difference of duration between start of cell construction and completion or oviposition among cells started at different hours in *Trigonisca*.

between two successive ovipositions made on the same day was measured as follows (in min.)

Date	No. ovipositions	Interval	Date	No. ovipositions	Interval
VII 22	2	104	VIII 3	4	38–281–34
24	3	180–63	9	3	5>–316
VIII 1	2	10>	11	3	180–32
2	2	0.5	12	2	300

The interval is usually long even though two or more completed cells occur (VII 24, 1.–2.; VIII 3, 1.–2., 3.–4., VIII 12), but occasionally short (VIII 1, 2, 9), always when two or more completed cells exist. If this pattern is formally interpreted, *Trigonisca* could be classified into singular type (B_s, **1.2.**). But, considering the poor colony conditions at observations as well as the rarity of B_s type in other groups under favorable conditions, it is possible that ovipositions are loosely batched (B_f type) under favorable conditions. The possibility of B_e is also not excluded but seemingly less likely, because of the appearance of two ovipositions fairly separated one another in spite of the presence of two completed cells as mentioned above. The basic oviposition pattern of *Trigonisca* remains, however, unsettled until observations are undertaken with stronger colonies.

Type of cell construction is also difficult to determine. Inspecting the colony in the morning (9 ~10:00) often both completed and half-built cells (1/4 to 1/3 high) are found side by side. If this co-existence is superficially interpreted, *Trigonisca* could be classified into successive type (S_c, **1.1.**). But the state mentioned is partly affected by the interruption of work at night. Start of construction in the daytime was observed four times in the presence of completed cells and 15 in the absence of such, indicating, at least partly, the inhibition of new construc-

tion, which is characteristic of synchronous type, (S_y **1.1.**). On the other hand, new construction began after the prior oviposition as follows: Within 30 min. (3 cases), 30 min. ~1 hr (1), 1 ~1.5 hrs (2), 1.5 ~2 hrs (2), that is, with much delay than in *Leurotrigona* belonging to S_y. Therefore *Trigonisca* could be regarded, tentatively, as belonging to semisynchronous type (S_m, **1.1.**), though the sequence is much irregular than in *Duckeola* and *Friesella* belonging to the same type, apparently in part caused by adverse colony conditions.

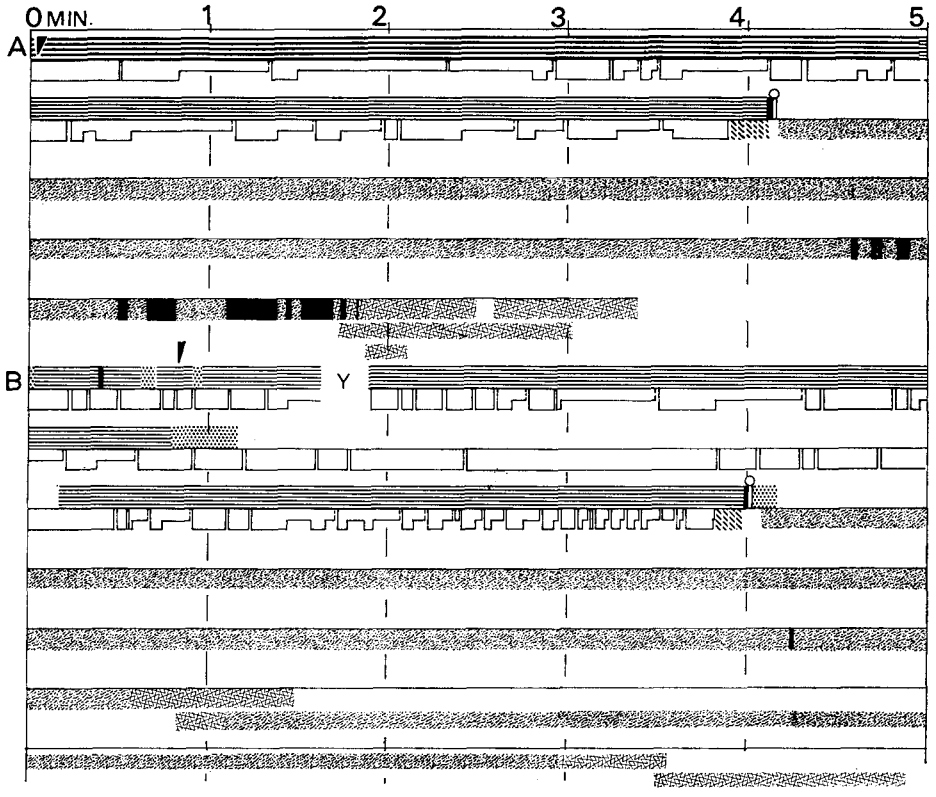
3.3. *Oviposition behavior*: Among 27 UOP observed, a rapid succession of two ovipositions occurred three times. All others were singular so that explanations will mainly be given with this singular type, followed by additional comments on batched cases if necessary.

As in other groups, the queen occasionally visits the cell cluster with growing cells, the number of which was usually only one to two. The visits become gradually frequent. Correspondingly workers concentrate around these cells. But the delimitation of patrolling and arousal phases ($\bar{P}$ and $\bar{A}$, **1.3.** ~**1.4.**) is difficult in *Trigonisca* for some behavioral peculiarities explained subsequently. Therefore, the temporal sequence before food discharge was analysed by using several behavior characteristics. As shown in Table 7, the *predischarge period* (N.B.,≠predischarge phase $\bar{r}$ in **1.6.** ~**1.7.**) is approximately divided into four stages. I (30 min. ~1 hr), II (10 mn. ~1 hr.), III (less than 30 min.) and IV (usually short). The correspondence of these stages to phases $\bar{P}$, $\bar{A}$ and $\bar{r}$ will be attempted after the description of predischarge behavior.

3.3.1. *Behavior before food discharge*: At the beginning of stage II, one or two workers attend the cell, making relatively prolonged body insertions each lasting 30 ~40 sec., often more than one min. (a prolonged case, 3 min. 35 sec.) At the arrival of the queen, workers gently avoid her by a mere body inclination, not by locomotion. At stages I and II the queen occasionally inspects the cell by inserting her head. Inspections are brief, mostly less than one sec. She stays by the cell for a while, either hanging on the cell or sitting on a neighbouring cell, then leaves the cell cluster often after taking a turn around the cell. In parallel with the repetition of her arrival-stay-removal, the number of workers attending the cell increases up to 4 ~5, the duration of each body insertion shortens and the alternation becomes more frequent, which indicate the beginning of stage III.

The behavior peculiarities in stage III are seen in the prolonged waiting of the queen by the cell and *ritualized presentation of mesosoma by workers* to the queen, a behavior pattern so far undescribed in stingless bees. The chronological sequence of queen and worker behavior in three relatively well recorded UOP is given in Fig. 24, taking the queen as the focus of observations. Case A and B are given approximately since the beginning of stage IV and case C since the final part of stage III but the behavior sequence does not qualitatively differ from that in the earlier part of III described below.

Contrary to *Leurotrigona* and especially to *Friesella* the attending workers do



not escape from the cell by the arrival of the queen. The change from actual to intentional insertions, seen frequently in these groups (cf. **2.3.1.**) is rare and gentle darting to the queen, frequently practiced by other groups, also seldom appears. Instead, the worker behavior in stages III and IV is characterized by the *presentation* mentioned above (Fig. 25). The worker approached by the queen raises the body by stretching legs perpendicularly to the body axis. The head and metasoma are lowered so that the mesosomal dorsum is raised. Keeping this posture, the worker thrusts the mesosoma to the queen. This behavior is distinctly *oriented.* When the queen turns around the cell, the worker at her way changes the orientation according to the movement of the queen, attempting to keep the presentation of mesosoma toward the queen (Fig. 25, C). When the queen approaches the cell from the upper side as is often the case, all cell attendants erect themselves by stretching legs and raise the mesosomas by lowering heads (Fig. 25, A, B). The worker in front of the queen directs the mesosoma to her, often keeping the body by mid and hind legs alone and occasionally exhibiting a very skewed posture (Fig. 25, B, H). The worker inserting her fore body into the cell also makes presentation

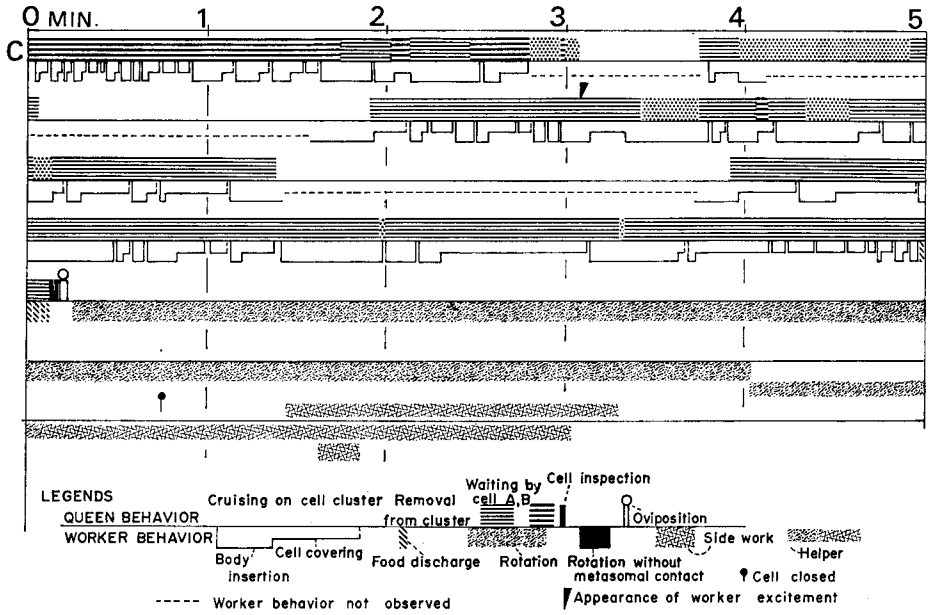


Fig. 24. Chronological sequence of three relatively well recorded UOP in *Trigonisca*. Y. Observation interrupted for 195 sec. Other explanations in legends.

after the removal from the cell, as if covering the cell by her body (Fig. 25, D, E). In Fig. 24 the duration spent by this *cell covering* is expressed with shallower parts of blocks which represent body insertions. But, when one bee is covering the cell, often another worker starts the insertion by creeping in the cell from the underside of the precursor (Fig. 25, G). Therefore the real duration of *post-insertion presentation* is longer than that mentioned in the figure. Further the figure gives only the presentation by the worker who performed insertion. Actually all other attendants are synchronously making the same act during the process as shown in Fig. 25, A, B and schematically in I.

The queen violently beats the presenter with antennae and fore legs. The presenter is passively beaten, keeping the posture and exhibiting no movements except for shaking the lowered antennae. Even in this act, the queen seldom exhibits wing beating, which was observed only once and very briefly among 13 precisely recorded UOP. Throughout this continuous coaction the queen rarely approaches the cell margin in stages III and IV (Fig. 25, A, B). Consequently the cell inspection by the queen is very rare, found only in three out of 13 UOP observed, in each only once (Fig. 24, B). Oriented presentation and cell covering after body insertion described above make an impression that these acts serve as barricades to defend the approach of the queen to the cell. But it is difficult to conclude that the queen cannot reach the cell disturbed by these acts. No positive

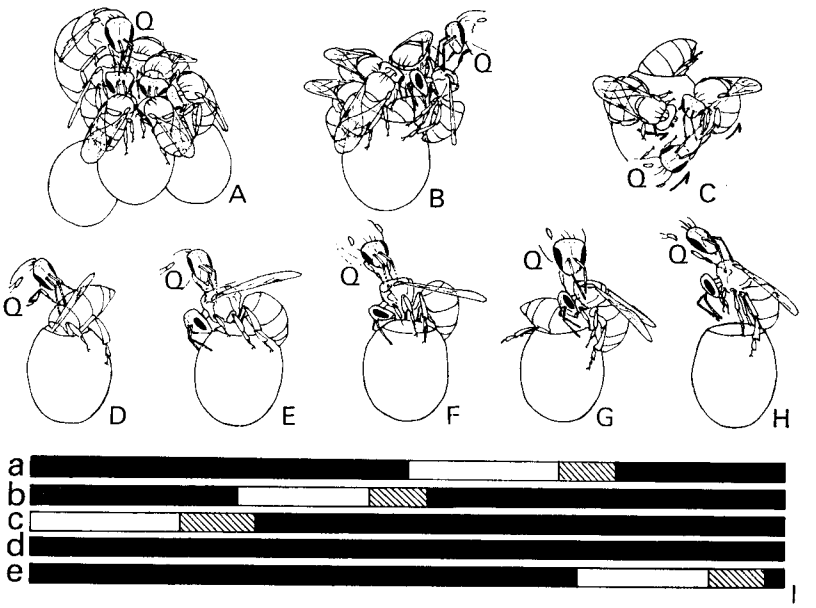


Fig. 25. Various postures taken by *Trigonisca* workers at *presentation of mesosoma to the queen*. Except A, the queen (Q) is shown only partly. I. Schematic expression of body insertions (white bars) and their relation to presentation (black bars) and cell covering (hatched bars) among five cell attendants during predischarge period.

attempt to surmount the wall by workers is taken by the queen. Her occasional arrival at the cell margin was observed four times but cell inspection did not take place in spite of the absence of any obstacles. The possible significance of this *presentation* will be discussed in Concluding remarks **3.**

During stage III the queen often turns around the cell, or exactly, around the wall built by workers, or changing her position from lateral to vertical (directing downward) waiting and *vice versa*. If there are two completed cells, the queen occasionally changes her waiting from one to another (Fig. 24, C) or even leaves the cell cluster, taking a rest at other places (Fig. 24, C). The presentation ceases by the queen removal and her long absence lowers the intensity of alternating body insertions. But the time spent by waiting is much longer than that by cruising. In this aspect *Trigonisca* is contrasting to *Leurotrigona*, and is classified into cW type (**1.4.**) as far as the colony observed is concerned (Cf. Figs. 18 and 24).

The final stage, IV, is more or less defined by the appearance of a frenetic body trembling in workers, which accompanies both insertions and presentations. The alternation of insertions becomes often frequent (Fig. 24, B). At insertion the worker occasionally rotates herself along the cell margin. The beating by the queen of workers becomes more violent and workers also themselves touch and

Table 7. Temporal structure of behavior sequence in oviposition process of *Trigonisca* before food discharge into brood cell

No. case observed	Duration of stages (min.)				No. arrival-removal by queen during		Duration of phases and their correspondence to stages		
	I	II	III	IV	III	IV	$\bar{P}$ (min.)	$\bar{A}$ (min.-sec.)	$\bar{r}$
1	(125+)	62	19	2	9	0	81(=II+III)	2-00(=IV)	
2	(28+)	27	25	0.5	6-7	0	52(=II+III)	0-30(=IV)	
3	(145+)	27	6	6	0	0	27(=II)	12-00(III+IV)	
4x	190	70	25	9	0	Y1	70(=II)	30-25	3-35
5	195	8	15	1	0	0	8(=II)	16-00(III+IV)	
6	(130+)	22	6	7	1	0	22(=II)	12-00	1-00
7	60	160	25	6	0	0	160(=II)	31-00(III+IV)	
8x	(70+)	70	32	22	0	0	70(=II)	54-00(III+IV)	
9x	64	30	4	19	0	Y5	30(=II)	0-40	18-20
10	(25+)	19	-----14-----		3, Y2	2	19(=II)	3-40	10-20
11x	20	6	3	15	-	-	-	-	-
12x	30	30	6	12	4, Y9	6, Y8	30(=II)	1-38	16-22
13	?	62	12	3	-	-	-	-	-

I. Stage from completion of cell to beginning of worker concentration. (Parenthetical cases=Cells already completed on the prior days. Time given is the duration from start of observation to beginning of stage II).
II. Stage from end of I to appearance of *presentation*.
III. Stage from end of II to appearance of high excitement.
IV. Stage from end of III to first food discharge.
x. Presence of two completed cells. Y. Change of queen waiting between two cells.

push one another. Even at this stage the queen occasionally leaves the cell and her absence can lower the worker excitement (Fig. 24, C).

3.3.2. *Temporal sequence of predischarge behavior*: Now the distinction of four stages is compared with that of phases developed in Section **1.** Besides poor conditions of colony observed, the difficulty to incorporate *Trigonisca* in our phase system lies in the effect of thermal conditions upon the cell construction cycle and the frequent removal of the queen from the cell or the cell cluster. By the first item, the completion of cell cannot be used to delimit the beginning of $\bar{P}$, because a completed cell can overnight and receive the egg on the next day. Another criterium, the appearance of worker excitement develops later, in stage III (presentation) or IV (frenetic trembling), which cannot be regarded as $\bar{P}$ judging from the behavior sequence.

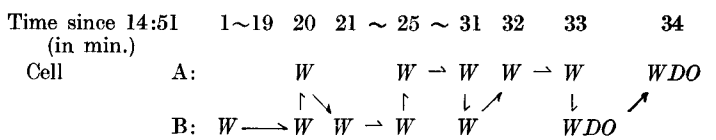
Therefore, the appearance of worker concentration could be adopted to delimit the beginning of $\bar{P}$, which more or less corresponds to the increased visits by the queen with the cell cluster. It would be a matter of opinion whether stage I is included in $\bar{P}$ or not, but here we exclude it from $\bar{P}$. Then, applying our definitions on the beginning of $\bar{A}$ and $\bar{r}$ given respectively in **1.4.** and **1.5.** to the data presented in Table 7, the durations of $\bar{P}$, $\bar{A}$ and $\bar{r}$ are given at the last three columns in the

same table, together with the correspondence to stages I~IV. The result is summarized: 1) $\bar{P}$ corresponds in most cases t_0 II but occasionally to II+III. 2) In many cases $\bar{A}$ and $\bar{r}$ are equivalent, corresponding to III+IV, occasionally to IV. 3) In some cases $\bar{A}$ and $\bar{r}$ are different for each other, without corespondence to stages.

The differences given above are caused by the presence or not of queen removal from the cell or the cell cluster. If the queen does not make such removal since the beginning of III until the food discharge, $\bar{A}$ and $\bar{r}$ are identical for each other and equal to III+IV by definitions, because the final arrival at the cell cluster and the cell means here the same event. Such is seen mostly in cases with only one completed cell (Cases 3, 5, 7) but not necessarily (Case 8). The similar situation is expected in *Melipona* characterized by predominant singular ovipositions (B_s cf. **1.2.** and **1.5.**). On the other hand, the appearance of queen removal from the cell, which would be more frequent in the presence of two completed cells (Cases 4, 9, 12), or from the cell cluster results in the discrepancy of phases and stages as seen in other cases in Table 7. The duration of phases is quite variable but $\bar{P}$ is longer, in average 0.5~1.5 hrs. while $\bar{A}$ and $\bar{r}$ are often less than 30 min., occasionally very short. However, the phase distinction must be corrected and supplemented by observations made with stronger colonies.

Finally the behavior sequence in the presence of two completed cells are referred to. In such case the queen alternately visits the cells so that it is difficult to predict which cell would first receive the larval food. But here also the waiting is distinctly longer than cruising, exhibiting type cW, not CW as in *Leurotrigona* (cf. **1.4.** and **2.3.1.**). In **3.2.** it was mentioned that the interval between two successive ovipositions becomes either very short or prolonged even though two completed cells occur. This depends on the continuation of worker excitement. If the excitement released by food discharges in the first cell is kept at a certain level for a while, the arrival of the queen at the second cell and her waiting there will reinforce the worker excitement, leading to a rapid succession of two ovipositions. On the other hand, if the excitement drops rapidly, the queen waiting at the second cell would be insufficient to release the food discharge rapidly, resulting in a delay of the second oviposition. Several cases are cited below:

Case 1 (=Case 8, Table 7, VIII 2). Cell B was 1/4 high at 9:45 when A was started. Both completed at 14:00. Distance between two cells was only 8 mm so that the queen could wait either at A or B by a slight change of position. Continuous observation was started at 41:51 and the queen changed her waiting (*W*) as follows (*D*. Food discharge, *O*. Oviposition:



In this case food discharges in two cells were successive and those in the second

cell appeared after queen waiting, that is, discharge type was D_c (**1.6.**). But the predischarge waiting at the second cell R_2 or $\bar{r}_2$ (**1.6.**) was very short, virtually obsolete, indicating a tight integration of two UOP in an IOP. It is interesting that a remarkable shortening of $\bar{r}_2$, secondary predischarge waiting, appeared in this very weak colony, which was never seen in *Leurotrigona* (**2.3.1.**). This might be an outcome of a close distance between two cells. But another case observed on VIII 1 took a similar sequence. Further observations under favorable colony conditions are required.

On the other hand, two ovipositions were independent for each other in the following three cases.

Case 2 (=Table 7, Cases 4 and 5, Fig. 24, A, VII 24): As shown in Fig. 24, the queen first waited by B, but later changed to A, which received food and egg at 16:06 (Case 4, Table 7). The queen soon arrived at B but left it after 30 sec. About 30 sec. later, she again arrived at B, but the worker excitement was already weak. Thereafter the queen visited B several times and oviposition took place at 17:08 (Case 5, Table 7).

Case 3 (=Table 7, Case 10, VIII 3): The queen waited by A. Worker concentration appeared also at B. Oviposition in A at 10:30, no worker concentration at B. Later (10:35) concentration appeared at B and oviposition took place at 11:08.

Case 4 (=Table 7, Case 12, Fig. 24, C, VIII 11): As seen in Fig. 24, the queen alternately waited by A and B. By oviposition in A at 15:49, a mild excitement appeared at B but it soon ceased. Oviposition in B took place at 16:25.

Although additional observations are indispensable, the characteristics in predischarge behavior of *Trigonisca* are summarized as follows: 1) Distinction of $\bar{P}$, $\bar{A}$ and O ($\bar{r}$) is difficult by gradual shift of behavior and frequent removal of the queen from the cell or the cell cluster. 2) Queen waiting is longer than cruising. 3) Cells can be batched or not, and shortening of secondary predischarge phase can appear or not. 4) Worker behavior at queen waiting is characterized by virtual absence of intentional insertions and the presence of peculiar behavior pattern, presentation of mesosoma to the queen. 5) Queen behavior is characterized by violent beating of workers and virtual absence of cell inspection.

3.3.3. *Food discharge, oviposition and operculation*: As in *Leurotrigona* each UOP in *Trigonisca* is articulated as $\bar{r}\cdot\bar{d}\cdot\bar{o}\cdot\bar{s}$ (**1.7.**), and each $\bar{d}$ is always successive. But as described in **3.3.2.**, each UOP was mostly independent, though rarely two UOP formed a batch, in which $\bar{r}_2$ could be shortened. Behavior sequence in $\bar{d}$ is simple. The first discharge evokes a violent excitement among workers attending the cell, who run around in all directions. A second and subsequent discharges immediately follow. The number of discharges per cell is 4 (8 cases), 5 (5) and 6 (4), in average 4.8, more or less comparable to other groups except *Leurotrigona* (**2.3.2.**). Each discharge lasts about 1 sec. and is made with a metasomal contraction as in other groups (Fig. 26, A). Postdischarge escape is distinct, traced to the distance of 3~4 cells (Fig. 26, A). No other behavior is inserted between successive discharges (Fig. 24) so that discharge subphase $\bar{d}_d$ (**1.7.**) is short

though only four cases were accurately measured: 6, 7, 9 and 13 sec., all with four *D.* The last discharge is soon followed by oviposition phase, $\bar{o}$, in most cases, but occasionally a few shallow insertions of mouth parts into the cell by workers appear as follows:

No. insertions		0	1	2	3	4	5
No. discharges	4	5			1	1	1
	5	3		1	1		
	6	4					
Total		12		1	2	1	1

The insertions tend to occur when the number of discharges is small. It is possible but not certain that these mean unrealized discharges inhibited by the sufficient amount of food already deposited in the cell. The insertions are very brief, not followed by escape. No worker oviposition takes place in postdischarge subphase, $\bar{d}_p$, which is also short, 4~5 sec. Total discharge phase $\bar{d}$ ($=\bar{d}_d+\bar{d}_p$) was precisely measured only three times, 11, 14 and 20 sec.

The oviposition is always preceded by a queen inspection of the cell, which once repeated twice without circumstancial particularity (Fig. 24, C). The inspection is shallow and brief, not exceeding 1 sec., but a longer and deeper insertion of the head was observed three times, lasting respectively 2, 3 and 10 sec. The last case apparently signifies the intake of larval food. The queen oviposition is short, ca. 2 sec., made without wing beating and any other particular behavior.

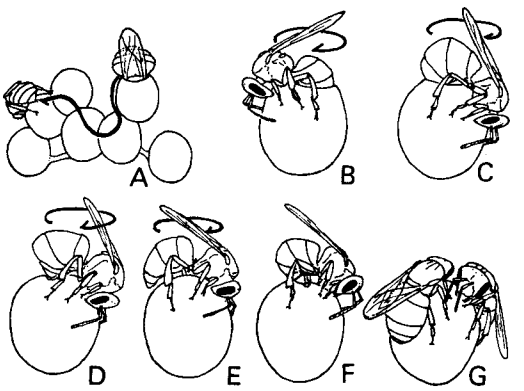


Fig. 26. Behavior patterns of food discharge (A, postdischarge escape at left) and cell operculation (B~G) in *Trigonisca*. Explanations in text.

The operculation of the cell oviposited starts either soon after (6 out of 16 cases) or with a delay (10/16). In the latter case, however, the delay is short, 1 sec. (1 case), 2 (3), 3 (1), 4 (1), 5 (2), 6 (1), 25 (1), so that preoperculation subphase, $\bar{s}_0$ (**1.7.**) is virtually absent. The last mentioned case (25 sec.) was caused by the cruising of the queen nearby. The operculation of *Trigonisca* is peculiar in two aspects, remarkable prolongation and persistence of rotation. The temporal sequence of operculation phase, $\bar{s}$, is given in Figs. 24 and 27. First the rotation

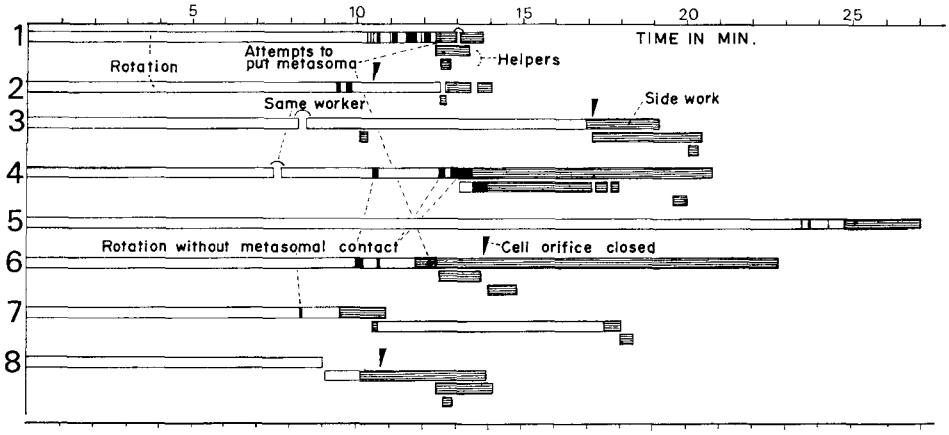


Fig. 27. Chronological sequence of cell operculation in *Trigonisca* in several precisely recorded cases. Explanations in text. Nos. 1, 7, 8 correspond respectively to A, B, C in Fig. 24.

subphase, $\bar{s}_r$ (Fig. 26, B ~D), is very long, lasting in average ca. 10 min. Cases measured are (min.-sec.): 5–30, 7–07, 7–30*, 8–16, 8–20*, 8–40, 8–45, 8–55, 9–25, 9–28, 9–45, 10–00, 10–23, 10–35, 10–40, 11–19, 14–50, 15–00, 23–00. Two asterisked ones were briefly interrupted by a departure of the operculator followed by an immediate return (cf. Fig. 27, Cases 3 and 4). By the continuous performance of rotation, the cell orifice becomes quite small already during $\bar{s}_r$. Then, the metasomal tip is removed from the cell orifice (Fig. 26, E). But this does not followed by sidework subphase, $\bar{s}_s$. Without putting the metasoma on the orifice, the operculator continues rotation on the cell. Attempts to put the metasomal tip on the orifice appear from time to time, in the maximum nine times (Fig. 26, F, Fig. 27, Cases, 1, 6), and occasionally the rotation with metasomal contact revives for a while (Fig. 27, Cases 2, 4, 6, 7). Thus, $\bar{s}_s$ (Fig. 26, G) is separated from $\bar{s}_r$ in most cases by transient subphase, $\bar{s}_t$ (**1.7.**) and the proportion of $\bar{s}_s$ to the total length of $\bar{s}$ is relatively small. The appearance of $\bar{s}_r$, rotation without metasomal contact, is known in *Tetragona*, *Cephalotrigona* and *Duckeola*, too. In these groups, however, the first withdrawal of metasomal tip occurs at an earlier stage, e.g. only 30 sec. to 1 min. after the start of rotation in *Duckeola*. In *Tetragona*, which is the unique group comparable to *Trigonisca* by the prolonged operculation, the first withdrawal appears later than in *Duckeola*, but never more than 5 min. after the start of rotation. On the other hand, $\bar{s}_r$ is very long in *Trigonisca*. The later part of $\bar{s}$ is also peculiar. At this stage, other workers "help" the main operculator as in other groups. But these helpers do not always work at cell sides. They occasionally make rotation, even putting the metasomal tip on the orifice (Fig. 27, Cases 7 and 8).

The closure of cell orifice was difficult to confirm, for the colony was observed

from the side, not from the top as in other groups, but in four cases (Fig. 27, Cases, 2, 3, 6, 8, shown with arrows), the time since the start of rotation to the closure was measured as follows: 10 min. 30 sec. (2 cases), 13–49, 16–06, being comparable to the average in *Tetragona*, 11.5 min. The time until the departure of the final participant was 13–40, 14–11, 14–15, 14–20, 14–40, 15–48, 16–00, 16–10, 18–00, 18–25, 18–40, 19–30, 21–00, 24–33, 25–05, 27–00, 32–00, in average much longer than the unique instance measured in *Tetragona* (14 min.). In spite of this prolonged operculation, the working manner does not give the impression of an inefficiency, which is very conspicuous in *Tetragona*.

The total duration of the oviposition process was measured in two ways (in min.): *A*. Since the start of worker concentration around the cell (=start of $\bar{P}$) to the end of operculation, and *B*. Since the start of *presentation* to the end of operculation, giving the following length (*A*/*B*): 33/33, 41/35, 46/38, 47/35, 49/30, 52/25, 70/43, 93/31, 138/47, 195/35. The duration is fairly constant in *B*. In the result mentioned, two cases are excluded in which two ovipositions appeared in succession. In one case (*Case 3* in **3.3.2.**), worker concentration appeared synchronously at two cells, A and B, the total duration of the process (in the way *A*) was 76 min. in A and 125 min in B. But the worker excitement at B once lowered before the oviposition in A, so that, calculated from the revival of worker concentration and *presentation*, the duration in B becomes respectively 49 and 30 min. In the other case (*Case 1* in **3.3.2.**), two ovipositions were made in rapid succession. The total duration since the synchronously appeared worker concentration (or presentation) to the end of operculation was respectively 60 (57) and 63 (60) min.

3.4. *Ethological characteristics of* Trigonisca: Although observations on *Trigonisca* are still incomplete due to poor conditions of the colony studied, the enumeration of ethological characteristics is tentatively given here as in *Leurotrigona* (**2.5.**). The characteristics given in *Leurotrigona* as common to all other groups are also shared by *Trigonisca*. The order of characteristics enumerated below is the same to that in *Leurotrigona*. Unless necessary, explanations of each item are omitted. Similarity to, and difference from other groups are abbreviated, usually referring to *Leurotrigona* (=*L*) alone. Symbols are same to those used in **2.5.**

1)≒*L*. 2)≒*L*. 3)≒*L*. 4) Unknown, probably≠*M*. 5)≒*L*. 6)≒*L*. 7)≒*L*. 8)≠*L*. 9)≒*L*. 10)≠*L*. Virtually absent, though not so completely as in *Melipona quadrifasciata*. 11)≒*L*. 12)≒*L*. 13)^{P}≠*L*,≒*D*, *F*. 14)≒*L*. 15) B_s in the colony observed (≒*M*), but probably B_f (≒*C*, *Tt*) in favorable conditions. 16)^{P}≒*L*. 17)≠*L*. Queen more waiting by cell than cruising (type cW≒*F*). 18) Difficult to conclude. 19) *L*<. Occasionally conspicuous, probably more in larger colonies (≒*Tt*)^{P}. 20)≠*L*. Appearing much later. 21)≒*L*. 22)≠*L*, ≒*M*, *F*. 23)≠*L*, *M*, *C*, *Tt*, *D*< 24) Characterized by *presentation of mesosoma to queen* (≠). 25)≒*L*. 26)≠*L*. 27)≠*L*. 28)≒L. 29)≒*L*. 30)≒*Tt*, *L*≤ 31)≒*L*. 32)≒*Tt*, ≠*L*, but rotation phase very long (≠). 33)≒*Tt*, ≠*L*.

The characteristics exclusive to *Trigonisca* are the presentation during arousal phase and prolonged and persistent rotation in operculation phase (23, 31). Further comparisons with *Leurotrigona* will be given in Concluding remarks. **2.**

Concluding remarks

Brief discussions were given in each section concerning the behavior described. Here general remarks are added to some aspects seemingly important for further studies of stingless bees in comparison with other social insects.

1. *Peculiarity of oviposition process in stingless bees*: The temporal articulation of the oviposition process in stingless bees was systematized in Section **1.** Taking the queen ovipositions as a focus, the daily life of stingless bee colonies proceeds through a rhythmic alternation of several phases, roughly divided into quiescent ($\bar{Q}$), transient ($\bar{T}$), patrolling ($\bar{P}$), arousal ($\bar{A}$) and oviposition phases ($\bar{O}$). The ovipositions are usually batched and each oviposition or UOP is further divided into several phases, basically formulated as $\bar{r}\cdot\bar{d}\cdot\bar{o}\cdot\bar{s}$. In other words, cell construction (*C*), food provisioning (*D*), oviposition (*O*) and cell operculation (*S*) form a tightly linked process, $C\cdot D\cdot O\cdot S$, which passes during a short time under high excitement, often reinforced by peculiar behavior. This basic pattern receives modifications in each group according to their group specificity. Obviously the phase distinction made in the present paper is still tentative, requiring further elaborations. But it is undeniable that the stingless bees are singular by this rhythmicity among social Hymenoptera.

In honeybees the temporal sequence in each cell is, if the process is traced, $C\cdot O\cdot D\cdot S$. But all these unit items are rather independent for one another, not forming an integrated process. The most remarkable difference lies in *D*, which appears in stingless bees before *O* and is passed in most cases within one minute under high excitement, while in honeybees it appears after *O* as a *diffused* performance lasting several days (=progressive provisioning). There are two other differences between these two highly developed eusocial bees. In honeybees, there is no distinction of storage pots and brood cells. The same cell can be used either for storage or for brood rearing and the same cell is repeatedly used for the latter purpose. Social wasps behave nearly identical with honeybees. Ants also behave basically similar to honeybees, though here the performance sequence is only $O\cdot D$ by the abandonment of *C*. Therefore these groups do not show the cyclicity of oviposition process characteristic of stingless bees. Certainly the queen oviposition may be performed through the alternation of laying and resting phases caused by a cyclicity of egg maturation. But this is not intimately linked with the cyclicity of worker performance.

Another social Hymenoptera, bumblebees, exhibits and interesting mixture of two types, called general (G) and stingless bee type (M), as follows (cf. Free and Butler 1959, Sakagami and Zucchi 1965):

1) Temporal sequence of performance is $C\cdot(D)\cdot O\cdot S\cdot n(DS)$[1]. The first half is well linked (≒M) while the later part, $n(DS)$, is diffused (≒G). 2) $C\cdot(D)\cdot O\cdot S$ is always exclusively made by the queen (≠M, G), especially C as a continuous performance, not as the summation of diffused activities as in M and G. 3) Oviposition is apparently rhythmic, but each batch is deposited into one and the same cell, which expands later in parallel with the growth of larvae (≠M, G). 4) By the separation of $n(DS)$ from the tightly linked earlier part, $C\cdot O\cdot S$, by the queen, the oviposition process proceeds differently both from M and G. 5) Operculation soon after O is exclusively made by the queen as a continuous work, being closer to M (=first by an worker as a continuous work, $\bar{s}_r$, later as somewhat diffused work by several workers, $\bar{s}_s$ cf. **1.7.**) than G (usually diffused work by several workers). 6) Differentiation of storage pots and brood cells and absence of cell reuse are similar to M.

Considering these items of information, it is understood that stingless bees retain the mass provisioning practiced by most solitary wasps and bees. Without adopting the progressive provisioning prevailing in all other higher social Hymenoptera, stingless bees transformed the mass provisioning into a peculiar system, proceeding through complicated behavioral coactions between queen and workers. In other words, it is regarded as a further elaboration of the archaic system instead of adopting a new system. It is interesting that they behaved oppositely in another aspect. The production of trophic eggs now confirmed in various groups is, in our opinion, the adoption of a new system instead of an elaboration of the archaic system, that is, the inhibition by the queen as practiced in most other social insects, though this system is in itself a new aquisition at the dawn of their social evolution, not a heritage since the ancestral solitary life such as mass provisioning (cf. Sakagami and Zucchi 1968, Sakagami, Camilo and Zucchi 1972). A similar comparison must be undertaken as to various aspects constituting a social system *separately*. The evaluation of relative heights of various social insects must be made through such comparisons, instead of making a premature assertion based upon a few characteristics.

Another point worth mentioning is an extreme diversity in oviposition behavior among various groups of stingless bees. It must be stressed that such diversity evolved with respect to the behavior sequence performed *within* the nest, i.e. in the place protected from various external factors, which serve in many animals as selection pressures.

2. *Ethological comparison between* Leurotrigona *and* Trigonisca: Both *Leurotrigona* and *Trigonisca* were erected by Moure (1950, cf. also 1951), together with *Celetrigona* and *Dolichotrigona* as the Neotropical subgenera of the *Hypotrigona* Cockerell. Later he confined the use of *Hypotrigona* to some species of the Ethiopean Region and erected other three 'genera', *Pariotrigona* and *Lisotrigona* for

1) Deposition of pollen before O or even before C is facultative. This requires further comparative observations.

some Indomalayan species and *Liotrigona* for some African species, all as belonging to the group *Hypotrigona*. (Moure 1961). In these papers, however, he pointed out repeatedly the possibility of their similarity due to a convergence achieved in parallel with the reduction of body size. Here the ethological comparison of two groups observed by us is made in order to give some additional information upon the relation within the group *Hypotrigona*.

Table 8. Ethological differences between *Leurotrigona* and *Trigonisca*

Characteristic	*Leurotrigona*	*Trigonisca*
1. Rhythmic wing movements of queen	Relatively rare but present at walk	Extremely rare
2. Cell construction	Synchronous (S_y)	*Semisynchronous (S_m)
3. Excitement at cell construction	*Relatively high	**Not conspicuous
4. Oviposition pattern	Exclusively batched (B_e)	*Singular (B_s) in the colony observed, probably facultatively batched (B_f) under favorable conditions
5. Pattern of predischarge queen behavior	More cruising than waiting (CW)	More waiting than cruising (cW)
6. Shortening of secondary predischarge waiting	Inconspicuous	*Occasionally conspicuous
7. Predischarge worker behavior	Simple, body insertions into cell replaced by intentional ones at queen waiting	Intentional insertions rare. Peculiar behavior, *presentation* of mesosoma to queen prevailing
8. Predischarge queen behavior	Simple, waiting short	Waiting long, with violent beating of workers with antennae and fore legs
9. Number of discharges per cell	Low, mostly 2	Normal, 4~6
10. Postdischarge escape	Inconspicuous	Normal, i.e. distinct
11. Duration of operculation	Normal	Extremely long, especially as to rotation subphase
12. Differentiation of rotation and sidework subphases	Distinct	Indistinct due to persistence of rotation.

* Those requiring further confirmation

The ethological characteristics common to both groups are as follows:

1) Cells arranged in cluster. 2) Involucrum absent. 3) Differentiation of honey and pollen pots absent. 4) Cells and pots prepared with little admixture of resin. 5) Waste materials thrown down from nest entrance. 6) Capacity of thermal regulation poor. 7) Queen walking with moderate speed. 8) Queen not taking prolonged geonegative resting. 9) Exaggerated queen-worker coactions out of oviposition process absent. 10) Food discharges in cells successive. 11) Excitement arousal relatively localized. 12) queen cell inspection in arousal and discharge phases rare, never ritualized. 13) During food discharges queen continuing to wait at cell. 14) Postdischarge subphase very short. 15) Worker oviposition during oviposition process absent. 16) Queen oviposition relatively short, though slightly longer in *Trigonisca*, not accompanied with peculiar behavior. 17) Cell operculation started without much delay.

Many characteristics are common to both but few are exclusively common to both. Exactly only characteristic 4) is regarded as such. Characteristic 5) is also rare in other groups but interestingly found in two cleptobiotic groups (**2.1.1.**). However, the nest structure as a whole (completely clustered≒*Friesella*, without differentiation of honey and pollen pots≒*Frieseomelitta**, and with little admixture of resin) could be regarded group specific. In other behavioral characteristics, the general similarity between two groups is apparent but many features are common to other groups, too.

The characteristics different between two groups are relatively many (Table 8), involving some conspicuous ones, e.g. 5, 7, 8, 9, 11, 12, suggesting two groups are distinct for each other in ethological makeup, too. This favors the opinion that these two groups are not so close as superficially interpreted by their morphological similarity. For the time being, however, we shall continue to consider them as the subgenera of the genus *Hypotrigona* until the polyphyly of these dwarf stingless bees would be confirmed. Anyhow, it is certain that they cannot be the groups directly ancestral of the main meliponine evolution. Their dwarfism accompanied with reduced wing venation denies any such possibilities, even if some of their particularities, either morphological or ethological, would represent primitive conditions.

3. *Conflict between fear and defense in workers at oviposition process. A hypothesis*: In **3.3.1.** a singular worker behavior in *Trigonisca*, the *presentation* of mesosoma to the queen was described. As already mentioned this behavior is seen as if bearing a barricading function toward the approaching queen. If such would be true, the behavior is assumed as linked with a defensive motivation. On the other hand, the exhibited posture suggests its submissive nature. The lowering of the head and antennae resembles the presumably ritualized submission expressed by workers of *Melipona* in front of the queen, though this has not explicitly been stated in our previous papers. Moreover, the temporal position of presentation also suggests its submissive nature. It arises at the situation where actual body

insertions into cells are replaced by intentional ones in many groups, *Melipona*, *Cephalotrigona*, *Tetragona*, *Duckeola* and notably *Friesella* and *Leurotrigona*. Although still far from the satisfactory conclusion, we tentatively regard this behavior as a submissive response secondarily transformed into a ritualized cell defense. To make this interpretation clearer, we must refer to our assumptions on the mechanism underlying the oviposition process.

In a previous paper (Sakagami, Camilo and Zucchi 1973) we presented a hypothesis without detailed explanation as follows: The core of the complicated oviposition process in stingless bees is a conflict in workers between fear to the queen and defense of the cell against her. Although still being premature, the outline of our hypothesis is sketched here: 1) In parallel with the cell growth, the food discharge motivation gradually increases in nurse age workers, stimulated by crop filled with larval food and by the presence of completed cells. 2) But actual discharges do not take place unless reinforced by further arousal of excitement. Frequent body insertions into completed cells at this stage are, in our opinion, still unrealized or intentional discharges (**1.4.**). 3) Arrival of the queen at the comb or the cell evokes two opposite drives in workers, the fear to the queen and the defense of the cell against her. 4) Fear is expressed by the replacement of body insertions by intentional insertions as mentioned above (cf. also **2.3.1.** and Fig. 16, M). In extreme case the escape from the cell is released (*Friesella*). 5) The role of the queen in the predischarge periods ($\bar{P}$, $\bar{A}$, $\bar{r}$) is to lower this fear and *to raise the defense of the cells by workers*, or *their ownership to the cells*, which is shown by the gradual recovery of body insertions in many groups of C<W type (**1.5.**), though this is relatively rare in *Friesella* and *Leurotrigona*, characterized by marked intentional insertions. 6) In the groups exhibiting C>W type (**1.5.**) the fear removalis probably achieved rather by generalized arousal than localized one (**1.5.**). 7) In some groups the cell defense or ownership is expressed by the appearance of aggressive darting to the queen by workers, which is, sometimes rather ritualized, conspicuous in *Scaptotrigona*, *Schwarziana**, *Scaura** and probably in *Nannotrigona**. 8) At the culmination of this process, e.g. the gradual prevalence of cell defense against the fear, the worker's ownership reaches the level to liberate food discharge, a token behavior for this *female* deprived of normal reproductive function. 9) Food discharge brings a momentary recovery of the fear once suppressed in the discharger as shown by postdischarge escape universally confirmed in all groups. 10) On the other hand, the discharge removes the final obstacles for food discharge in other workers, resulting in a rapid succession of discharges, also universally confirmed in all groups.

We are ourselves still not contented with this hypothesis, but it can explain, at least partly, the singular behavior sequence in oviposition process. The hypothesis also explains why the worker ovipositions, a phenomenon so characteristic in stingless bees, are intimately linked with the oviposition process in most groups. If this hypothesis is valid, we could again stress the singularity of stingless bees among social insects mentioned in Concluding remarks **1.**

Returning to the presentation in *Trigonisca*, however, this curious behavior is still not fully explained by our hypothesis. In particular, why a submissive posture transformed into a ritualized defense remains unsettled. Probably the excitement arousal is reciprocal between queen and workers. While the queen functions to lower the discharge threshold in workers, worker's increased cell defense would stimulate the queen to reinforce her role as activator, and the presentation have possibly been ritualized to increase such reinforcement.

4. *Some comments on laying workers in stingless bees.*: The appearance of laying workers in orphan colonies is an event widespread in social insects. In stingless bees this event is of particular interest. Because in many groups worker ovipositions arise even in queenright condition. Further the oviposition process in queenright colonies takes a definite temporal sequence, integrated in a well articulated structure, where the queen seems to behave as pace maker. How such temporal structure is modified in orphan colonies is interesting in relation to the analysis of oviposition behavior. Keeping these particularities in mind, some additional comments are given on our observations in *Leurotrigona* (**2.4.**).

Compared with the normal process, which goes through reciprocal stimulations between two castes, the disintegration of the process is obvious in worker oviposition in orphan colonies. But the disintegration arises not homogenously throughout the process, some parts being conspicuously modified while others relatively intact as explained below: 1) Synchronous cell construction becomes indistinct but its tendency is still retained. 2) Batched ovipositions are obviously absent because there are many layers. Nevetheless, it is interesting that both food discharges and ovipositions tend to concentrate in a limited period. Two assumptions, which are not mutually exclusive, are held to explain this. A. Temporal fluctuation of the number of workers ready to discharge food and to lay, and B. Spread of excitement caused by a food discharge in a cell to workers staying nearby. 3) The role of the queen as pace maker is partly replaced by potential laying workers. Interestingly they exhibit some behavior patterns reminescent of the queen in other groups, such as A. Rather persistent waiting by cell with less cruising (=*Friesella*), B. Repeated body insertions in cells, somewhat resembling ritualized cell inspections by the queens of *Cephalotrigona*, *Tetragona* and *Duckeola*, and C. Violent beating of other workers inserting bodies in cells (≒*Melipona*, *Trigonisca*), all not exhibited by the queen of *Leurotrigona*. 4) The role of laying workers is amphipotential, for she plays the roles of both queen and workers in the normal process. 5) Number of food discharges necessary to release the oviposition as well as discharge behavior do not differ from those in the normal process. But the postdischarge escape, being characteristic to all groups so far studied, does not appear. This is remarkable in relation to our hypothesis held in Concluding remarks **3**, but its interpretation is postponed until further comparative studies would be achieved. 6) The sequence after food discharge is quite variable, either culminating in oviposition or releasing food ingestion. 6) Performance of

operculation soon after oviposition by egg layer itself, as well as the differentiation of operculation phase into rotation and sidework subphases ($\bar{s}=\bar{s}_r+\bar{s}_s$, cf. 1.7.) are very rigidly linked, forming the most stable part of the whole process.

Due to its erratic nature, the oviposition process in orphan colonies has been observed only in a few other groups, *Cephalotrigona*, *Partamona*, *Scaptotrigona** and *Lestrimelitta*,* all rather casually. The disintegration of the process accompanied with endless continuation of alternate body insertions and ingestion of once discharged food with congestion of workers around cells are common to these groups and *Leurotrigona*. On the other hand, there are some remarkable differences as follows (*P*=*Partamona*, *C*=*Cephalotrigona*, *L*=*Leurotrigona*): 1) Postdischarge escape is observed in *P*. 2) Worker eggs are laid at cell margin in *P* and *C* as in queenright condition, not always into the cell as in *L*. 3) Appearance of some behavior patterns indicating potential workers such as erratic body shaking and wing vibration in *L* are not seen in *P* and C (This might be due to the lack of precise observations). 4) In *C* cells are not always operculated soon after oviposition and cells receiving food but still not egg are sometimes operculated. In such case, a few workers attempt to operculate the cell, standing at cell sides as in *Friesella*, but much more erratically, that is, not by rotation. 5) A tight linkage of oviposition and operculation seen in *L* is not observed in *C* and *P*. Consequently oophagy arises commonly in these groups.

Summarizing, the behavioral disintegration appears in *L*, but not so conspicuously as in *C* and *P* and, though precise analysis is still not undertaken in *Scaptotrigona*.* It is pointed out that workers of *Leurotrigona* do not develop their ovaries in queenright colonies as in *Cephalaotrigona* and *Partamona*.* Whether any causal connection exists between these two items or not is interesting for further studies.

5. *Importance of observations under diverse colony conditions*: In the present study the colony of *Trigonisca* and some colonies of *Leurotrigona*, especially the former, were inevitably observed under adverse colony conditions. The results obtained must be supplemented by additional observations under favorable conditions. However, the incomplete results suggest themselves the presence of some important problems. Comparing the results of *Trigonisca* with those of other groups, usually obtained under favorable condition, some aspects of oviposition process seem to vary considerably according to colony conditions, while others to remain relatively stable. Judging from the previous experience by us and our colleagues, elementary behavior patterns are relatively stable under various conditions while its integrated whole such as various types in cell construction, batch formation, etc. are more susceptible, and the quantitative apsects of such integrated complex, e.g. frequencies and durations, are most vulnerable. Some related results have been presented in *Tetragona* and *Friesella*. Camilo came to the same conclusion by comparing the colonies of *Melipona rufiventris* Lepeletier under favorable and unfavorable conditions (unpub.).

Our observations with various groups, both already published and still not, have mainly been executed with the colonies reared under presumably favorable conditions. This is inevitable because many groups are rare, being difficult to obtain and to rear artificially. But for a thorough understanding of the oviposition process, it is necessary to observe the colonies under diverse conditions, at least as to the groups relatively easy to obtain and to rear. Obviously there are numerous conditions which affect colony activities. But, colony size, amount of food and thermal protection are enumerated as principal factors. Secondly the presence or not of the queen affects much the oviposition process as discussed in the preceding section. Finally the oviposition process may vary according to the age of the queen. Especially the time since the fertilization of a virgin queen to the complete maturation of her oviposition behavior is a short but important period to understand the behavioral ontogeny (cf. Lemasson Naves da Silva *et al.*). The opposite instance, the senility of the queen is usually difficult to produce artificially but the casual observations with the queen at her final days must carefully be collected. Consequently a thorough understanding of the oviposition behavior of a given species requires comparative observations of colonies at least in the following four conditions: 1) Colony with numerous workers, ample food, a gravid queen, and reared in heat conditioned hive. 2) Queenright colony reared under unfavorable conditions which result in the contraction of oviposition. 3) Orphan colony and 4) Colony with a virgin queen.

Summary

1) The oviposition behavior of two dwarf Brazilian stingless bee species, *Hypotrigona* (*Leurotrigona*) *muelleri* (Friese) and *H.* (*Trigonisca*) *duckei* (Friese) were observed. Both species are cluster makers with little use of resin in preparing brood cells and storage pots.

2) Some comments are given on the temporal structure of stingless bee oviposition process, in order to supplement and to correct our previous explanations. The daily colony life is divided into five phases; quiescent ($\bar{Q}$), transient ($\bar{T}$), patrolling ($\bar{P}$), arousal ($\bar{A}$) and oviposition ($\bar{O}$) phases.

3) Oviposition phase, $\bar{O}$, usually forms an integrated oviposition process, IOP, involving several unit oviposition processes, UOP, or ū, which is further divided basically into four phases, predischarge ($\bar{r}$), discharge ($\bar{d}$), oviposition ($\bar{o}$) and operculation ($\bar{s}$), some of them involving subphases.

4) Oviposition behavior differs considerably among various stingless bee groups. Several types are distinguished as to cell construction, batch formation, excitement arousal, food discharges and phase differentiation.

5) *Leurotrigona* exhibits synchronous cell construction, exclusive batch formation, excitement arousal more by queen cruising than waiting, and successive food discharges in cells. The oviposition behavior is characterized by its general simplicity, in particular by excited cell construction, low number of food dis-

charges per cell and inconspicuous postdischarge escape. Observations on laying workers show an obvious disintegration of oviposition process though not so conspicuously as in some other groups.

6) *Trigonisca* exhibits semisynchronous cell construction, no or facultative batch formation and excitement arousal more by queen waiting than cruising, with frequent removal of the queen amid the oviposition process. But these characteristics must be confirmed by further observations under favorable conditions. The oviposition behavior is characterized by a singular predischarge behavior, presentation of mesosoma by workers to the queen, and prolonged operculation with a persistence of rotation movement.

7) Some considerations are given as to the singularity of oviposition process of stingless bees among social insects, ethological comparison of *Leurotrigona* and *Trigonisca* and importance of observations under diverse colony conditions for a thorough understanding of oviposition process. A conflict in workers between fear to the queen and cell defense against her is postulated as a hypothesis to explain the mechanism underlying the oviposition process.

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References

Araujo, V. de Portugal 1958. A contribution to the bionomics of *Lestrimelitta cubiceps* (Hymenoptera, Apidae). J. Kansas Ent. Soc. **31**: 203–211.

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

Camilo, C. 1972. Alguns aspectos do comportamento de *Melipona rufiventris rufiventris* Lepeletier. In Cruz-Landim *et al.* (ed.) "Homenagem à Warwick E. Kerr". 317 pp., Rio Claro: 57–62.

Free, J.B. and C. G. Bulter 1959. Bumblebees. xiv+208 pp., Collins, London.

Kerr, W. E., Sakagami, Sh. F., Zucchi, R., Araujo, V. de Portugal e J. M. F. de Camargo 1967. Observações sôbre a arquitetura dos ninhos e comportamento de algumas espécies de abelhas sem ferrão das vizinhanças de Manaus, Amazonas (Hymenoptera, Apoidea). Atas Simpósio Biota Amazônica **5**: 255–309.

Lemasson Naves da Silva, D., Zucchi, R. and W. E. Kerr 1972. Biological and behavioural aspects of the reproduction in some species of *Melipona* (Hymenoptera, Apidae, Meliponinae). Anim. Behav. **20**: 123–132.

Lévi-Strauss, C. 1955. Tristes Tropiques. Plon, Paris (From a Japanese translation).

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

Moure, J. S. 1950. Contribuição para o conhecimento das espécies brasileiras de *Hypotrigona* Cockerell. (Hymen.-Apoidea). Dusenia **1**: 240–261.

——— 1951. Notas sôbre Meliponinae (Hymen.-Apoidea). *Ibid.* **2**: 25–70.

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

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

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

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

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

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

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

——— and S. Laroca 1963. Additional observations on the habits of the cleptobiotic stingless bees, the genus *Lestrimelitta* Friese (Hymenoptera, Apoidea). *Ibid.* **15**: 319–339.

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

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

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

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

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

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

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

Schwarz, H. F. 1948. Stingless bees of the Western Hemisphere. Bull. Amer. Mus. Nat. Hist. **90**: xiv+546 pp.

Zucchi, R. e Sh. F. Sakagami 1972. Capacidade termoreguladora em *Trigona spinipes* e em algumas outras espécies de abelhas sem ferrão (Hymenoptera: Apidae: Meliponinae). In Cruz-Landim *et al.* (ed.) "Homenagem à Warwick E. Kerr". 317 pp., Rio Claro: 301–309.