230626 230623 230622 230620 230614

LA MARIOLA

TINY WORKERS AND A HUGE QUEEN

The hidden behaviour of the colony-forming bee *Tetragonisca angustula*
a film by Lucas L.M. de Bruijn and Marinus J. Sommeijer

This film is about a tiny bee with spectacular behaviour, living in a nest that is more complex than that of the honeybee.

INTRODUCTION
Tetragonisca angustula is a small species of the large group of fascinating stingless bees. Stingless bees (Meliponinae, “meliponids”) occur exclusively in the tropics. Where they are mostly, because of being numerous, important pollinators.
Their greatest diversity is in tropical Central- and South America, continents where the honeybees, the only other group of highly social bees, originally did not occur.

SOCIALITY
All of the hundreds of meliponid species have highly complex social behaviour. Knowledge about this behaviour has remained unknown for a relative long time. This is probably due to the fact that, in addition to their tropical occurrence, the nests are always built in well protecting cavities. The Japanese biologist Sakagami was the first who systematically investigated the nest biology. He used details of behaviour, e.g. types of comb-building and striking interactions between queen and workers, to improve the classification within this group.

Taxonomically, meliponids are related to honeybees and bumblebees. All these transport flower-collected pollen in characteristic pollen baskets on the hindlegs. All other species of bees carry pollen to their nest in other ways.

In this film we will focus particularly on the behaviour inside the nest.

DISTRIBUTION
This tiny stingless bee occurs widespread in the tropics of Central- and South America. and can be common in areas where also other species of stingless bees occur. For example it is one of the 64 meliponid species in the country of Costa Rica where it is called Mariola or Mariquita.

Mariola nests are built within diverse substrates. We can find nests in hollow trees and in natural cavities in rocks and hill-sides. It is remarkable that this species also is adapting very well to human environments: we have seen nests in hollow lightpoles, plastic containers and in a diversity of other cavities, that all serve well as a nesting base.

NEST EXIT TUBE (dit subtiteltje wordt niet gesproken)
STINGLESS BEES HAVE A RUDIMENTARY NON FUNCTIONAL STING, BUT CERTAINLY WELL DEVELOPED MANDIBLES.
As most stingless bees Mariola has a narrow entrance tube that leads to the inside of the nest. Such a restricting opening protects against robbers interested in brood and stored food.

The entrance tube itself is of a delicate construction, resembling loose finely woven lace. The opening of the tube can be closed when predation pressure is high, and under certain conditions at night.

PARTICULAR DEFENCE

Most species of stingless bees have guard bees in their species-specific entrance tunnel. This is also the case in Mariola.

But, very different from nearly all other stingless bees is that they in addition have another unique nest defence force. This consists of a group of workers guarding the nest by fly-hovering in a loose group just in front of the entrance. Any unwanted visitor is attacked by these helicoptering guards. Although being small, these bees are very effective defenders. Together they can bite in the wings of an attacker and keep an even much larger enemy immobilized at the floor for some time. Recent research revealed that these specialized Mariola guards are a little larger than workers that carry out other nest tasks.

OPEN NEST
As is visible when we open a nest cavity, storage pots are separate from the brood nest. The brood itself is well protected by a multi-layered system of waxy sheets. This coverage is called the involucrum.

POTS
Pollen and honey are stored in separate pots. These pots are much larger than the brood cells. In general they are situated more in the periphery of the nest.

The construction of storage pots is dynamic. Pots are directly removed when empty and the building material can be used for new constructions. Also, when new foodplants start blooming new pots can rapidly be built.

Here we see two empty, newly built honey pots together with darker coloured pots that are filled with honey and sealed.

We can see here workers entering a honey pot either to deposit or to extract honey.

PROPOLIS
In addition to nectar and pollen, foragers can also collect plant resin from suitable plants and trees. Such foragers use their pollen baskets for transporting this. Collected plant resin is stored at specific storage dumps, where we call it propolis.

All stingless bees, honeybees and bumblebees use bees-wax as a building material. Typical for the meliponids is that their wax producing glands are positioned on the dorsum of the abdomen. Stingless beeswax is always mixed with propolis to be used as a building material. This mixture is called cerumen. The degree to which wax and propolis are mixed depends on what nest structure is built.

Here we see workers building on the involucrum.

Pure propolis is used as a coating for the nest cavity and to fill up cracks. Some other stingless bee species make extensive use of mud, to shape their nest entrance, or to partition the space for their nest in a hollow tree.

WASTE DUMPS
The occurrence of waste dumps in the nest is another typical feature of stingless bees. Such special areas that serve for the keeping of all sorts of debris do not occur in honeybee hives. Remains of pupal material from the inside of brood cells, becoming available when hatched cells are removed, and body parts of dead bees are deposited on this dump.

Most remarkable is that the waste dump is also frequently used as a latrine. We have seen that in Mariola, worker bees visit the waste dump regularly for defecation.

We can see that deposited fecal excrements remain first as wet filaments over the dump surface.

The sides of the waste dump are neatly kept by workers, who in a shoveling manner collect small particles and place these on the dump.

Other workers take with their mandibles large lumps of dump material and extensively work on such solid chunks.

Subsequently they carry these to the outside, where they are dropped in flight.
The complete function of the waste dump is still not fully understood. We hypothesize that the waste dump also may have a microbiological function.

BROOD NEST
Hidden within the protecting involucrum is the real brood nest. Mariola broodcells are arranged in combs. These combs are, as in most other stingless bees, horizontal single layers of cells. Very typically, each brood cell is used only once, so for each new egg a new cell is built.

BUILDING OF COMBS
The first cells of a new comb are built on top of the centre of the previous comb, leaving some space between the new and the earlier comb. More cells are added sideways so that combs grow gradually towards the perifery. In Mariola cells under construction occur always in different stages.

Cerumen from cells from which bees have emerged is re-used. In this way old combs disappear and new combs can be built there. This results in the cyclic comb construction which is typical for all comb-building meliponids. Some other meliponid species build brood cells in cluster arrangements, which can either be loose or more compact.

CALLOWS
In older parts of the mature combs we can see that new bees are ready to emerge.
They can remain in the cell for a time with the heads visible, when the cell cover has already been removed by other workers.

Recently born workers have a whitish colour and are called callows. It takes a few days for their skin to harden and to obtain the darker coloration of adult bees.
They soon enroll in nest activities, such as cleaning the comb surface. They also interact with the queen in the royal court and in food exchanges with other workers.

P.O.P. PHASES
The behaviour of cell building, provisioning and egg laying can be divided into several phases. In the first phase new cells are being built. The bottom of a new cell is started as a small platform at the edge of the comb.

CELL BUILDING
Starting from the bottom, the cell is gradually built up during a period that may last for a few hours. Here, the cell in front is approximately 1/3 of its final size.

And here, the cell in front is ¾,

Eventually the cell is provided with a collar that protrudes above the comb surface. On the cell on the right we can see the beginning of this collar.

With this cell the same situation. The worker is adding building material in order to finish the rim. On the cell in the back we clearly see a finished rim. Now, the cell has reached the stage to receive the larval food and an egg.
In the end we see here four completed brood cells provided with a collar.

QUEEN CELLS
The queens of Mariola are reared in special cells, that are much larger than the regular brood cells. In well populated colonies these cells are generally constructed at the edge of a full grown comb.
In some colonies it is possible to find four or even more queen cells at the same time. They are always located at some distance from each other, mostly at different combs. Because of the larger cell size containing more larval food, queens of Mariola emerge much larger than workers.

QUEEN PRODUCTION AND DIFFERENCE WITH MELIPONA
This occurrence of typical queen cells is remarkably different from queen production in stingless bees of the genus *Melipona.* in this group of stingless bees workers and queens are produced in the same standard type of brood cell. And consequently, *Melipona* queens are not larger than workers at the moment of emergence from the cell. At that moment hey are even a bit smaller.
After having mated, the eggs in the ovaries start to develop and as a result only the size of the abdomen increases drastically. Eventually, developed queens of *Melipona* are just as queens of Mariola very much larger than the workers.

QUEEN ACTIVITY DURING CELL BUILDING
During cell building the queen usually rests on the involucrum or on the older combs. From time to time she may be surrounded by workers that form a royal court. During these periods of relative rest, the queen spends a lot of time autogrooming. In the presence of workers she rhythmically spreads her vibrating wings. Such a resting period usually ends when workers vigorously start to interact with her. The queen then starts walking again and so she may visit the comb where cells are being built.

TROPHALLAXIS
Oral food transfers between workers are a common behaviour both in honeybees and stingless bees. This is called “trophallaxis”. In Mariola, such food transfers are initiated by the begging act of one of the participants. This is similar in bees of the genus *Melipona*. Differently, in the honeybee trophallactic transfers can also be started by workers who spontaneously offer food to another worker or to the queen.

As we have also seen in *Melipona* species, the incidence of trophallactic interactions increases significantly reaching the completion of a cell building period. We have found that this is a means by which cell provisioners collect enough food to be able to discharge food into the cell. This larval food is a mixture of pollen, honey and glandular secretions.

EXCITEMENT PHASES
Phase 2 temporal excitement

At the end of the cell construction phase, there are several cells present with a collar that protrudes from the comb. Periodically the workers gather around one or more of these cells and behave frantically. They alternatingly dive into a ready cell, while some workers may freeze above it. Notably, workers that engage in this excitement have extended abdomens,

probably filled with larval food. Usually, in Mariola, such periods of excitement are followed by periods of much more quite behavior.

Phase 3 Final excitement
In the final excitement phase the workers are again clearly hyperactive. And now the queen arrives on the comb. She moves very fast, making many turns and interacting intensively with workers. The queen-worker interactions now appear remarkably aggressive.

This means that workers dart or jump towards the queen and she in turn may walk over workers as if they were not there. Excited workers perform quick body insertions in the ready cell(s).

PROVISIONING
Phase 4 provisioning and oviposition
In this phase the cells are provisioned with larval food. The cell marked with an arrow is the first cell to be provisioned.

The cell is filled with larval food in seconds through 5 – 6 discharges by different workers. These discharges are easily to be seen by the contractions of the abdomen. When the cell is filled with larval food up to 2/3 of its volume, the queen arrives for her final visit.

The actual egg laying by the queen is always preceded by her uptake of a little of the larval food. After the queen's laying the cell will be closed.

TYPICAL MASS PROVISIONING
The fact that the whole amount of larval food for complete larval development is brought into the cell in a very short moment is characteristic for all meliponids.

MORE CELLS AT SAME TIME
Typically for Mariola is that the queen does not have to remain by the cell during this provisioning, as is the case in *Melipona* species. Therefore it is possible that two different cells are being provisioned more or less simultaneously.

In this footage the first discharges take place in the cell on top, but the cell on the left will also be filled.

CELL CLOSURE
The duration of the closure of the cell can be short, since the cells were already provided of a collar. This collar can be immediately folded inwards when the queen withdraws after laying. The first part of the closure is done in a rotating movement by a single worker who persistently remains at this cell until it is nearly closed. From this moment other workers may join in the completion of cell closure.

QUEEN FEEDS ALSO ON EGGS LAID BY WORKERS
As mentioned, usually the queen of Mariola does not stay consistingly near the cell during the whole provisioning process. Cells that have obtained the appropriate amount of larval food may be surrounded by a group of workers. And now, when the queen keeps on cruising excitingly on the combs, a worker may lay an egg.
This worker egg is quickly placed at the inner rim of the cell where it is not in touch with the larval food. Such a worker egg is called "trophic eggs" since the queen eats this worker egg as soon as she returns to this cell. Directly after that, she lays her own egg on the surface of the larval food.

In all cases the queen will eat something prior to her own oviposition. Since laying workers only lay eggs in a few cells, the food uptake by the queen consists mostly of larval food that the workers have deposited into the cell.

MELIPONICULTURE
Since stingless bees store pollen and honey in long-living nests, they have been used, and even been domesticated, by various human cultures.
Here we see an indian woman of the indigenous Bribri tribe in Southeast Costa Rica, inspecting her small colony of Mariola, kept in a calabash in her kitchen.

Before the European honeybees were introduced, honey production in the Americas was only possible with stingless bees. Particularly in the old Maya region, there was a developed beekeeping practice with meliponids. One colony of the large sized *Melipona beecheii* could produce here, and in other Central American areas up to several litres of honey. Such traditional “Meliponiculture” still exists, but it is now mostly a rural activity and unfortunately it is in danger of extinction due to several ecological limitations.

BEEKEEPING WITH MARIOLA
Other species of stingless bees are still being kept in Tropical America, and also in other continents. Our small *Tetragonisca angustula* is also kept in a wide area of its distribution. And this why the Mariola from Costa Rica has in neighbouring countries different names like Mariquita, Doncella, Chumelo, Virginitas, Angelitas, Jatai etc.

The honey production of Mariola colonies kept in boxes, hollow bamboo tubes or even empty softdrink bottles, is just about a teacup full of honey. However, this fine tasting honey is highly valued everywhere! In some places a Mariola colony is considered as a living medicine box. The honey is used for various health problems. Fore example, it is commonly recommended as a treatment for eye diseases.
.

CONCLUSION
In this film we have seen that this tiny bee is interesting because of its elaborate and remarkable behaviour. Within the large group of about 20,000 species of bees in the world, such level of sociality occurs exclusively in the stingless bees (Meliponinae) and in the honeybees (Apinae). Only there, we can see complex colonies with workers and queens with specific behaviour as well as with different morphology. *Tetragonisca angustula* is a good representative of the stingless bee group. To appreciate the beauty of this kind of sociality further, we can, as a conclusion, compare our small Mariola with the much better known honeybee.

SOME MAJOR DIFFERENCES BETWEEN TETRAGONISCA ANGUSTULA AND APIS MELLIFERA:

1. Size of the queen
   Mariola queens are already huge at hatching

2. Nest entrance
   As in all stingless bees Mariola has a narrow nest opening

3. Brood cell arrangement
   Combs of stingless bees are singly layered

4. Food for the Queen

Mariola queens eat larval food and worker eggs

5. W-Q interactions

Worker-queen interactions in Mariola are seemingly aggressive

6. Larval feeding

In stingless bees, all larval food is brought into the cell just before egg laying

7. Q cell

Huge queen cells are regularly present in Mariola, not only when swarming is going on

8. Queen laying

Mariola queens lay eggs only in cells that are filled with larval food

9. Eggs layed by workers

Mariola workers deposit trophic eggs at the inner rim of a provisioned brood cell

THE END

This film would not have been possible without the help and cooperation of numerous researchers, students, cooperators and friendly beekeepers all over the world.

Particular thanks to:

Henry Arce, Costa Rica

Johan van Veen, Costa Rica

Kees Zwakhals

Harriet de Jong

Ingrid Aquilar, Costa Rica

Luis Sanchez, Costa Rica

Dick Koedam, Brazil

Louwerens-Jan Nederlof

Hekke Groenendijk

Chris Degenaar

Bartjan Bakker

Frans Meeuwsen

Peter Francke

Bert Agterhuis

Frouke Hofstede

Marc Coemans

Pascalle Bonnier

Universiteit Utrecht

Universidad Nacional de Costa Rica

Uyttenboogaart-Eliasen Stichting

Diergaarde Blijdorp Rotterdam

MUSIC: Remmel Begeer

==========