



Importance of studying nesting biology of stingless bee *Tetragonula iridipennis* Smith (Hymenoptera; Apidae; Meliponini) in India

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Abstract

Stingless bees are eusocial insects like honey bees that live together in permanent colonies. *Tetragonula iridipennis* nests are permanent fixtures that can be found primarily on brick walls, in tree cavities, and in empty logs. The nest of *T. iridipennis* consists of a nest entrance, an entrance tube, brood pots, honey and pollen pots, batumen and involucrum. The store pots (honey and pollen pots) and brood pots are arranged separately. The useful products from nests are propolis, honey, pollen, and beeswax. Exploration of this information could be helpful in improving knowledge about nesting biology and its importance.

Keywords: stingless bees, *Tetragonula iridipennis*, nesting biology, honey, pollen, beeswax

Introduction

The stingless bees are one of the most important bee species in terms of ecological, economic and cultural importance (Bhatta *et al.*, 2019) [4]. Stingless bees mainly inhabit the tropical and subtropical regions of America, Australia, Africa, and some regions of Asia continent (Roubik, 1989) [30]. The *Tetragonula iridipennis* also known as the Indian stingless bee have been included in the Tribe Meliponini, under Family Apidae, into Superfamily Apoidea of the order Hymenoptera (Taye, 2019) [39].

Stingless bees are taxonomically different from honeybees. The honey bees are included in the Apinae subfamily, whereas the stingless bees are included in the Meliponinae subfamily of family Apidae. Two tribes, Trigonini and Meliponini, are included in the subfamily Meliponinae (Francoy *et al.*, 2009) [12]. In tribe Meliponini two genera are included: *LisoTrigona* and *Trigona* (Michener, 2000) [20]. The subfamily Meliponinae has two tribes, Meliponini and Trigonini, which are divided into *Melipona* and *Trigona* genera, respectively. (Wille, 1983; Francoy *et al.*, 2009) [40]. [12]. Most *Trigona* species are relatively long-winged and small. Frederick Smith first described this species in 1854. Previously *Tetragonula iridipennis* was known as *Trigona iridipennis* but from 2013 onwards the name *Trigona iridipennis* was given to the *Tetragonula iridipennis* (Rasmussen, 2013) [28].

The names '*Trigona*' and '*iridipennis*' refer to their triangular shaped abdomen and iridescent wings, respectively (Devanesan *et al.*, 2009) [10]. Sometimes *Tetragonula iridipennis* also known as 'Dammer bee' because it gathers a specific type of resin and wax produced from its body to build the nest (Batra, 1977) [2].

Tetragonula is the most common genus in the Indo-Malayan region, ranging from India to the Solomon and Caroline islands. In the Indian subcontinent, the diversity of stingless bees was summarised by Rasmussen (2013) [28]. The southernmost regions of India along the coast of the Bay of Bengal have the greatest populations of stingless bees but

can also be locally common elsewhere. In central region of India, stingless bee colonies are less common. Stingless bees can also be found in other countries of the Indian subcontinent like Nepal, Bhutan, Pakistan, and the majority of Bangladesh (Taye, 2019) [39].

Stingless bees are the smallest honey-producing bees. These bees are known as stingless bees because of their absence of sting ability, but they have an equally effective biting mechanism to defend the nest (Raju *et al.*, 2018). Like honey bees, Stingless bees are eusocial insects that live in colonies and build their nests in cavities in tree trunks, empty logs, brick walls etc. Only the entrance of the hive is exposed outside, while the colony is hidden inside. Stingless bees live in their nests, where they mate, feed and go through all of their developmental stages. Inside the nest, there are many configurations of brood compartments and pollen and honey pots.

The pollen and honey are stored in distinct pots and surround the brood region (Roubik, 2006) [32]. Permanent stingless bee colonies comprise of hundreds or thousands of workers, a queen and some drones (Wille, 1983). Stingless beekeeping is known as Meliponiculture and *T. iridipennis* has been kept in India since ancient times for the medicinal value of its honey, propolis and bee wax. (Choudhari *et al.*, 2012; Kumar *et al.*, 2012; Rasmussen, 2013) [6, 16, 28].

Nesting biology of *Tetragonula iridipennis* Smith

Nesting biology is a significant aspect of stingless bee behaviour. *Tetragonula iridipennis* nest are the main place of their activity. Nests are a notable point of the colonial existence of social insects, which is crucial for providing shelter against environmental disturbances.

According to Mohan and Devanesan (1999) [23], the nest of *Tetragonula iridipennis* Smith is made up of mainly 5 parts - nest entrance, brood cells, store pots (honey and pollen pots), involucrum and batumen. Besides them, waste and resin dumps are also present.



Fig 1: Photo of *Tetragonula iridipennis* Smith nest at the entrance gate of Department of Zoology, University of Rajasthan, Jaipur (302004).
Photo credit – Corresponding author.

Nesting site and Nest orientation

T. iridipennis nests are typically found in cracks in building stone or brick walls, inside the hollows of tree trunks and cavities in the ground. In addition to these, nests are also found inside hollow blocks, in a drawer, in empty tanks and boxes, in a vase, on the ground in social insect's active colonies like termite, ant, wasp or other stingless bee colonies (George, 1934; Schwarz, 1939; Michener, 1961; Rasmussen, 2004) [37, 19]. The bee uses cavities for nesting to regulate nest temperature, avoid exposure to sunlight and protect itself from predators (Raju, 2009) [26].

The sun is the most important signal at each stage of orientation because nest orientation frequently affects how much solar radiation is absorbed and the time when the nest receives the most radiation. Some species orient their nests to receive solar radiation in the cold early hours, while others orient the nests to expose the lowest possible solar radiation in the midday hours. As a result, nest orientation is emphasised when assessing nest-site preferences because it determines the length of time the nest is exposed to sunlight, which in turn greatly affects the temperature inside the nest. Nest orientation also has a clear and causal relationship to nest microclimate (Pavithra *et al.*, 2013) [25].

Nest constructing materials

The material used in the construction of the nest in *T. iridipennis* has a very specific, distinctive function. According to its physical properties, the amounts of various materials used vary on the substrate, habitat conditions and the availability of those resources (Horvath, 1963) [13]. As a result, some nest entrances had higher mud deposits, whereas others had an accumulation of resin, wood pieces, stones, pollen etc (Pavithra *et al.* 2013) [25].

As these bees are also known as Dammer bees, the main materials favoured around the nest among the resources was mud and resin (Batra, 1977) [2]. Dammer bees collect a form of resin in addition to the wax that their bodies naturally make to utilise in the construction of their nest. Sand is

another component that can be seen on the surface. Because sand granules can be measured easily and have rigidity, they make an excellent building material for walls. Stingless bees use a lot of plant material in their nests in addition to endogenously produced wax. Wax also undergoes intermolecular modifications at remarkably low temperatures, which makes it strong and usable. When compared to other materials used at the nest entrance, the build up of mud was discovered to be present in higher amounts (Pavithra *et al.* 2013) [25].

Nest entrance

The nest entrance acts as a channel between the interior and exterior environments. In terms of nest characters, the nest entrances are important. The simplest stingless bee nest entrance sticks out a little from the base of the entrance hole (Roubik, 2006) [32]. Nest openings are connected to physiochemical regulation in addition to defence and foraging (Biesmeijer *et al.*, 2005) [5]. The size of the nest entrance varies in different nests. It might be oval, slit-like, round, irregular-shaped etc. (Roubik, 2006) [32]. It is built of soil elements, resin, and dried plant materials. The newly constructed entrance had a soft, light brown colour and kept its shape throughout the year. With time, the entry tube's colour changed, becoming hard and darker.

The nest entrance showed that stingless bees preferred middle-sized nest entrances over large and small-sized nest entrances. They also showed a preference for the design of the entrance, favouring an oval shape over a circular or irregular opening. Small or plain nest entrances are cryptic; they serve as the only access point for adult bees that might be defensive. The entrance wall could serve two functions. First, nest wall has served as a barrier against adverse weather and natural enemies and second, it can be used as infrastructure to control the colony. The arrangement of the walls will determine the area they will allot to themselves, which will control their density, encounter rates, and possibly even how tasks are distributed (Pavithra *et al.*, 2013) [25].

The external entrance tube of *T. iridipennis* continued into the internal nest cavity, also known as the internal tunnel. There was only one distinct entrance tube per colony, however, the size and form varied from colony to colony. The entrance tube opening was elliptical in most cases and in a few cases it was slightly round (Choudhary *et al.*, 2021) [7].

Nest aggregation

The majority of bee colonies in *T. iridipennis* consisted of a single colony per site, followed by two colonies per site. But there were certain places where three, four and more than four colonies are present. However, compared to other bee colony sites, the majority of them had an aggregate of one or two colonies. There were therefore often one or two bee colonies developed per site (Choudhary *et al.*, 2021) [7].

Brood cells

The brood chambers are smaller in size, oval in shape and are mostly used for brood rearing (Raju, A.J.S. 2009) [26]. Worker bees initially build wax pillars that serve as the foundation for the brood cells. The worker bees begin grouping to build brood chambers on these pillars. Construction of the cells begins at the base of the pillars and moves upward (Mohan, 2000) [22]. The cells expand as a result of workers contributing in successive and irregular cycles. They either have an oval or spherical form (Danaraddi *et al.*, 2009). One layer of the multi-layered combs is built on top of the other, with each layer increasing both vertically and horizontally. Before provisioning, the queen walks over the brood comb cells and examines them. After walking, she settles down near a cell that has been collared for egg laying (Raju, 2009) [26]. While moving around the queen, several employees load the chosen cell with food. The queen places her abdomen inside the cell and lays an egg inside once the workers have provisioned it. The egg is kept perpendicular to the liquid food in the centre. After oviposition, the queen quickly departs the cell. The employees have engaged in provisioning, and one worker is responsible for sealing the ovipositing compartment (Devanesan *et al.*, 2009) [10]. Sakagami (1982) [35] noted that it takes roughly two hours to construct a single cell, 1-2 minutes for provisioning alone, and 10 minutes or so for provisioning, oviposition and cell closure. The process of provisioning and operculation of all cells operated simultaneously and 4-9 workers take participation in it (Bentham *et al.* 1995) [3].

The brood cells were exclusively used for brood rearing; the cells were used only once for raising the offspring. The cells contained a variety of larval and pupal stages. When pupae reached maturity, emerging adults shed their cell walls, exposing their cocoons. The new adults were brown to black, in contrast to the larvae and early stages of the adult, which were creamy white (Salmah *et al.*, 1996; Wittmann *et al.*, 1991) [36, 43]. The time required for the emergence of an adult from the egg is 21-25 days (George, 1934). The complete process of formation of a brood cell, providing larval food and laying an egg is known as the Provisioning and Oviposition Process (POP) (Roopa, 2002) [33].

The cream to ash-coloured young adults began grooming their bodies immediately after their emergence, and they subsequently became darker after eight days. After that, they began clearing away the trash, and dead bees, building cells, providing food, and manning the entrance for 20 to 25

days. Then they started foraging after 35 to 40 days old (Roopa, 2002) [33]. In roughly 80-87 days, the deceased marked bee was discovered, usually inside the nest itself. The 20-25-day-old bees remove the dead bees (Raju, 2009) [26].

Store pots (honey and pollen pots)

In a colony of *T. iridipennis*, food chambers are situated outside the brood cells and are made up of honey and pollen pots. The size of honey and pollen pots is much larger than that of brood cells (Lindauer, 1957; Kshirasagar and Chauhan, 1977; Crane, 1992) [17, 15, 8]. These store pots are around the same size and can be found all across the site. The pots are constructed adjacent to one another so that they resemble a bunch of grapes (Dollin 1996) [11]. When filled, the pollen pots were sealed after being efficiently loaded with pollen. Only a few open pots were present that were used to store incoming pollen. Similar to pollen pots, honey pots were also sealed after the ripening of honey. The honey pots were oval-shaped, dark brown coloured and slightly larger than the pollen pots. They were situated at the inner edge of the colony and some were often mixed in with pollen pots (Danaraddi *et al.*, 2009).

Involucrum

Brood involucrum is one or more lamellar coverings that surround the brood area. These layers are shiny and brown in colour and are formed of propolis, resin, and sometimes plant components. Around the brood comb, it is arranged in alternating layers to permit bee movement. Normally, involucrum is absent in *T. iridipennis* nests, but the bee will make these wax sheets when the nest is exposed to sunlight or in weak hive colonies (Muthuraman *et al.*, 2013) [18].

Batumen

Batumen is known as the outermost covering of the nest. When a cavity is too large for a colony, it is temporarily sealed up at both ends with layers of batumen membranes or, in the case of an open or underground nest, entirely covered by multiple layers. It is made of mainly mud, resin, and wax. Batumen is useful in controlling the hive's volume, keeping the hive's temperature stable, and protecting the nest from rain. In addition, a coat of batumen is also applied to the nest's interior wall. Bees often leave their waste products, plant fibres, and mud between the layers of batumen in exposed nests. These materials eventually crashed into a structure in a different area of the hive. This will serve as a supporting structure for the nest (Willie, 1983; Mohan, 2000; Rasmussen and Camerago, 2008) [22, 29].

Waste and resin dumps

Resin dumps are fresh resinous material around the entrance tube opening that appears as a few scattered drops of a creamy or yellowish colour (Lindauer, 1957) [17]. Stingless bees generally deposit their waste products on top of the trash pile, which also includes dead or partially dead bees, parts of brood, cocoons and feces. Trash piles can be found both inside and outside the nest. (AICRP, 2004) [1].

Colony defence behaviour:

Stingless bees do not have sting as a defensive method, but they have a variety of other mechanisms to defend the nest from threats.

The following are possible threats to stingless bee colonies: (Kumar *et al.* 2012) [16]

- Predators: birds, lizards, toads, spiders, centipedes, ants etc.
- Intruders: wax moths, robber bees, flies, hive beetles, other animals and man.

Some stingless bee species exhibit protective behaviour. Only when nests are the protective behaviours are visible, such as an attack or a fast retreat, as well as the internal nest construction. *T. iridipennis* uses its equally efficient biting mechanism to protect its nest. They irritate people and release a toxic substance when biting by snooping into the nose, eyes, ears, and hairs. The most common type of strategy for the defence of a nest is to make it and the entrance invisible to intruders (Bruijin, 1996). The design of the nests allows for the gathering presence of defending bees, which frequently enter the nest from the entry area. One of the defence mechanisms is the release of alarm chemicals from the mandibular gland into the air both inside and outside of the nest (Wittmann *et al.*, 1990). Then, a massive force of flying defenders emerge from the nest, turn to face the entrance, and engage in aerial combat with non-nest mates or charge at larger animals from the front.

The "scutellum nest" of *Trigona* is a notable meliponine defence structure. The exposed-nesting *Trigona* forms this hard and thick barrier. The scutellum is primarily composed of bee faeces. Layers outside of scutellum are covered by a few thin batumen coverings. These could be easily destroyed by predator attack and serve to allow the discharge of numerous defence bees from all around the nest surface. A defence tactic used by a variety of meliponines is to block the entrance to the external nest at night with a loosely knit curtain (Nogueira-Neto, 1962)^[24].

Hive products

Propolis

Propolis is a resinous substance that bees make from plant sources such as stems, leaves, and flower discharges mixed with wax and mandibular secretions. Bees employ this substance to shield their colony from physical and biological factors. It can be obtained from the hive by scrapping with a hive tool or knife. Propolis is placed at the nest's entrance and in cracks on the outside to keep out other organisms, inside the hive to keep the interior at the proper temperature and at the location where the eggs are laid to maintain asepsis. It is also used to fix dead organisms in nests to stop microbial growth.

Propolis is a potent natural antibacterial and antiviral food. This food keeps them resistant to bacterial and viral infections that can otherwise wipe out their colonies. Scientific testing has shown that propolis is useful in treating a wide range of medical conditions. It works as a natural antibiotic to treat infections and wounds in the body. In the past, propolis was used to treat rashes, skin infections, and internal and external ulcers. Regular use of propolis is proven to lower blood pressure and strengthen the immune system (Taye, 2019)^[39].

Honey

Stingless bees make honey from the nectar of flowering plants. It is stored in pots made of wax cerumen. Honey is mostly composed of glucose and fructose, and contains vitamins, minerals, and other nutrients. It is the primary source of energy for stingless bees and can also give people more energy. Honey from stingless bees can be used to

make alcoholic and non-alcoholic drinks, as well as to make bread, cookies, and biscuits.

The honey produced by stingless bees has medicinal value due to resinous substances produced from storage pots inside the hive (Boon 2002). Stingless bees are not utilised for honey production or pollination in India, but their honey is still used for cancer treatment, wound healing, ulcers, kidney disorders, intestinal infections, and other ailments in Ayurveda (Devanesan *et al.* 2009; Bruijin, 1996)^[10]. Honey from stingless bees is well known for its anti-inflammatory and antibacterial characteristics, which contribute to its potency in treating wounds and preventing both internal and exterior infections.

Pollen

Stingless bee workers gather pollen grains from flowers and store them as Beebread in pots inside the nest. Pollen is rich in proteins, minerals and vitamins and provides these nutrients to the bees. In addition to being added to infant food, pollen can be packaged and used as a dietary supplement. Numerous cosmetic preparations also employ it.

Beeswax

Wax is produced by glands in the worker bee's abdomen and utilised to make pots. The store pots can be turned into wax by hot water extraction after being used to extract honey or Beebread. Beeswax can be used in makeup products to improve the health of the skin and body. Beeswax can be used to make lip balms, body and facial creams, and lotions (Taye, 2019)^[39]. Beeswax can also be used to make industrial products like textiles, polishes (for wood, floors, and leather), and candles in addition to these health products. Industrial beeswax is used in the pharmaceutical industry to cover tablets and capsules.

Conclusion

Stingless bee is a eusocial insect that lives together in colonies. The nest of *Tetragonula iridipennis* Smith is the main centre of their activity. In the pollination of crops, stingless bees are very useful. Besides that, from the nest many useful products like – honey, pollen, beeswax, and propolis are received. So *Tetragonula iridipennis* is very important for human life. Artificial hives can be planted near agricultural fields for its management. Their social organization attracts several unexplored aspects to be resolved. This review paper will provide essential cues for the first time towards the importance of understanding the nesting biology of *Tetragonula iridipennis* in India. It will further disclose relationships between the behaviour of different castes and colony populations that may influence the amount of food stored within the nest. Thus present review paper will promote the Artificial Domestication and Management Programme (ADMP) of *Tetragonula iridipennis* Smith in India.

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