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NESTING ETIQUACY OF STINGLESS BEE TETRAGONULA "IRIDIPENNIS" SPECIES GROUP

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ABSTRACT

In this study a total of 42 feral colonies of *Tetragonula "iridipennis*" were domesticated and observed for their nesting behaviour in feral nest and also in hive. Observation on the nesting behaviour revealed that, they generally inhabit old mud and stone walls, and the shape and colour of nest entrance were predominantly elliptical and black, respectively. The length and width of the entrance tube ranged between 9-13 and 3-9 mm, respectively on different substrata. The external and internal tunnel length ranged between 0-18 and 3-156 mm, respectively. The orientation of hive entrance was mainly towards northeast and east. Number of brood pots constructed/ batch and time required for a worker to construct the single brood pot were recorded.

Key words: *Tetragonula "iridipennis"* species group, feral colonies, dammer bee, habitat, behaviour, nest characteristics, nest camouflage, brood pots, mud, stone walls, nest entrance, tunnel length

Beekeeping with stingless bees is called Meliponiculture, which has been practiced for many centuries in various parts of Latin America, where these bees are considered as very valuable domestic species. Worker bees possess weak or vestigial stingers hence the term "stingless" is used to designate this group of bees. Currently, it is most widely practiced in Neotropical realms, including Asia, with many species (Chuttong et al., 2016; Rahman et al., 2018). Stingless bees are most likely to live in perennial colonies and their nesting pattern depends on direct or indirect interactions of nest mates sharing pheromones, stimuli and environmental cues which activate nest building as morphogenetic process (old nest act as platform for new nest construction) (Michener, 1974; Leonhardt et al., 2007).

Stingless bees are small (few mm in length) and resident species which nest among old walls, dead trees, tree cavities, nests in the ground, crevices of culverts, among orchid roots, in empty tanks, boxes, etc. Nests are primarily found inside the forest cover (Brown and Albrecht, 2001). The nest comprises of entrance, brood pots, honey and pollen storage pots, waste and resin dumps and nest envelope like involucrums and batumens (Pooly and Michener, 1969; Alves et al., 2018). Cerumen is the mixture of wax and resinous material, which is used for constructing brood pots, food pots and involucrum. In *Tetragonula carbonaria*, for building the pillars, workers carry the bits of cerumen to an elevated point and they deposit the loads of (cerumen) building material (Michener, 1974). For well protected nesting space, bees cover the cavity with batumen layer. Strong layer batumen is the combination of mud, plant material and more resin (Roubik, 2006; Gruter, 2020). Unlike in honey bees, brood cells/pots were never reused by the stingless bees. They build new ones for each new egg. During larval development they spin their cocoons inside the brood pots and the workers remove most of the cerumen (cerumen plate - trochoblast) from brood pots before adult emergence (Ihering, 1903; Van Benthem et al., 1995). The behaviour of stingless bees was not much revealed as they are cavity nesting bees. This study was done to explore the behavioural aspects of stingless bees and their nesting characteristics from the colonies which are present in certain districts of Tamil Nadu.

MATERIALS AND METHODS

Survey was conducted for the availability of feral colonies of stingless bee in selected districts of Tamil Nadu viz., 1. Mettur (Salem), Kunjandiyur (11°48'14"N, 77°51'28"E); 2. Pennagaram (Dharmapuri), Anumandhapuram (12°19'04"N, 78°06'48"E); 3. Morappur (Dharmapuri) Dhodamapatti (12°03'22"N, 78°29'56"E); 4. Vridhachalam (Cuddalore), Aanandhakudi (11°27'44"N, 79°19'57"E); and 5. Vanoor (Pudhucherry), Aurobindo Ashram (12°00'42"N, 79°44'30"E). From these locations, five, twenty, fifteen, and two colonies were recovered, respectively. Feral colonies recovered were housed either at the Department of Entomology, Faculty of Agriculture, Annamalai University or Aurobindo Ashram's 420 acres of organic farm, at Pudhucherry. While capturing the feral colony and after domestication into hive, colony nesting behaviour, nesting pattern and its structure in relation with nesting habitat were observed visually. By using a standard measuring tape on cm scale, the nest entrance and colony size were measured. Feral colonies nesting site, orientation, elevation and their nesting attributes data were collected by following Sheetal and Basavarajappa method (2009).

RESULTS AND DISCUSSION

Actual location of colony collection sites from different districts led to survey of 42 colonies on various substrata. Most of the nesting sites of *Tetragonula* sp. were found in cavities of old walls and tree trunks. The major sites among all substrata were mud wall, stone wall and dead tree logs (Fig. 1). Other than these, few sites were also found inside electric box, pipes and in termitarium. The nest physical characters such as shape, colour, orientation, nest enclosure material and nesting elevation preferred by the feral colonies were listed (Fig. 2). The colour of the nest entrance merged with the substratum. Nests in brick wall had the orange nest entrance, nest in tree trunks had brown-black colour entrance (Fig. 3). The nest elevation varied from below ground to 15 feet above ground level. Nest entrances had additional deposits of stone, mud, pollen particles, leaf bits and grease to protect the nest from enemies. Most preferred enclosure materials were grease and mud particles (Fig. 4). The colony entrance was observed to be in different orientation such as north, south, east, west, northeast, northwest, southeast and southwest but the preferred nesting orientation was east and northeast (Table 1).



Fig. 1. Nesting habitat and structure; (a, b) Mud wall, (c, d) Stone wall, (e, f) Palm tree



Fig. 2. Shape of nest entrance; (a) Round, (b) Elliptical

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Fig. 3. Camouflage (colour) of nest entrance; (a) Grey colour entrance in stone wall and (b) Orange colour nest entrance in brick wall



Fig. 4. Deposition of particles on Hive entrance (a) dried leaf bits on entrance tube, (b) grease on entrance tube

S.No.	Nest characters	Observation/ criteria		Most preferred				
1.	Shape of nest entrance	Round, elliptical, irregular		Elliptical				
2.	Colour of nest	Black, grey, orange, yellow		Black				
3.	Nest orientation	North, south, east, west, northeast, northwest,		East, northeast				
		southeast, southwest						
4.	Nest enclosure material	Wax, resin. Grease, mud, stone, leaf bits,		Grease, mud and sand particles				
		pollen						
5.	Nest height from ground	Below ground to 15 feet above ground level		6 to 7 feet				
Nesting habitat								
1.	Places visited	Roadside, parks, residential buildings or		Residential buildings or old				
		house, agroforestry area		houses				
2.	Colony location	Interior, exterior (from the roadside)		Interior regions				
3.	Substratum (habitat)	- Stone, mud and brick walls		Wall of stone and mud				
	1. Old walls	- Iron, electrical and telephone wire pipes						
	2. Iron pipes	- Cement plastering						
	3. Electrical pipes	- Wooden door rims						
	4. Tree trunk	- Mud						
Size of natural colony and substratum*								
		Palm tree	Mud wall	Stone wall				
Cavity dia (mm)		356.0	670.0	756.0				
Nest e	ntrance (mm) L	9.4	10.4	9.4				
	W	5.2	6.8	5.8				
External tunnel (mm)		6.0	5.6	13.0				
Inner tunnel (mm)		47.8	34.2	156.6				

Table 1.	Nesting	parameter	of natural	Tetragonula	colony
	0	1		0	2

*Values mean of five colonies

Out of the 42 colonies recovered, 25% were found in old mud wall, 23% in tree trunk and 18% in stone wall cavities that were located inside and not visible outside and also away from main roads and more in rural areas. The least preferred nesting habitats were electric pipes, wooden door (each 5%) and iron pipes (2%) (Fig. 5). It was contradictory to the findings of Pavithra et al. (2013) where they reported that walls made of bricks



Fig. 5. Nesting habitat preference

were more preferred. The possible reasons for the increased colonization in mud wall might be due to the high preference of this habitat by stingless bee or unused mud wall might have been commonly available in that locality. In districts like Dharmapuri and Mettur of Tamil Nadu, villages have unused mud houses and stone walls for partitioning the fields that is mainly used as a base source of colonies. Nest entrance colour merged with nest substratum (such as nest in stone, mud and brick walls, palm tree, etc.) colour i.e the entrance colour was as that of the surrounding colour like black, grey, orange and black respectively. These observations were like the findings of Lima et al. (2013) and Mythri et al. (2018). The most preferred colour and shape of nest entrance is black and elliptical respectively. Nest entrance was observed with mud particle and grease (which we normally apply on hive stand to prevent ant attack) deposition to prevent predators' entry inside. This observation is partly in accordance with the findings of Pavithra et al. (2013) who reported more of mud accumulation around the nest entrance.

Observations revealed that, out of 42 colonies, 25% colony were facing East i.e., Sun rise and particularly nest entrance facing the floral resource. Mostly East and Northeast directions were preferred by feral colonies (Fig. 6). It was contradictory to the findings of Marhold et al. (1997) and Pavithra et al. (2013) who reported that it preferred southern and northern directions, respectively. Preferable nesting elevation in feral colonies were 6 to 7 feet from the ground level.



Fig. 6. Nest orientation of feral Tetragonula colony

This finding is contradictory to the report that the most preferred nesting elevation was 11-20 feet from the ground level (Slaa, 2006; Pavithra et al., 2013; Layek and Karmakar, 2018). These contradictions might be due to the changes in nesting substratum and climatic conditions with respect to the findings of Pavithra et al. (2013). The entrance was made of cerumen, that was soft in the beginning turned rigid later. A distinct entrance tube was found in many nests in stone and mud walls but absent or simmer down in tree trunk nests. The length and width of the entrance tube ranged between 9-13 and 3-9 mm respectively. The length and width of entrance tube on palm tree, mud wall, and stone wall were 9.4, 10.4, 9.4 mm and 5.2, 6.8, 5.8 mm respectively. The external and internal tunnel length ranged between 0-18 and 3-156 mm respectively. Most of them had an external and internal tunnel length of 6.0, 5.6, 13.0 and 47.8, 34.2, 156.6 mm, respectively in palm tree, mud, and stone wall. The internal tunnel was connected with inner parts of the nest (Table 1; Fig. 7). Depending up on the stages of brood development, brood pots colour varied. Newly constructed pots were brownish and later (before adult emergence) it became straw coloured (Fig. 8). Workers constructed the brood pots in batches of 7-11. Single worker constructed one brood pot of three fourth size within 1.30-2.00 hr. It is contradictory with the observation of Roopa et al. (2017) who reported that 4-6 brood pots in a batch and for each brood pot construction it took 2.0-2.5 hr.



Fig. 7. (a) External entrance tube (b) Internal entrance tube

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CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

B.S.V: For collection and domestication of feral colonies and observation on behavioural studies; drafting the manuscript. S.M: Advisor for the research work and for drafting the manuscript. B.A: For drafting the manuscript.

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