ECOLOGY, BEHAVIOR AND BIONOMICS

Nesting Behavior of *Centris* (*Hemisiella*) *vittata* Lepeletier (Hymenoptera: Apidae) in an Area of the Cerrado in the Northeast of the State of Maranhão, Brazil

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ABSTRACT - The nesting behavior of *Centris* (*Hemisiella*) *vittata* Lepeletier was studied in the Urbano Santos Cerrado in the northeast of Maranhão State, Brazil. To date, this species has only been studied in trap-nests. The nesting behavior of this species in a natural condition is recorded for the first time. Nesting occurred in preexisting holes in dead trunks of aroeira, *Astronium myracrodruon* (Anacardiaceae), at the end of the rainy season. The cells were constructed with a mixture of sand and oil. After finishing the nests, females used only oil to line the entrance wall. Two females collected sand at the same time to build their nests, and another one was seen collecting resources at *Byrsonima* sp. (Malpighiaceae). The pollen load of one bee was analyzed and was found to contain mainly *Hymenaea courbaril* (Leg-Caesalpiniaceae). Development from egg to adult took about 60 days. Protandry was observed, being males smaller than females. The nests were parasitized by Coleoptera and Diptera. However, the females only showed aggressive behavior against females of the same species or genus.

KEY WORDS: Solitary bee, nest, preexisting hole, bionomy

Centridini bees are solitary, medium to large sized species which use different kinds of nesting substrata. The tribe includes species that excavate simple or compound nests in flat ground or in vertical banks (Vinson & Frankin 1988, Batra & Schuster 1977, Rozen & Buchmann 1990, Aguiar & Gaglianone 2003). Other species build their nests in holes of wood, such as tree trunks, inside termitaria or use preexisting cavities, such as cells abandoned by wasps or other bees (Coville *et al* 1983, Silva *et al* 2001, Aguiar *et al* 2006, Ramos *et al* 2007a,b), old beetle boring (Michener & Lange 1958) and trap-nests (Garófalo *et al* 1989, Pereira *et al* 1999, Aguiar & Garófalo 2004, Mendes & Rêgo 2007).

These bees are considered effective pollinators for a variety of plant species, particularly "oil plants" such as Malpighiaceae, Krameriaceae and Scrophulariaceae (Vogel 1974, Neff & Simpson 1981, Buchmann 1987, Rêgo & Albuquerque 1989, 2006).

Due to the essential ecological role played by these bees in a wide range of floral communities, studies of the biology of these species are important for conservational efforts to be developed for solitary bees and the plant species they pollinate. The aim of this study was to provide data on the nesting behavior of *Centris vittata* Lepeletier, a species distributed in several Brazilian ecosystems.

Material and Methods

The study was carried out in an area predominated by Cerrado fragments in the municipality of Urbano Santos (3º12'28'' S, 43º24'12'' W), northeast of Maranhão State, in Santo Amaro farm, owned by Comercial & Agrícola Paineiras, about 500 km from São Luís. The main soil types are sandy (well drained and with low natural fertility), quartz sand and red-yellow latosol. The dominant vegetation is that typically found in the Cerrado, although seasonal semideciduous forests and patches of mixed vegetation may also be found (Brasil 1984). The area, where the study was carried out, is in a clearing in a mesophytic forest, approximately 300 m from one of the main rivers in the municipality (the Mocambo River), with a riparian forest bordering it. An eucalyptus plantation is located nearby. The climate of the region is tropical megathermal (corresponding to Aw' according to the Köppen classification), annual average rainfall of approximately 1,800 mm. Most of the rainfall is concentrated in the period between December and May, with the months of July to October being very dry. The annual average temperature is between 26°C and 27°C (Brasil 1984).

The nests were located in preexisting holes in dead trunks of *Astronium* sp. (Anacardiaceae), commonly known as

"Aroeira". When females were observed to be active, the nests were marked and numbered. The remaining holes were inspected with the aid of a torch to identify if other nests had been built. Fifteen nests of *Centris vittata* were marked; five of them had already been completed, and females were observed to be active in the remainder. A few days after the nests had been finished, the trunks were cut into transverse sections with a chainsaw and taken to the laboratory. Tubes were placed to cover the holes until the adults emerged. The nest architecture was studied and the individuals measured. The activity of females was recorded by direct observation from December/2003 to February/2005.

Pollen grains found in the scopae of one of the females were subjected to acetolysis (Erdtman 1969) to identify the plant species in which *C. vittata* foraged. The quantitative analyses of the pollen types was accomplished by means of consecutive counting of 100 grains (Ribeiro *et al* 2008) to determine the percentages and occurrences of each pollen classes according to Louveaux *et al* (1978)

To determine if there was a significant difference in head and thorax width between males and females the Mann-Whitney test was applied. The chi-square test was applied to check if there was a significant difference between the expected proportions of males and females and with those obtained in the field; and the mean and standard deviation were used to compare the main measurements of the nests and the individuals (width of the head and thorax) as well as the time spent outside and inside the nest.

Results

Nesting frequency, substrate used and nest architecture. The trunks were exposed to sunlight for most of the day, and females were observed building nests at the end of the rainy period, during the months of May and June (Fig 1a).

The holes had a long cylindrical shape and measured from 7.5 to 9.9 mm ($x = 8.7 \pm 0.7$ mm; n = 14) at their narrowest point and from 9.4 to 19.7 mm ($x = 13.1 \pm 2.4$ mm; n = 14) at their widest point. They were located at heights that varied from 0.53 to 3.08 m ($x = 1.87 \pm 0.87$ m; n = 14), and the diameter of the trunks ranged from 48 to 57.5 cm ($x = 53.1 \pm 9.5$ cm; n = 12).

The nests had horizontal cavities with galleries branching vertically upwards or downwards or at an angle to the grain. The cells were laid out in a line one after the other and followed the vertical, horizontal or oblique path taken by the galleries.

The trunks were cut transversely (Fig 1b) until cavities that contained cells were exposed. A large number of cells were found (Figs 1c, d), some of which contained pieces of cocoon woven by larvae (Fig 1e); however, it was not possible to measure the cells, nor was it possible to determine exactly how many cells a nest could have. The material used to build the cells was a mixture of sand and oil, which solidified and became firm after several days. The cells appeared to be molded within this damp soil, and a space near to the nest entrance was filled with sand, without any other substance added to it. When the last cell was completed, females built a wall similar to an incomplete cell (plug) at the entrance of the nest and cover it with oil. This wall would become stiff within several days.

Behavior of the females. The behavior of four females was monitored while they were building their nests; a total of six nests were built. Before start building the nests, females flew around the trunks and went in and out of the cavities, some of which contained nests that were being built or that had been finished, as well as old nests from which the adults had already emerged. Once a hole had been chosen, the female stayed inside it, and a scraping sound could be heard, possibly caused by the female cleaning the cavity, as small particles were expelled from inside the hole.

Two females were seen in a small piece of open countryside 31 m away from the nesting area collecting sand together to build their nests. They were scraping the sand with their middle legs, but also at times with their anterior ones, and transferring it to their scopae with the aid of the middle legs and abdomen. When they returned to the nest with some material, they would first put their heads into the nest, would come out and go in again immediately afterwards, but this time with their abdomen first to unload the collected material. The trips to collect food for the larvae lasted from 1 to 31 min ($x = 12.5 \pm 8.2$ min; n = 21) and the length of time females remained inside the nests varied from several seconds to 24 min ($x = 3.2 \pm 5$ min; n = 22).

To close the entrance of the nest, females collected sand to mold a plug, and rubbed the forelegs against each other after collecting oil to produce a whitish foam that would stick to the plug (Fig 1f).

These activities finished around 5:30 pm, and females would spent the night inside their own nests (n = 1). Sometimes females flew around the trunks, going in and out of cavities until they selected one of them near to their nests, where they would spent the night (n = 3), always with their abdomen pointing to the entrance of the hole. Once the nest was completed, females (n = 2) selected another cavity in the same trunk or in nearby trunks where they cleaned their whole body before starting to build a new nest.

Centris vittata were seen twice exhibiting aggressive behavior while defending their nests: once from another smaller *Centris* species (possibly *Centris tarsata* Smith), and in another occasion to defend her nest against a *C. vittata* female intruder.

Sex ratio, size of adults and parasitism. The bees emerged from eight out of the 15 marked nests, with two bees emerging from each of three nests, three bees emerging from one nest and only one bee emerging from each of the remaining nests, with a sex ratio of 1.6:1 ($x^2 = 0.69$; gl = 1; P = 0.5791). In nests where both males and females emerged (n = 2), males always emerged first, indicating protandry. There were also nests where only males or females emerged (n = 4 and n = 2, respectively). In three nests (N4, N5 and N6) that were completed on June 12th, 2005, the first cells in which adult emergence was observed (N4 and N6 - August 5th; N5 - August 7th), produced one male each. A female emerged from N6 on August 12th, 2005. The adults had a development period of approximately 60 days.

The width of the head of males varied from 4.9 mm to 5.2 mm ($x = 5.1 \pm 0.1$ mm; n = 7) and the width of the thorax

Fig 1 a) Female *Centris vittata* nesting in a dead aroeira trunk in the Cerrado at Urbano Santos, MA, Brazil; b) Transverse section of aroeira trunk with *C. vittata* cells; c and d) Detail of *C. vittata* cells; e) Cocoon and cell of *C. vittata*; f) Plug covered with oil and the foam produced by the *C. vittata* female after the nest has been completed.

from 5.4 mm to 5.8 mm (x = 5.6 ± 0.1 mm; n = 7); while females were from 5.7 mm to 6.2 mm (5.9 ± 0.2 mm; n = 4) and 5.8 mm to 6.5 mm (x = 6.1 ± 6.3; n = 4), respectively. Although only a small number of individuals emerged, there was a significant difference in the size between males and females, with the males always being smaller (head: Z = 2.6458, P = 0.0082; thorax: Z = 2.5513, P = 0.0107).

Four nests were parasitized: three by Meloidae (Coleoptera) and one by Bombyliidae (Diptera). When nesting was taking place, one female of the Diptera was observed flying over the trunks and placing a large number of eggs around the hole entrances.

Pollen analysis. A female was found dead in one of the cavities with pollen on its scopae. Analysis of the pollen revealed that it came mainly from *Hymenaea* (Fabaceae). Although some grains from *Tetrapteris* (Malpighiaceae) and *Caesalpinia* (Leg-Caesalpiniaceae) were also found, the amount was considered insignificant. Females were also seen collecting material in *Byrsonima* (Malpighiaceae) flowers near the nesting area.

Discussion

There are few studies on *Centris vittata* (Frankie *et al*

1988, 1993, Thiele 2005), and none provide any details on the nest architecture or the materials used in building the nests. The only study available on the nesting biology of this species used trap-nests in Cajuru (São Paulo State, Brazil) and includes a detailed description of the nest architecture and building materials (Pereira *et al* 1999). However, the present study is the first to record nests of this species in natural conditions.

Centris species nesting in preexisting cavities in wood were shown to prefer shaded areas, as high temperatures can limit their larval development. *Centris vittata* was the one with the most limited range of habitats and preferred humid environments, such as riparian and mesophytic forests, as they would provide the most suitable temperature for their development. In addition, the species did not nest during what are considered the hottest months of the year (Frankie *et al* 1988).

In the municipality of Cajuru at São Paulo State, the nesting activity of *C. vittata* took place during the hot, humid season (Pereira *et al* 1999) and in Costa Rica during the driest season (Frankie *et al* 1983). In the present study, nesting was observed to take place at the end of the rainy season. Pereira *et al* (1999) pointed out that differences in the nesting season reflect the different blooming periods. According to Frankie *et al* (1998), variations in nesting frequency may be a normal characteristic of species that nest in preexisting cavities and may be determined by factors such as the variation in the natural mortality, patterns of annual climatic extremes or disturbances in marginal habitats. They also found that some *Centris* species have a diapause in the last larval stage in July and November; however, this was not observed by either Pereira *et al* (1999) or the present authors. Pereira *et al* (1999) suggested that males and females that come out lastly, hide in places that were unknown to the researchers for the whole period until nesting activity started again

Centris vittata nests were found in cavities with larger diameter holes (1.1 cm) (Frankie *et al* 1988, 1993, Pereira *et al* 1999) confirming that females nesting in preexisting cavities select diameters according to their body size, as larger orifices would require them to spend more energy preparing the cell and filling the empty spaces (Garófalo *et al* 1989, Jesus & Garófalo 2000, Aguiar & Martins 2002). In addition, the arrangement of the cells in the nests depends on the spatial limitations imposed by the cavity (Michener & Lange 1958, Jesus & Garófalo 2000). Pereira *et al* (1999) found that the number of cells in a complete nest varied from one to eight. Vinson *et al* (1996) however, observed a much lower number (1-2 cells). In the nests studied in the Urbano Santos Cerrado, the maximum number of individuals that emerged from a single nest was three. However, it should be taken into account that one of the females built more than one nest. As the architecture, the number of cells also depends on the space available in the cavity (Pereira *et al* 1999).

The protandry observed in *C. vittata* is a characteristic of many solitary bees and wasps (Rozen & Buchmann 1990, Camillo *et al* 1993, Morato *et al* 1999, Pereira *et al* 1999).

It is well known that *Centris* species (including those in the *Hemisiella* subgenus that nest in preexisting cavities), collect sand to fill their nests (Michener & Lange 1958, Coville *et al* 1983, Buchmann 1987, Morato *et al* 1999). Michener & Lange (1958) suggested a gregarious behavior for *C. vittata* reporting that at least 75 females of these species were recorded collecting loosen sand in bare soil over a six-month period in the same place in the Barro Colorado Island, Panama. According to them, four females were seen working together in a 20 cm^2 area, although there was an area of more than five square meters that could be used. In addition, ten bees were seen at the same time, arriving, leaving and collecting sand. They visited various sections of a large area, and after one female had decided to land, the others landed nearby (Michener & Lange 1958). Although the number of females collecting together was not that large in our study, this behavior was observed in two females who were collecting sand together.

The function of the plug covered in oil was previously discussed by Pereira *et al* (1999), who suggested that it provided protection against natural enemies. In the area under study, a female of Diptera was observed laying eggs around all the entrances, particularly those in which *C. vittata* females were active. No aggressive behavior from *C. vittata* females against female Diptera was observed. Females of *C. vittata* would only remain at the entrance to their nests while watching the Diptera female moving around the nest entrance. Some studies show that other parasites, such as some species of Coleoptera, are relatively rare (Carballo 2002), although reports show that these can attack various species that nest in preexisting cavities (Morato *et al* 1999, Pereira *et al* 1999, Aguiar & Martins 2002).

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