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TECHNIQUES FOR THE OBSERVATION OF BEHAVIOUR AND SOCIAL ORGANIZATION OF STINGLESS BEES BY USING A SPECIAL HIVE [1]

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The stingless bees are one of the ideal materials for the comparative study of behaviour and social organization, because of the large number of species and corresponding ethological diversity. Recently, our knowledge on this interesting group has been greatly enriched, mainly by the contributions made by the Brazilian school (*Cf.* Moure, Nogueira-Neto & Kerr, 1958). But there still remain many problems to be explored, especially when compared to the enormous accumulation of information on the honeybees.

The techniques for the rearing of stingless bees in rational hives of a special type were developed by Nogueira-Neto (1948, 1953, 1957 and 1964). For closer studies of their behaviour and social activities, however, we need a special type of observation hive designed for such purpose. Kerr and his collaborators had already developed an observation hive, a single flat box covered by several pieces of transparent glass. The hive was protected by a black cloth when no observation was going on, and communicated with the outdoor by means of a tube (Lindauer & Kerr, 1958; Hebling, Kerr & Kerr, 1964). I made some improvements on this hive and obtained the type described in the present paper, which was used for observing various species and yielded satisfactory results. Also some necessary cautions and techniques in handling and observing stingless bees in such hive are briefly described.

The species which I reared in this hive were the following (supra-specific classification system follows that of Moure, 1951, 1961):

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Trigona (*Trigona*) *spinipes* (Fabricius)	Rio Claro, SP
T. (*Tetragona*) *clavipes* (Fabricius)	Cosmópolis, SP
T. (*Frieseomelitta*) *silvestrii* (Friese)	Cosmópolis, SP
T. (*Geotrigona*) *mombuca* Smith	Cosmópolis, SP
T. (*Duckeola*) *ghilianii* (Spinola)	Manaus, Am
T. (*Tetragonisca*) *jaty* Smith	Rio Claro, SP
Cephalotrigona femorata (Smith)	Manaus, Am
Hypotrigona (*Leurotrigona*) *muelleri* (Friese)	Martinópolis, SP
Plebeia (*Plebeia*) *droryana* Moure	Rio Claro, SP
P. (*Friesella*) *schrottkyi* (Friese)	Rio Claro, SP
Partamona (*Partamona*) *testacea testacea* (Klug)	Manaus, Am
P. (*P.*) *cupira* (Smith)	Rio Claro, SP
Nannotrigona (*N.*) *testaceicornis* (Lepeletier)	Rio Claro, SP
N. (*Scaptotrigona*) *postica* (Latreille)	Piracicaba, SP
Melipona quadrifasciata anthidioides Lepeletier	Rio Claro, SP
M. marginata marginata Lepeletier	Cabreuva, SP
M. compressipes manaosensis Schwarz	Manaus, Am
M. seminigra merrilae Cockerell	Manaus, Am
Meliponula bocandei (Spinola)	Luanda, Angola

Most of these species, even the subbterranean species such as *T. mombuca* and *P. testacea testacea,* lived in the hives without any particular harm, at least during the observation period, which lasted from one month to more than half a year. Observations made by me and several collaborators on certain species, have been already published (Beig & Sakagami, 1964; Sakagami & Oniki, 1963; Sakagami, Beig & Akahira, 1964; Sakagami & Zucchi, 1963; Sakagami, Beig & Kyan, 1964; Sakagami, Montenegro & Kerr, in press). Observations on the other species will successively be published. Besides the species mentioned above, some other ones were introduced in the hives by those collaborators after my departure from Brazil. One hive was also applied to a colony of a bumblebee, *Bombus* (*Fervidobombus*) *atratus* Franklin, and yielded many interesting results (Sakagami & Zucchi, unpub.; Zucchi unpub.).

Observation hive

It consists of: a) Outer case; b) Inner case; c) Corridor.

a) Outer case. It is a flat wooden case (Fig. 1). Its size can be modified according to that of the inner case, which again can be changed to fit the size of the colony that will be introduced. However, it is better to fix the width up to 55 cm for the observa-

tion case (*cf.* later). If the larger size is necessary, the length can be increased instead of the width (the maximum length I used was 82 cm, with which the length of the inner case was 61 cm).

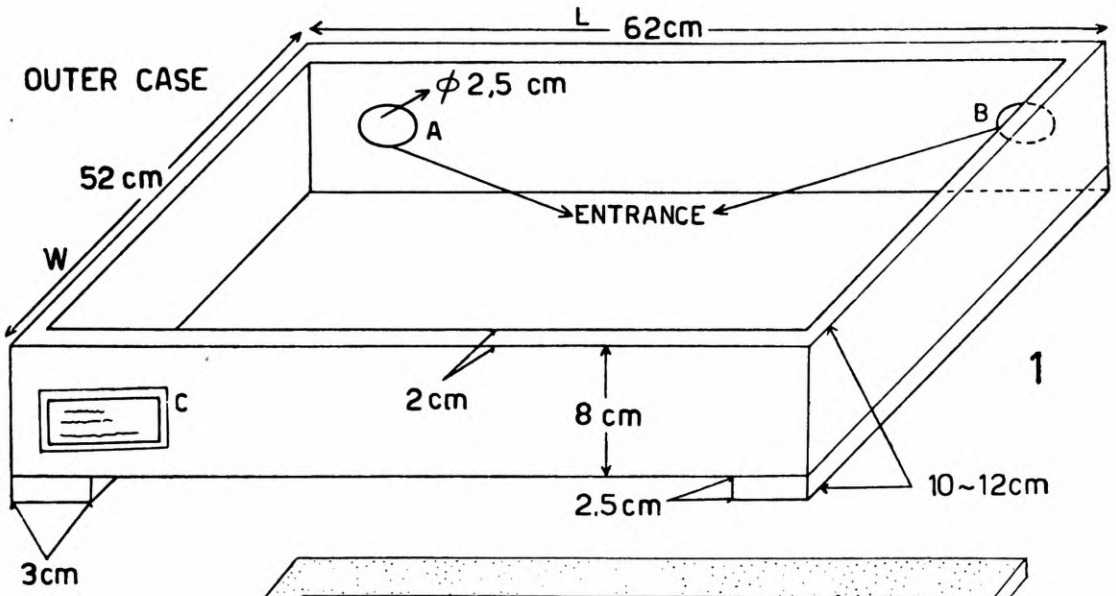
The outer case is provided with: 1) supports at bottom; 2) an entrance hole and 3) an electric heater. The entrance hole can be at any place according to the research plan. However, the corner of the side opposite to the observer is most appropriate. It is recommended to make two holes (Cf. Fig. 1, A, B) in order to: 1) facilitate the modification of the entrance for diverse purposes; 2) keep two colonies within the same inner case (of course separated one from the other by a middle partition). Such arrangement for two colonies is for: 1) keeping two colonies under the same conditions; 2) comparing them closely (I kept *P. schrottkyi* and *N. testaceicornis* in such a way). Of course, the surplus hole must be closed if unused.

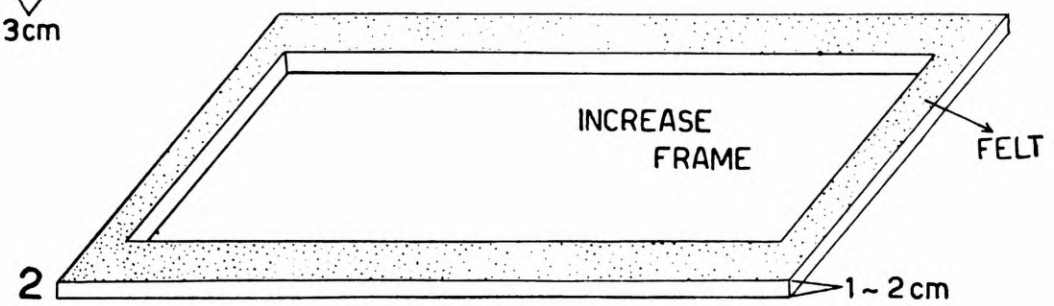
The instalation of an electric heater is seen in fig. 6. The purpose of it is naturally to mantain a proper nest temperature. This is important because the colony, being forced to live in the observation hive, cannot maintain the nest in a form best suited to heat conservation. Further, artificial heating decreases the formation of excessive involucrum in some species (*N. postica*, etc.), which disturbs observation.

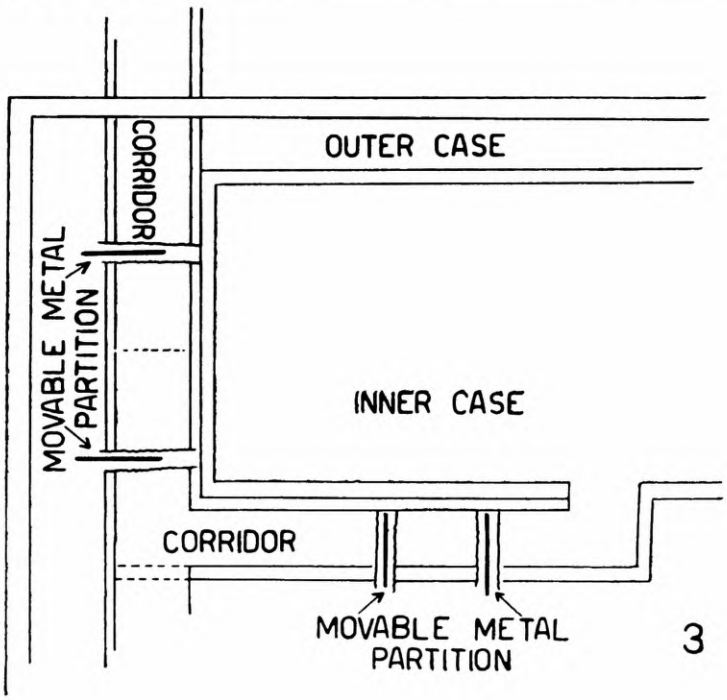
The glass cover of the outer case should be at least 3 mm thick, because it also serves as a desk to take notes. The top margin of the outer case must be provided with felt, in order to improve the fit between glass cover and case (Fig. 4). It is recommended to cover the margins of the glass cover with cellotape, in order to protect it against unexpected breakage and the resulting injuries to fingers, etc. (Fig. 4). Further, it is always advisable a spare cover. It may be advantageous to attach a metal frame to receive a card recording case no., colony no., date of introduction, etc (Fig. 1, C.).

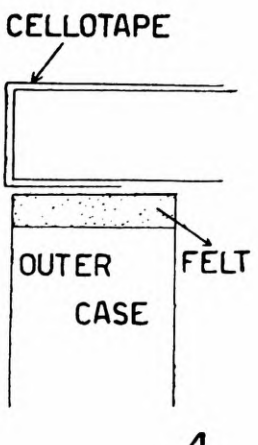
b) Inner case. The inner case is also made of flat wood board (in both inner and outer cases, the bottom can be replaced by synthetic boards instead of wood). It is a flat box, the size of which can be changed in accordance to the size of the colony to be introduced. However, the size mentioned in fig. 5 is adequate in most cases. If the colony is larger, it is recommended to increase the length *but not the width* (important!). For a smaller colony, the case can be reduced by the application of a partition (Fig. 6, A). For some species prefering narrow slits and crevices, especially those that do not build regular combs, it is recommended to give a considerably narrow space. For instance, one colony of *H. mulleri* refused to establish the nest in the inner case but used the corridor as the nesting space.

With this size, the distances between inner and outer cases are about 7-9 cm laterally, 2-3 cm at top and 1.5-2 cm at bottom. This spacing seems to be the minimum requirement to keep the thermal circulation (Fig. 7). The optimum height of the inner case varies according to the storage pots of the species introduced. For this purpose, it is better to fix the height of the case at about 3 cm and to prepare several wooden frames of the same width and length, each with the height of 0.5-1 cm. These may be inserted between case and glass cover, singly or doubly according to the condition. The height of the outer case can also be adjusted by the insertion of such frames (Fig. 2).


L 62cm
OUTER CASE
φ 2,5 cm
A
B
52 cm
W
ENTRANCE
C
2 cm
8 cm
2.5 cm
10~12cm
3cm
1


INCREASE
FRAME
FELT
1~2 cm
2

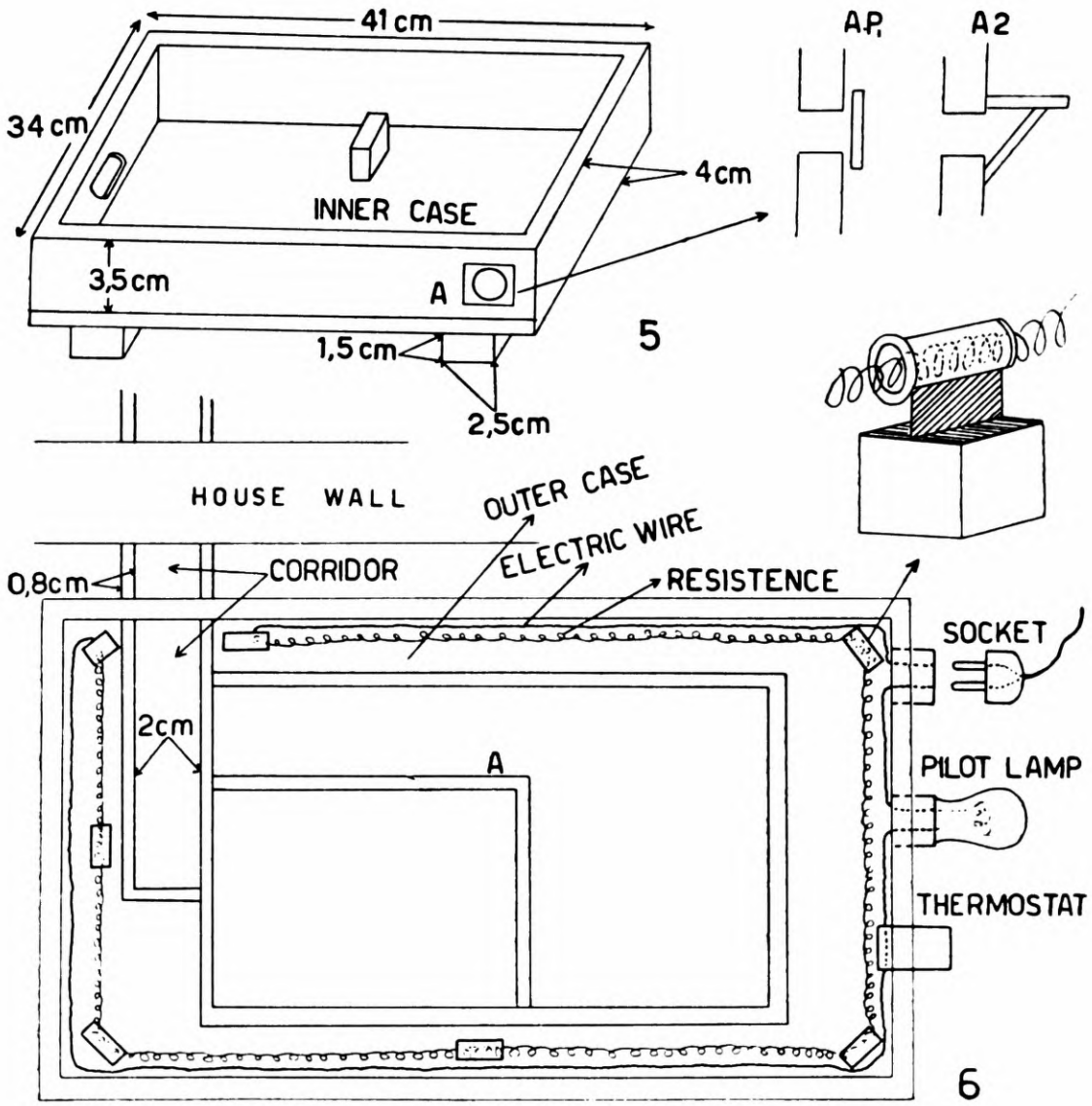

CORRIDOR
OUTER CASE
MOVABLE METAL
PARTITION
INNER CASE
CORRIDOR
MOVABLE METAL
PARTITION
3


CELLOTAPE
OUTER
CASE
FELT
4

The inner case is provided with an entrance hole open to the corridor. There is no limit to the size of this hole, but 3 cm may be more or less convenient. The decrease of size, if necessary, can be made simply by the insertion of a perforated card.

It is convenient to perforate a small hole near one corner of the case (Fig. 5, A) for several purposes (or it may be better to make a pocket as in A 2). The Nogueira-Neto feeder may be used there. It consists of a strait glass tube, filled with 1/3 water and 2/3 sugar, with the open end plugged with cotton, and placed horizontally.

The inner case is also covered with glass, either with: 1) a single large piece, or 2) with several smaller ones (Fig. 8), or 3) with one large and a few smaller ones. In the species which do not much use resins the use of several pieces is convenient for different reasons. On the other hand, in the species abundantly using resins the application of such propolis begins from the pieces



of glass and extends from them, resulting in a decrease in transparency. In such species alternative 3) is the most convenient. In any instance, the cracks between glass pieces or between glass and case are filled by the bees with resins. They often fix things very tightly, so each piece should exceed a little, at least at one side, the margin of the inner case, in order to facilitate the opening of it if necessary (Fig. 9). If several pieces of glass are used, each junction must be supported by small pieces of wood (Fig. 8, A) (see Hebling, Kerr & Kerr, 1964), the height of which is the same as that of the inner case.

At any rate, several replacements of glass must always be ready. Besides breakage, many species put resins on the glass, so that the latter must be changed from time to time. The removed glass must be put in water and cleaned within one or two days, in order to be ready for immediate reuse.

c) Corridor. The corridor provides communication of the inner case with the outside world, passing through the holes of the outer case and the wall of the room (Fig. 3, 6). The type of corridor depends on the purpose of the research work. If only life within the colony is important to be observed, the insertion of a simple plastic or gum tube is sufficient. But if the behaviour of foragers is the aim, a wooden corridor with glass cover is recommended, or a side wall also of glass. In such case, the corridor can be elongated to open inside the inner case, as seen in figs. 3, 6, to facilitate observation. Several slits are made in one side of the corridor wall, through which a metal piece may be operated to open or close the corridor (Figs. 3, 10). By this, the traffic of bees can easily be regulated or, if necessary, sample bees can be taken with a minimum of confusion.

If the nuptial flight of a queen must be observed, a framed piece of wire mesh can be inserted, through which only workers can pass. Using this, the occurrence of a nuptial flight in the absence of the observer is avoided. (Of course, this mesh can be used only in a species possessing queens larger than workers).

Location of hive

The observation hive is set on a table. The relative height of table and chair is very important (Fig. 11). As the observation hive is about 10-12 cm high, the table on which the hive will be placed must be correspondingly lower than the usual table. Or the chair must be higher than the common ones. This trifling aspect is of great importance, because observations may often continue during several hours without interruption. The prevention of physical fatigue of the observer is indispensable. Because of the same reasons: 1) The chair must be reasonably comfortable, 2) The margin of the table must be in the same perpendicular line of that of the hive (Figs. 11, 12, 3) The table must be of a type that facilitates the accomodation of legs. The length of the table is better if longer than that of the hive, to leave extra space for all things necessary. During the observation, often there is no time to leave the hive to search them.

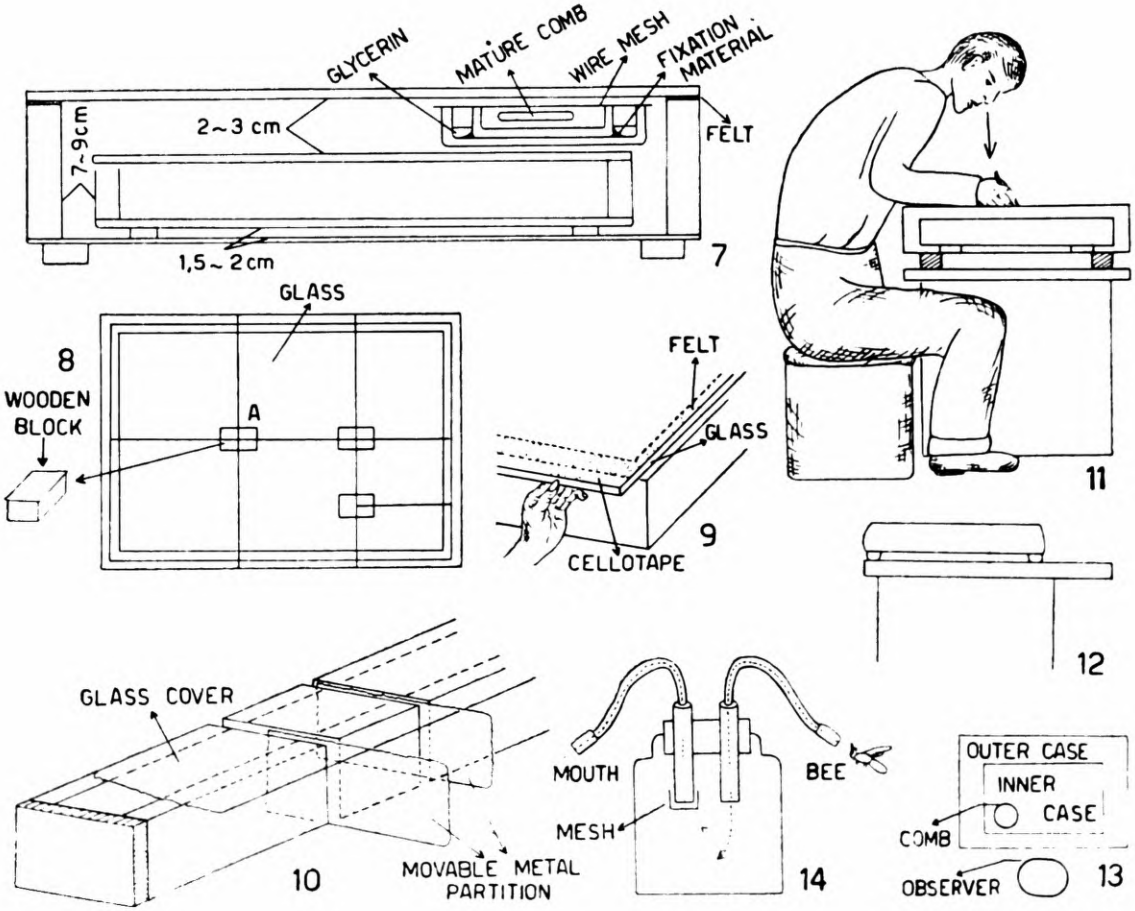
The room to place the hive is also important. The hive can be set near a window. But the window must be of a type which completely shuts out sun rays. Direct tropical insolation is enough to kill the whole colony within a very short time.

Introduction of colony

If the colony must be transferred to an observation hive near the previous nest site, the colony must first be transferred about 1-2 km distant and kept there some 2 weeks, to prevent the return of flyng bees to the original site. Even after this procedure, often many bees return to the original site. But if the purpose of the observation hive is not to have a strong colony, the loss of some bees is tolerable.

a. Before the introduction, the electric heater and the thermostat must be tested in order to prevent the overheating. The placement of at least two short thermometers, one within the inner case, the other in the outer case, is very useful.

b. All cracks must be inspected, and filled with propolis or wax, to prevent the invasion of phorids. The bees do this by themselves, but phorids often invade before the bees have time to establish order within the new hive.



c. At first the queen must be sought and, if discovered, she must be put in a small container, to be released later, after the majority of bees and nest elements were introduced.

d. The arrangement of nest elements within the hive depends on the research to be made. The element most important to the research plan, for instance, combs, must be put at the left corner, near to the observer (Fig. 13), in order to facilitate the observations (very important). This is the reason by which I recommended to restrict the width of the hive.

e. Do not break the pots, etc.

f. The use of a fine brush is verv good to handle delicate species such as *T. jaty, T. silvestri, P. schrottkyi,* etc. A kind of pipette jar (Fig. 14) is recommended to use with species which do not carry much resin on their corbiculae.

g. It is advisable to close the entrance at least one day after introduction. And also to cover the glass with paper, to make adaptation easier.

The colony management after the introduction depends on the kind of research work planned. If the marking of individuals is not performed, then change of contaminated glass, removal of garbage (which very easily becomes a cradle of phorids) and infested pots, feeding of the colony (according to the deficiencies noted), and changing of the location of nest elements (for the purposes of the research) are the main routines. It is recommended to inspect the hive at least once every day, to forecast any unwanted results.

Observation

The procedure of observation is variable, according to the different kinds of research. The following are some general cautions:

a. Observations are made under artificial ligth. The usual electric lamp is sufficient (bees get accustomed very rapidly, although there is difference among species). But if the observation continues during a relatively long time, say, more than 1 hour, the fluorescence lamp has two faults: 1) The reflexion on the glass cover, which makes it difficult to observe. Therefore, the lamp must be of a type which can be moved over the glass cover, not the fixed type; 2) It is very sensitive to the changes of voltage. It often stops or is difficult to work at a lower voltage. Use a simple type of transformer and also prepare the usual electric lamp, too, for such situation.

b. The observer must have an agreement with his colleagues or with any other persons in the laboratory for his tranquility. The best is to interdict the room during the observations. If this is impossible, the observer must not allow anyone to speak him, or to stand or to walk around him. As observations are made under the highest concentration of attention, the mere presence of other persons is often already inconvenient.

c. Training for rapid and accurate noting. It is recommended to write, in any observation, the date and hour of the beginning and end of observation. Often this becomes very important. The observer must be trained to take notes without seeing a note-book, and to see the stop-watch and the bees at one and the same time.

The use of abbreviations is imperative. For instance. "23 S c 7" means that bee 23 stored cell c during 7 seconds. The use of a tape recorder is recommended when accurate recording of many items of information is necessary.

For the observation of small species or of fine behaviour texture, the use of a binocular microscope or of a low magnification standlens is useful.

Marking of cells

Combs are marked by puting a transparent celluloid film over the glass cover of the inner case, and tracing the cells with glass pencil or some appropriate ink. Several cautions: 1) Each cell must always be seen vertically from above. 2) To add some reference points. The best is to mark the margins of the hive. It is also good to destroy 2-4 cells as reference points. If all cells must be recorded individually, the boundary of the construction work of each day is traced on the celluloid with line. In this way, the same number can be repeated each day. Of course in such case the protocol must be written as 15, Nov.-1, etc.

In the small species, such as *T. jaty,* the individual recording of a cell is very difficult. In this case, each cell is plotted on the celluloid film with paint and the records are retraced with enlargement. The alternative use of several colours (each day using a different one) is also recommended. (Two colours may be sufficicient in most cases).

Marking of individuals

The marking is made by using colour paints. To mark one or two individuals is not difficult and can be done in any way. But if several hundreds of individuals must be marked, some cautions are necessary each day. The best is to mark a number of individuals immediately after emergence. The following remarks concern this.

a. Preparation of a mature comb in which the emergence is taking place. Put it in a doubled-Petri dish or any comparable container. The interspace is filled with glycerin to prevent an attack of ants. In tropical regions where the stingless bees are flourishing, the destruction by ants often is a serious problem. This double dish may be put in a "warm box", or simply over the case of the observation hive (Fig. 7). The use of a super frame mentioned previously is convenient in such case. The top is covered with wire mesh. From this container, the emerged individuals are taken every day (preferably at the same hour) and the necessary number of them is marked. If there is an excess of newborns, these can be introduced in the observation hive without marking, or in other hives, *but do not leave them in the Petri-dish.* If they remain, the significance of the daily marking is lost.

b. Many species are so small, that they can be marked safely only after being immobilized. The best way to do this without an after-affect is by freezing. (Ether and CO^2 gas are not always re-

commended). The bees must be inspected from time to time and taken from the ice case, if necessary, in order to prevent over--freezing.

c. Marking systems of different types can be used. However, von Frisch's system is the most convenient, with a slight modification (Frisch, 1922). In marking each day, it is better to put day and individual marks separately, that is, to put day marks on thorax and individual marks on other parts. In *Apis*, the individual mark is put on the abdomen. But in Meliponids, the mark on the abdomen is not adequate because: a) the wings are often very long and dark, so that marks are difficult to see. b) Meliponids secrete wax-plates on the abdominal terga, and marking disturbs normal secretion. We succeeded in puting individual marks in the midleg and in the base of the wing, a double check. The system is the same as with day-marks. By this way, we can mark up to 10 individuals each day. More individuals can be marked by a sligth improvement of the system. However, 10 individuals each day are usually sufficient. If excess individuals can be taken each day, it is recommended to mark a further 10 individuals with the day-mark only. Hence, 10 with day and individual marks and 10 with day-marks only.

d. The marking paint is most important. To make easy and rapid markings, each paint must be put in a small glass tube about 3.5 cm long and 1 cm wide (not too long), all in a common stand. Blue and green paints are often very dark, so that it is better to mix them with white (for blue) and yellow (for green) in order to obtain a highter colour. The condition of the paint is important. If too hard, it will not stick, if too soft, it will spread on the body, even kill the bees. Therefore, every day the condition of the paint must be checked before marking. If too hard, it must be diluted with a thinner. (Always prepare a good thinner, which is more important than the paints themselves). To mark, a toothpick is adequate, but the condition of the tip is important. If too pointed, or too rounded, the marks cannot be done well.

e. The most rapid and best way to mark bees is as follows: A) Place them in an ice box and immobilize the bees on a cloth. B) Put a droplet of a rather soft paint on one side, and paint this colour on all bees. For instance, if the day mark is 29, all bees should receive red on thorax, left posterior, and blue, right anterior. At first put red marks on all bees, then blue on all. C) The individual marks are put thereafter, grasping wings legs with fine tweezers. Any blows or grasping, except on the wings and on the tips of legs must strictly be avoided. If the bees become too active, refrigerate them once more.

f. Marked individuals are temporarily transfered to another bottle. After all are marked, inspect the bottle. If there are individuals erroneously marked or the marks are indistinct, remove them and mark other individuals as substitutes.

g. After all are satisfactorily marked, transfer them to the observation hive. In some species, for instance, *N. postica*, marked individuals are attacked when introduced in the corner of hive, but not when on the comb. In such a case, open the glass cover, invert the bottle and give a strong tap to the bottom of the bottle containing the marked bees. When they fall on the comb, close the glass cover.

h. Of course, the observer must be trained to translate rapidly the colour marks to numbers.

i. An important advice. Often one thinks over the necessity of improving the adopted mark-system. But if one is already accustomed to one systen, for instance, that of von Frisch, do not change it, even if eventually it proves to be inconvenient in some aspects. When changed without caution, the new and old systems confuse the memory and then accurate recording becomes impossible. On the other hand, it is recommended to use another colour, for instance pink, for any other special purpose. If it is necessary to distinguish experimental bees from control ones, each already having day and individual marks, put pink marks only on the experimental bees. This facilitates a rapid distinction between two lots.

The marking paint must be of a quick drying type.

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Abstract

A new type of observation hive is described. It consists chiefly of: a) a low inner case, b) an outer case, which completely encloses the inner one; c) a corridor connecting the inner case with the outside.

In the space between both cases, an electric heater regulated by a thermostat mantains the desired temperature.

Details of the construction of the observation hive are given, as well as instructions on how to use it and the best way to mark and observe the gees and their cells.

Resumo

Um novo tipo de colméia de observação foi descrito. Consiste principalmente em: a) uma caixa interna; b) uma caixa externa, que cobre completamente a interior; c) um corredor, ligando a caixa interior com o exterior.

No espaço entre ambas as caixas, uma resistência elétrica, regulada por um termostato, mantém a temperatura desejada. Ambas as caixas são cobertas por chapas de vidro.

Detalhes de construção da colméia de observação foram apresentados, assim como instruções sôbre a maneira de usá-la e o melhor modo de marcar e observar as abelhas e suas células.

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