



## RESEARCH ARTICLE - BEES

## Bionomy and Nesting Behavior of the Bee *Epanthidium tigrinum* (Schrottky, 1905) (Hymenoptera: Megachilidae) in Trap-Nests

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### Abstract

About 85% of pollinating bees are solitary, but few are used for agricultural pollination. The Anthidiini tribe looks very promising in Brazil, in the light of ecosystem utility, but it is still a scientifically poorly researched species. In order to fill this gap, studies on the bionomics of these species may collaborate on future efforts for the use of *Epanthidium tigrinum* as a pollinator. The presented research results aims to describe the bionomy and the biological cycle of the bee *E. tigrinum* (Schrottky, 1905) in pre-existing cavities. Conventional trap-nests inserted in wooden blocks were placed in covered spaces and daily observations of spontaneous nesting as well as individually tagged bees and nests incubated in B.O.D. were carried out during 11 months in Fortaleza-CE. The results showed that the competition with other species of bees for the pre-existing cavities and the lack of food resources or nest building materials are limiting factors for the establishment of *E. tigrinum* populations. However, once these problems were remediated, the bees showed multivoltine behavior, nesting throughout the experimental period. Data from 16 nests studied showed that bee foraging began on average at 7:50 a.m., foragers collected pollen in the morning and resin in the afternoon, took on average 15 days to complete a nest and there was a negative correlation with the temperature for the number of nests and for the number of cells constructed. The offspring emergency mean time was 43 days, the sexual ratio was 1.2: 1 (n = 66 emerged bees) and the mortality rate was 18.5%. The number of nests constructed per female ranged from 1 to 4 with an average size of 7.97 mm. It is concluded that *E. tigrinum* shows potential to rational rearing in trap nests aiming crop pollination.

### Introduction

There is an estimation of 25,000 to 30,000 bee species in the world, of which 85% show solitary living habit (Roubik, 1989; Griswold et al., 1995). Although solitary bees are very well represented in terms of numbers, studies regarding their nesting habits and biological cycle are scarce and restricted to few species. However, many of those bees have potential for agriculture as pollinators, as in the case of *Nomia melanderi*, *Peponapis pruinosa* and some species of the genera *Xylocopa*

and *Centris*, efficient pollinators in crops such as passion fruit (*Passiflora* spp.) (Freitas & Oliveira-Filho, 2003; Silva et al., 2007), West Indian cherry (*Malpighia marginata* DC) (Vilhena & Augusto, 2007; Magalhães & Freitas, 2013), cashew (*Anacardium occidentale* L.) (Freitas & Paxton, 1996) and cucurbits (Shuler et al., 2005; Julier & Roulston, 2009).

In the Megachilidae family some species like *Osmia* spp and *Megachile rotundata*, are already used in the pollination of crops such as pear (*Pyrus communis* L.), apple (*Malus domestica* Borkh), almonds (*Prunus amygdalus* Dulcis), cherry



(*Prunus avium* L.), apricot (*Prunus armeniaca* L.), peach (*Prunus persica* (L.) Batsch), and alfalfa (*Medicago sativa* L.) in the European Union, Canada, United States and Japan (Matsumoto et al., 2009; Pitts-Singer & Cane, 2011; Sedivy & Dorn, 2013; Sheffield, 2014).

The Megachilidae family is a very diverse group of bees. Occurring worldwide, such bees can be easily differentiated by their distinct morphology, presenting a ventral scopa as the family's main characteristic, and therefore the absence of scopa on the hind legs (Silveira et al., 2002; Michener, 2007). Megachilidae is comprised of two subfamilies: the Fideliinae and the Megachilinae. Megachilinae is composed by the tribes Osmiini, Dyoxini, Lithurgini, Anthidiini, and Megachilini with over 4,000 species described, mostly presenting solitary behavior (Nates-Parra & Gonzalez, 2000; Michener, 2007).

In Brazil, the Megachilidae family is represented by the subfamily Megachilinae, moreover, from their tribes, only Lithurgini, Anthidiini, and Megachilini hold described species in the country (Silveira et al., 2002; Michener, 2007). Among those, Anthidiini assembles a diversified group, which occurs in all continents, with a wide geographical distribution. Despite that, locally, they often show little expressiveness of diversity and abundance (Silveira et al., 2002; Michener, 2007).

The Anthidiini build their nests in pre-existing cavities in wood, soil, or exposed on rocks, branches or leaves (Morato & Fields, 2000; Michener, 2007). Although there are still few biological and ecological data on Anthidiini in Brazil (Camarotti-de-Lima & Martins, 2005), it is known that building material used by these bees in their nests are very diversified, and varies according to the genera and species. Some bees gather plant fibers in order to be used for coating their cells; some, utilize resin, often cobbled with other materials, such as pebbles, leaf pieces or clay. Despite the little knowledge about the nesting habits of those bees, and moreover, the information on that regard are exclusively based on groups that have built nests in trap-nests (Alves-dos-Santos, 2004; Alves-dos-Santos et al., 2004; Camarotti-de-Lima & Martins, 2005), yet a few biological and ecological data are related to the Anthidiini in Brazil (Camarotti-de-Lima & Martins, 2005).

An interesting group of Anthidiini bees for further studies is the one whose species use resins in building their nests. Among them, bees of the genus *Epanthidium*, with 23 neotropical species (Urban & Moure, 2012). The resin, used by those bees is secreted from plants, collected still in a viscous state, and moreover, carried in the female's mandibles to the nest. Despite the little knowledge about their chemical characteristics, it is known that these resins possess fungicide and bactericide properties (Alves-dos-Santos et al., 2004). However, there is little information about the bionomy and nesting behavior of these species and how to handle them in nurseries. Therefore, aiming to contribute to the knowledge and potential breeding and management of Anthidiini, the present study aims to describe the bionomy and nesting

behavior of the *Epanthidium tigrinum* bee (Schrottky, 1905) (Hymenoptera -Megachilidae) in trap-nests.

## Material and Methods

### Experimental area

The experiment was conducted at the Bee Unit of the Department of Animal Science, at the Federal University of Ceará (UFC), in the city of Fortaleza (Latitude: 3°44'33.70"S; Longitude: 38°34'45.46"W), from December 2014 to October 2015. The local climate is characterized as Aw' by the Köppen classification (1948), sub-humid hot tropical, rainy season from January through May, average rainfall of 1338.0 mm and average temperatures that variety of 26°C to 28°C (IPECE, 2015).

The area has a small portion of Tableland and Forest surrounding the place where some species of native and exotic fruit trees are found, such as coconut (*Cocos nucifera* L.), mango (*Mangifera indica* L.), cashew (*Anacardium occidentale* L.), West Indian cherry (*Malpighia marginata* DC) and nance (*Byrsonima crassifolia* L.).

### Trap-nests and sampling

The trap-nests were made of black cardboard paper with 12 cm of length. Initially, only nests with 5.5 mm diameter were used. However, due to the occurrence of high competition with other species of bees and wasps, nests with smaller diameters, 4.5 mm, were used. These nests were distributed in six wooden blocks, totaling 354 cavities, and then, installed in structures where they stayed protected from rain and direct sun rays.

The trap-nests (TN) were inspected every day and when nests were finished, they were removed, labeled and stored individually in transparent plastic tubes, then closed with voile and cork stopper. The tubes containing TNs were taken to the Bee Laboratory at the UFC Bee Unit, where they were kept inside a B.O.D. (Biochemical Oxygen Demand) at 27°C and 60% relative humidity until the emergence of adults. At every observation, the nests removed to the lab were replaced by empty ones with the same diameter to assure the constant availability of nests.

Aiming to avoid robbing and abandonment of the nests of *E. tigrinum*, as well as to attract bees to the nesting place, a mixture of resin and wax of Meliponinae was provided, same material used to close trap-nests. This mixture was extracted from the nests of bees *Melipona subnitida* and *Scaptotrigona* sp. nov. present in the meliponary at the Bee Unit. The material was available to the bees soon after being collected, by distributing small portions around the trap-nests.

### Nesting Behavior

Five females of *E. tigrinum* were marked with non-toxic paint as soon as they had begun building the nest. The bees were captured with plastic vials at the entrance of the trap-

nests, then placed in a freezer until dormancy in order to be marked, and released afterward to go back to their nests. Each bee was observed for an interval of five days. Observations were performed during the whole day, starting at 5:00 am and ending at 6:00 pm, in order to assess the behavior of those bees, regarding the time they start foraging, the permanence time inside nest, in the field, the resources collected, and the time taken to finish a cell.

Subsequently, after the end of the observations, the bees received new identification, consisting of a numbered tag that was made of A4 sheet paper. The tags were fixed in the dorsal part of the thorax, between the wings, using Super bonder LOCTITE® glue brand. Then the bees continued to be watched as to be determined the total of nests that each female have established; the number and the size of cells constructed; the time of cells construction and offspring emergence; the sex ratio, mortality and parasitism rate.

*Statistical analysis*

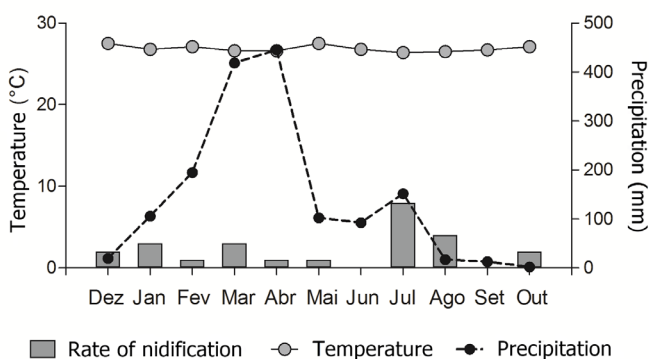
The descriptive statistics were used for nesting data, seasonality, total of nests settled by each female, number and size of cells built, building time, emergence, sex ratio, mortality and parasitism rate. In order to determine the relation between climatic factors (temperature, humidity, precipitation and wind speed) with the departure and arrival of bees, and the number of nests and cells built, the Spearman Correlation test was used because data did not show normality. The statistical program PAST 2.17 was used for performing the analysis.

**Results**

*Seasonality and nesting*

*Seasonality*

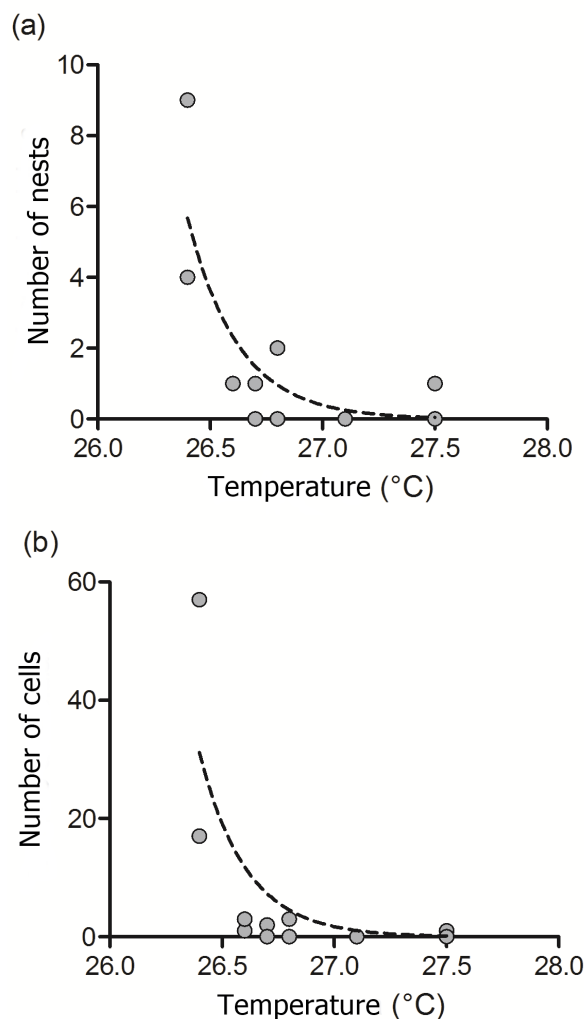
During all period of experiments, the trap-nests were occupied by bees *Epanthidium tigrinum*. However, during the first months, the bees hardly ever completed their biological cycle because most of them abandoned the nests without finishing any cell. Competition with other bee species for



**Fig 1.** Nesting of bees *Epanthidium tigrinum* in trap-nests in function of temperature and pluvial precipitation, December 2014 through October 2014, at the Bee Unit of the Department of Animal Science of the Federal University of Ceará.

nesting sites and the robbery of nesting resources by other bees have been observed as the cause of a small number of nests during this period. Aiming to overcome these problems, we replaced the trap nests used for narrower ones, only 4.5 mm in diameter in order to hinder the nesting of bees *Centris analis*, the main competitor for nesting sites, and offered to the *E. tigrinum* females resin collected from nests of meliponine bees, preventing robbery of nests from each other. These procedures allowed the bees to nest, build their cells and complete their nesting cycle.

There was no significant correlation ( $p < 0.5$ ) of the precipitation and temperature with the occupation of nests, even though the highest occurrence of *E. tigrinum* females was observed during the months of July and August, after the rainy season (Fig 1). When assessing the correlation between climatic factors with the number of nests established and with the total of cells built, a negative correlation was observed with temperature ( $r_s = -0.639$ ;  $p = 0.034$ ) for the total of nests and cells ( $r_s = -0.702$ ;  $p = 0.016$ ) (Fig 2).



**Fig 2.** Correlation between temperature and (a) total number of nests established, and (b) total number of cells built by bees *Epanthidium tigrinum* in trap-nests, at the Bee Unit of the Department of Animal Science of the Federal University of Ceará, December 2014 through October 2015.

### Nesting

During the experimental period, 16 nests were completed, belonging to 10 different bees, with the distribution varying from 1 to 4 nests per bee ( $1.6 \pm 0.97$ ). From those nests, 67 individuals emerged (37 males and 30 females) (Table 1) resulting in a sex ratio of 1.2:1. There was no occurrence of parasites in the observed nests and the non-development of larvae was the main cause of mortality, resulting in a mortality rate of 18.5%. Another factor responsible for the mortality was the attack of ants that occurred in two nests.

The females built their nests in a linear fashion following the shape of the trap-nests and using mainly resin, which sometimes was mixed with sand or clay because these nests were constructed inside trap nests previously occupied by wasps or bees (*Centris analis* and *Tetrapedia diversipes*).

The bees spent one day to build each cell, starting the collection of resin in the afternoon and collecting pollen and nectar in the following morning, before oviposition. When each cell was completed, the cycle restarted. In five nests we observed the occurrence of vestibular cells. These structures are empty cells which are larger than a regular one and located between the last provisioned cell and the nest closure. In the present study, the vestibular cell measured an average size of  $28.66 \pm 9.10$  mm.

The bees took in average  $15.44 \pm 5.93$  days to complete one nest. From the first cells built, i.e., the more internal ones, emerged males, and from the more external cells emerged females, being the females the first ones to emerge. The sizes of cells varied regardless the bee's sex, and measured an average size of  $7.97 \pm 1.31$  mm. The offspring took an average time of  $43.69 \pm 4.64$  days to emerge.

**Table 1.** Data of nests of *Epanthidium tigrinum* collected in trap-nests, from December 2014 to October 2015, at the Bee Unit of the Department of Animal Science of the Federal University of Ceará.

Bees	Number of cells	Bees emerged		Construction duration (days)	Emergence duration (days)	Closing distance (mm)	Vestibule (mm)
		♂	♀				
1	6	1	4	11	46	24.67	-
1	6	4	0	19	48	38.62	17.61
2	5	2	2	11	43	20.35	-
2	5	2	2	17	43	20.91	29.75
3	5	4	0	15	49	23.30	42.44
3	9	1	4	17	44	21.13	-
5	9	5	3	25	40	31.62	29.06
6	6	2	3	25	36	17.56	24.45
7	6	3	3	15	43	20.35	-
8	6	3	3	12	49	13.98	-
10	7	3	3	14	43	23.51	-
10	2	1	0	7	49	64.75	-
10	2	2	0	15	45	26.52	-
10	2	1	1	7	44	21.02	-
11	2	1	1	11	45	26.31	-
12	3	2	1	26	32	31.31	-
<b>Total</b>		37	30				

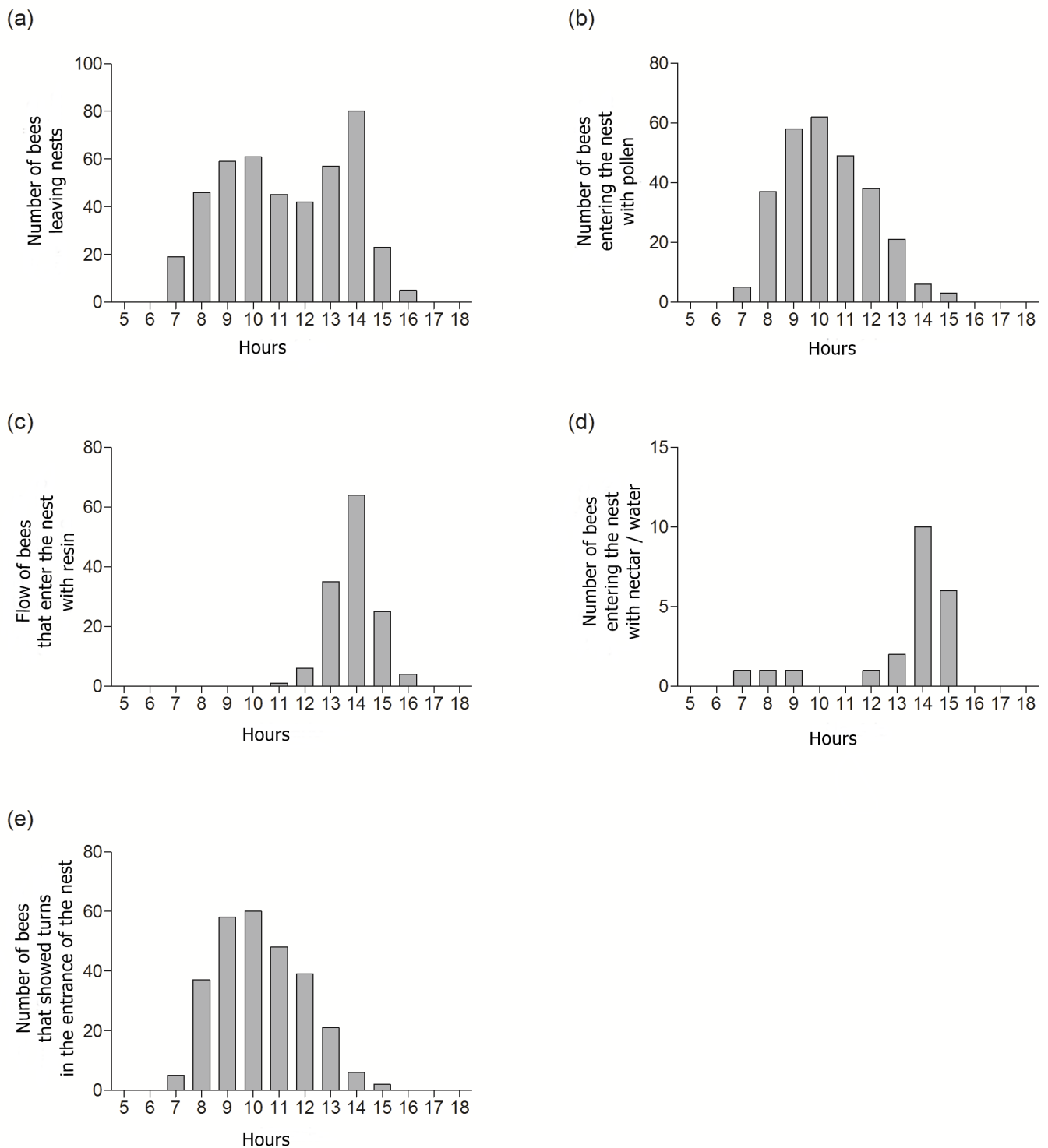
### Nesting behavior

The observed *E. tigrinum* bees started foraging, on average, at 07:50 am  $\pm$  30 min. In the morning hours those bees collected pollen and nectar, and resin in the afternoon hours. Such collecting behavior was influenced by the temperature and relative air humidity, as well as the stage of nest building, since the bees have always started nest building by collecting resin, regardless of the time and definite nesting location.

The bees remained in activity until 4:00 pm, although once a bee has finished activity as late as 4:51 pm. The bees remained inside their nests between field trips for an average  $9 \pm 5$  min, and in the field, they spent in average  $19 \pm 7$  min,

varying according to the resource collected. The moment of greatest bee activity occurred at 2:00 pm, which coincided with the greatest flight movement to collect resin, with a higher constancy of flight during the rest of the hours (Fig 3).

A behavior that has affected considerably the time bees executed their tasks along the day was the change of resource collection, which according to the observations, is the moment of oviposition and generally coincides with the last trip for pollen collection. But, in some cases, it took place after the first or second trip to collect resin: At the time of oviposition, the bees have come to spend over an hour inside the nest, and after that the female left and returned to nest a few times, making turns around its entrance but not flying. Eventually, the bees



**Fig 3.** Flight activities of *Epanthidium tigrinum* bees and gathering of resources in trap-nests at the Bee Unit, Department of Animal Sciences of Federal University of Ceará, December 2014 through October 2015; a) Departure of bees from nest; b) Pollen arrival; c) Resin arrival; d) Nectar/water arrival; e) Turnings at the nest' entrance.

returned to inside the nest and positioned themselves very close to the entrance, sometimes placing the antennae out of the nest, and remained like this until reassuming the foraging flights.

The morning flights of *E. tigrinum* for pollen collection have taken over an hour on some of the observation days. The collected pollen, loaded on the abdominal scopes, was very noticeable due to the yellowish coloration. In two days, two of the bees spent over 2 hours in the field. The time bees

spent in the field has varied throughout the sampling period. Every day, the first foraging flight was the longest one when comparing to the other foraging flights of the day, varying from 15 min to 2 hours and 30 min. In rainy days, the foraging behavior was affected with foraging flights much longer than usual. In case the bees were still in the field when the rain started, they would not return to the nests until the rain stops and the sun shines again.

The behavior observed when these bees returned from the field is described as follows: first, they entered straight into the nest with the head facing in, then they came out backwards with the abdomen facing out. After that, the bees made a turn without flying and returned into the nest still with pollen in the scopa, but this time with the abdomen towards the inside of the nest. Then, they unloaded the resource inside the cell. The sequence of movements entering and leaving the nest and “turning” backwards inside the nest only happened when the bees collected pollen, and it would take from 1 up to 5 min to be completed.

Flights for resin collection were shorter and took 30 min at the most, when bees were going to the field in search of this resource. However, in most cases, they returned empty and ended up collecting the supplied resin in a way that the flights were even shorter, varying from 1 up to 5 min only.

The permanence of the bees inside the nest varied greatly, generally 1 to 11 min, although some have stayed inside the nest up to 42 min between flights. Observations suggested that the individuals were collecting resin and quickly depositing it inside the nest and returning to collect more resin, only shaping it at the end of foraging hours, when they had collected all the resin they need for that day. Only in a few cases the bees have slightly worked on the resin between the collecting flights. Resin collection was easily noticeable because the bees carried the resin held in the mandibles in shapes of bulky pellets.

Pollen collection showed a negative correlation with humidity and rain precipitation (Table 2). Resin collection was not influenced, since the bees used mainly the supplied resin of meliponines, which was placed on the proximities of trap-nests in a covered place, which hindered the interaction of those bees with the environment during foraging.

**Table 2.** Correlation of bee departure, pollen arrival, resin arrival, nectar/water arrival, and turning behavior with climatic variables of bees *Epanthidium tigrinum* in trap-nests, at the Bee Unit of the Federal University of Ceará, December 2014 through October 2015. Where *rs* is the value of correlation and *p* is the significance.

	Temperature		Humidity		Precipitation		Wind speed	
	rs	p	rs	p	rs	p	rs	p
Exit	0.201	0.335	-0.319	0.120	-0.154	0.462	0.003	0.990
Pollen	0.340	0.097	<b>-0.477</b>	<b>0.016</b>	<b>-0.481</b>	<b>0.015</b>	-0.301	0.143
Resin	-0.062	0.767	0.126	0.548	0.260	0.210	0.186	0.372
Nectar/Water	-0.131	0.531	-0.045	0.830	-0.071	0.735	0.205	0.326
Turning	0.348	0.088	<b>-0.481</b>	<b>0.015</b>	<b>-0.440</b>	<b>0.028</b>	-0.297	0.149

## Discussion

### Seasonality and nesting

Most of the studies about the Megachilidae species had been carried out in the Northern hemisphere and have found seasonal species which individuals only are active during a short period of the year and the offspring show diapause, emerging as adults only when conditions are favorable again (Torchio & Tepedino, 1980; Pitts-Singer & Cane, 2011; Rinehart et al., 2013). In Brazil, seasonality has already been seen for some species of Megachilidae such as *Megachile* (*Melanosarus*) *nigripennis* Spinola (Marques & Gaglianone, 2013), *M. benigna* Mitchell, and *M. maculata* Smith (Cardoso & Silveira, 2011), *Anthodiocetes lunatus* Smith (Camarotti-de-Lima & Martins, 2005) and *Anthodiocetes megachiloides* Holmberg (Alves-dos-Santos, 2004) at greater latitudes, however Morato (2001) suggests that *Anthodiocetes moratoi*, in the Amazon region, nests throughout all year. This study demonstrates that the species *Epanthidium tigrinum* remained active during the whole experimental period of 11 months varying only in number of nests constructed, and in quantity of cells built per nest. Therefore, there is no seasonality for this species under the studied conditions.

Some studies suggest that seasonality in bees can be associated to the availability of specific resources for nest

building, adult feeding and larvae provisioning (Viana et al., 2001; Mendes & Rêgo, 2007). In this study, the first factor may have been relevant, because few of the observed bees completed nest building and showed high abandonment rate in the beginning of the study. In one case, a bee has robbed all material collected by another bee of the same species, which has resulted in the abandonment of both nests. The nidification, permanency of these individuals in nests and successfully conclusion of them, was only possible when the resin was provided close to the trap-nests. Henceforth, it was possible to see many bees collecting this resin, both the marked bees that were observed and others of the same species that were not nesting in the trap-nests. These behaviors perhaps suggest the lack of plants that provide resin in the area, which makes nesting unviable for these bees. In this way, the availability of vegetal resin would be, indeed, a limiting resource for the establishment and maintenance of populations of this species in an area and constitute an important factor when considering the rational breeding of these bees

The sex ratio in a bee species may vary and is associated with conditions of food availability or other local factors (Marques & Gaglianone, 2013). A work performed by Sugiura (1994) with *Anthidium septem spinosum* Lepeletier found that mother bees of this species are able to manipulate, as an adaptive way, the sex ratio of descendants in relation to

their ability to invest in the offspring. In this work, the offspring position inside the nest is also described, demonstrating that males are reared in most internal cells while females are produced in the most external ones, as also observed in the present study. The author reports that this distribution is directly related to the reproductive life of these bees because larger males have a higher capacity for reproduction and require greater parental investment. The lower ratio of females' production in comparison with males have also been reported for other bee species such as *Megachile* (Moureapis) *benigna* studied by Teixeira et al. (2011) and *Megachile* (Pseudocentron) *gomphrenoides* (Torretta et al., 2012). Also, some nests of *Epanthidium tigrinum* presented production of a single sex, fact already reported for some species of *Megachile*, such as *M.* (Austromegachile) *orbiculata* Mitchell (Morato, 2003), *M.* (Moureapis) *anthidioides* Radoszkowski (Cardoso & Silveira, 2003) and *M.* (Moureapis) *maculata* Smith 1853 (Cardoso & Silveira, 2011).

The bees of the genus *Epanthidium* are in the group of the Anthidiini, which collect resin for building their nests. During the experimental period, before resin was supplied, an odd material was noticed being collected by those bees and used along with resin to build and close some nests; however, it was not possible to have this material analyzed. After supplying with resin, their nests started to be built basically from this material. This may suggest that, depending on the quantity of resin available, these bees may use other mixed materials to build their cells. Cilla and Rolón (2012) described the nests of *Epanthidium* aff. *sanguineum* that made use of clay, resin and vegetal fibers in their construction. Another important factor noticed was that some of these bees have built their cells in trap-nests previously occupied by bees *Centris analis* and *Tetrapedia diversipes*. Cilla and Rolón (2012b) also reported the occupancy of unoccupied nest of *Centris muralis* Burmeister by bees *Epanthidium* aff. *sanguineum*. This behavior suggests a flexibility of these females in relation to the choice of nesting sites, not having specific requirements of places and conditions.

#### *Nesting behavior*

Apparently, environmental variations, resources availability, type of resource used, and intrinsic differences of the species influence the nesting behavior of Megachilidae species. The bees *E. tigrinum* have collected pollen and nectar in the morning, and resins in the afternoon. Such behavior optimizes usages of resources in the area, as the surrounding plants provide pollen, a limited resource, early in the morning and, once removed, there is no new supply until the next day. On the other hand, the resin becomes more malleable, therefore, easy to handle and be transported during the hottest hours of the day, which justifies collection only from noon on. Moreover, at noon, there is not enough pollen available in the field anymore.

In this study, while the bees *E. tigrinum* were making short flights for collecting resin and long flights for collecting both pollen and nectar, therefore spending more time in the nest when returning from the flights for pollen and nectar than from the flights for resin, Alves-dos-Santos (2004) reports that *Anthodioctes megachiloides* Holmberg, in São Paulo, made longer flights when collecting resin and spent more time inside the nest; whereas when collecting pollen, the flights were shorter and the permanence inside nest, shorter. The unlimited supply of resin we offered very close to the nests of *E. tigrinum* has made this resource as much, or more available, and much more accessible and of easier collection than the resins that *A. megachiloides* had to search and collect in the surrounding vegetation of their nests.

Finally, the results presented in this study show the possibility of rearing *Epanthidium tigrinum* bees in a rational way regarding the management and maintenance of the species in the desired area, pointing to crucial aspects to success such as the availability of resins and cavity size to prevent competitors. The establishment of managed populations of this bee will allow the further development of studies of the species, and a better understanding of the tribe Anthidiini.

#### **Conclusions**

The availability of resources for nest building and cell provisioning are limiting factors directly related to the obtainment of nests of *Epanthidium tigrinum* and to the establishment of this bee species.

The supply of stingless bee resin is an efficient alternative to meet the nesting needs of the *Epanthidium tigrinum* bee in resource-limited settings.

The competition with other species that nest in pre-existing cavities can be avoided using nests of smaller diameters, suitable for *Epanthidium tigrinum*, but not for larger species.

The little demand for nesting places, polylectic behavior, non-existence of seasonality, and little or no predation by enemies and parasites, make it possible to establish nurseries of *Epanthidium tigrinum*.

There is still a large deficit of biological information about the tribe Anthidiini, so that further studies are needed for future research aiming to extend the collected information.

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